



## Monitoring wheat root development in a rainfed vertisol: Tillage effect

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### ABSTRACT

Good root system development is essential for optimum wheat (*Triticum aestivum* L.) grain yield, especially under water-limiting conditions. Published information about the influence of tillage system on root dynamic and their effect on grain yield in Mediterranean rainfed Vertisols is scarce. A three-year field study was conducted on a typical Mediterranean rainfed Vertisol to determine, using a minirhizotron system, the effects of tillage system on root growth and grain yield in wheat. Tillage treatments were no-tillage (NT) and conventional tillage (CT). The parameters measured were root length (RL) and root diameter (RD) for 6 depths. Minirhizotron measurements were performed at 5 wheat growth stages. The RL was greater under NT than under CT for most growth stages and depths, this being the key to its greater grain yield (3.2 vs 3.0 Mg ha<sup>-1</sup>, respectively). The RD was not significantly affected by the tillage treatments, but was lower from stem elongation onward and during the dry years. The key to the development of a good wheat root system is the rainfall received during the tillering stage, regardless of soil water content at planting and rainfall before or after this growth stage. Under the rainfed Mediterranean Vertisol studied, wheat productivity is greater under NT due to better root system development.

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### 1. Introduction

Little is known about root dynamics, despite their importance, as root systems are difficult to access. The study of the root growth pattern may help to determine how it is affected by rainfall distribution and when it is ready for the maximum quantity of N fertilizer. Pregitzer (2002) suggests that our knowledge and understanding of fine roots (<2 mm Ø), their length and diameter, structural and functional diversity, longevity and renovation times are still highly deficient. The study of plant root systems under field conditions is rather difficult and complicated, because the soil limits access to the roots for observation purposes. Root length density is an important parameter for the characterization of the root system, in particular, the behaviour of fine roots, and for the prediction of its response to changes in the environment (Fitter, 1985). Root diameter is one of the most important parameters for rhizosphere modelling (Himmelbauer et al., 2004). At the plant level, large-diameter roots account for most of the root system biomass. Small-diameter roots account for most of the root system surface area and are the site of the soil-plant exchanges responsible for water and nutrient uptake (Eissenstat and Yanai, 2002; Waisel and Eshel, 2002). Wheat root growth decreases or ceases after anthesis; however, this pattern may vary as a function of soil water content and N status (Campbell et al., 1977). Gooding et al. (2005) and Andersson et al. (2005) argue

that roots growing after anthesis may compete with grain for C and N, or play a major role in N translocation to the grain.

A number of studies report greater water storage under NT than under CT, and their effect on grain yield increase (Power et al., 1986; Unger, 1984). However, López-Bellido et al. (2007a,b), under Mediterranean conditions, reported that NT was not more efficient than CT in soil water accumulation and productivity. Merrill et al. (1996) recorded an appreciably greater increase in root growth under NT when compared to CT, attributing this increase to improved soil water use and storage, particularly in the area close to the soil surface. They also reported that spring wheat roots penetrated the soil to a greater depth under NT, due to the existence of a more favourable topsoil environment. Clark et al. (2003), however, suggest that the topsoil may be harder under NT, while conventional tillage operations may cause soil compaction just below the ploughed layer, leading to the formation of a plough pan, which may affect root system development.

Root studies face a constant effort to reduce the time, labour and cost of traditional methods, and chiefly to overcome the shortcomings of destructive sampling needed to obtain roots. Besides preventing repeated measurements and simultaneous observation of related phenomena at the same site, this bears important consequences on the quality of root data collected at each sampling time, since the number of samples is often lower than required for statistical significance and high-resolution studies are not feasible (Amato et al., 2009). In recent years, increasing use has been made of non-destructive techniques, such as the minirhizotron technique, to study the root system in situ. Hendricks et al. (2006) report that

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the minirhizotron is one of the best tools available for obtaining higher-quality data on the root system and thus, furthering our knowledge of root growth, demographics and dynamics. However, this method is not without its constraints: (i) root diameters greater than 2 mm, making this method best suited to the study of fine roots (Zeng et al., 2006), capable of detecting root diameters as small as 0.09 mm (Pierret et al., 2005); (ii) heavy textured soils, soils with argillic horizons, or loose structureless soils, which pose greater challenges for the installation of minirhizotrons (Hendrick and Pregitzer, 1996); (iii) wet clay soils which can smear the outside surface of the minirhizotron tubes, reducing visibility; and (iv) cracks which develop when some clayey soils dry, allowing light to penetrate deep into the soil and possibly into the minirhizotron tubes (Dubach and Russelle, 1995). Most studies report that the minirhizotron underestimates root length in the upper soil layers compared to root length density, and overestimates root length in the deeper soil layers when compared to the soil core method (Heeraman and Juma, 1993; Wiesler and Horst, 1994; Liedgens and Richner, 2001; Merrill et al., 2002; Machado and Oliveira, 2003). Underestimation has been attributed to poor soil/tube wall contact and inhibition of root growth caused by light entering the topsoil. In deep soil layers, root growth tends to be overestimated because of preferential root growth along the tube.

In light of previous studies carried out in the same experiment by López-Bellido et al. (2007a,b) and Muñoz-Romero et al. (2010), it is difficult to understand why NT is sometimes more productive than CT in a Mediterranean rainfed Vertisol. Both studies showed that soil water at planting was not a key factor. As a result, our hypothesis was that the Muñoz-Romero et al. (2010) study was not performed at a depth deep enough to prove that the root system is not a key factor in grain yield differences between tillage systems. Therefore, our objective was to perform a deeper wheat root system study to: (i) determine the effects of tillage system on seasonal growth, (ii) determine the relationship between the root system and grain yield, and (iii) determine the key growth stage for good root system development with respect to rainfall.

## 2. Materials and methods

### 2.1. Site

Field experiments were conducted in Córdoba, southern Spain (37°46'N, 4°31'W, 280 m a.s.l.), on a Vertisol (Typic Haploxererts) typical of the Mediterranean region, where rainfed cropping is the standard practice. Weather-related parameters for this area are as follows: average annual rainfall, 584 mm (39%, October–December; 37%, January–March; 19%, April–June; and 5%, July–September); average annual evapotranspiration, 1000 mm; average duration of dry period, 4–6 months; average annual temperature, 17.5 °C; average temperature in the coldest month, 9.5 °C; and average temperature in the warmest month, 27.5 °C.

### 2.2. Experimental design

The study took place over a 3-year period (2003–2004, 2005–2006 and 2006–2007) within the framework of a long-term experiment started in 1986. No data was recorded for 2004–2005, since a severe drought prevented the minirhizotron from being installed in the soil. Treatments were NT and CT. The experimental design was a randomized complete block with three replications. The area of each experimental plot was 10 m × 5 m (50 m<sup>2</sup>). Wheat was grown in a 2 year-rotation with faba bean (*Vicia faba* L.). The rotation was duplicated in reverse crop sequence in order to obtain data for both crops on a yearly basis.

### 2.3. Crop management

Weeds were controlled with glyphosate [N-(phosphomethyl) glycine]+MCPA [(4-chloro-2-methylphenoxy) acetic acid] at a rate of 0.87 + 0.60 kg a.i. ha<sup>-1</sup> before planting. Conventional tillage treatment included mouldboard ploughing (25–30 cm depth) and disk harrowing and/or vibrating tine cultivation (10–15 cm depth) several times to grind clods. The crop residues were not removed from either tillage method. Wheat cv. Gazul was planted in 18-cm-wide rows between 1 and 10 December of each year of the 3-year period at a seeding rate of 150 kg ha<sup>-1</sup>. Wheat was harvested between 1 and 15 June. Nitrogen fertilizer (100 kg ha<sup>-1</sup>) was only applied to the wheat plots as ammonium nitrate; one half was applied at tillering and the other half at stem elongation. Each year, the wheat plots were also supplied with P fertilizer at a rate of 65 kg ha<sup>-1</sup>; the fertilizer was incorporated in CT and banded with a drill in the NT plots. Soil test K (ammonium acetate) was adequate ( $\geq 530$  mg kg<sup>-1</sup>) and therefore K was not applied. During the wheat growing season, weeds were controlled using diclofop methyl [2-(4-(2,4-dichlorophenoxy)phenoxy)propanoic methyl]+tribenuron {methyl-2-[(N-4-methoxy-6-methyl-1,3,5-triazin-2-yl)-N-methylamino]carbonyl amino sulphonyl} benzoate} at 0.9 + 15 g a.i. ha<sup>-1</sup>, respectively.

### 2.4. Measurements

One soil sample was taken to determine bulk density (Blake and Hartage, 1986). Soil sampling was performed prior to planting. Samples were taken of four different layers using a manual Eijkelkamp auger: 0–15, 15–30, 30–60 and 60–90 cm. Soil water content was determined with two measurements per wheat plot at planting and at harvest to a depth of 0.9 in 0.3-m increments using a ThetaProbe mL2x soil moisture sensor (AT Delta-T Devices, Cambridge, UK). Root measurements were made with the CI-600 root growth monitoring system (CID Bio-Science, Camas, WA, USA), fitted with a scanner head for collecting images, a laptop computer and 1.8 m standard clear soil tubes (50.8 mm internal diameter) with end caps. The images were processed with WinRhizotron® software (Regent Instruments Inc., Canada), which provided RL (mm cm<sup>-2</sup>) and RD (mm) values for each plot and each wheat growth stage for both tillage systems tested. After wheat emergence, a plastic tube was installed on a permanent basis to harvest at the centre of each plot on the sowing line, 45° off vertical as recommended by Johnson et al. (2001). An auger of the same external diameter as the tube was used to facilitate close tube-soil contact. In turn, the scanner was inserted into each tube to a depth of 100 cm; images were captured at 6 depths with the aid of an automatic indexing handle, equivalent (given the angle of the tube at 45° off vertical) to 0–15, 15–30, 30–50, 50–65, 65–80 and 80–100 cm. Images were captured at the following Zadoks growth stages (Zadoks et al., 1974): tillering (21), stem elongation (31), booting (41), flowering half-way (65), and ripening (81). Measurements were made between late January and mid-May of the three study years. The wheat grain yield (harvested in early June) was determined using a 1.5 m wide Nurserymaster Elite Plot Combine (Wintersteiger, Ried, Austria) (30 m<sup>2</sup> per plot). At harvest, a 0.5 m<sup>2</sup> portion was sampled at the centre of each wheat plot. The yield components (head m<sup>-2</sup>, grain head<sup>-1</sup>, and 1000 grain weight) were determined from this sample.

### 2.5. Statistical analyses

Annual data for each variable over the total 3-year period were subjected to analysis of variance (ANOVA), using a randomized block design combined over years and the error term according to McIntosh (1983). Tillage system and year were considered fixed

effects. Growth stage and soil depth were considered as repeated-measure variables. Means were compared using Fisher's protected least significant difference (LSD) test at  $P < 0.05$ . LSDs for the different main effects and interaction comparisons were calculated using the appropriate standard error terms. The Statistix v. 8.1 (Analytical Software, 2005) package was used for this purpose.

### 3. Results

#### 3.1. Weather conditions and soil water content

The wettest year was 2003–2004 (704 mm; rainfall from September to August), while the other two years had a low rainfall (2005–2006: 402 mm; 2006–2007: 414 mm). Average annual rainfall for this area is 584 mm. The rainfall prior to planting was 373, 120 and 137 mm for each year, respectively. Soil water content before planting and at harvest for each year was significantly different. The values for each year at sowing and harvest were  $0.36, 0.24,$  and  $0.32 \text{ m}^3 \text{ m}^{-3}$  and  $0.30, 0.12$  and  $0.22 \text{ m}^3 \text{ m}^{-3}$ , respectively. Significant differences between tillage systems were observed only in soil water content before planting in the second year (2005–2006):  $0.28$  and  $0.21 \text{ m}^3 \text{ m}^{-3}$  under NT and CT, respectively.

Rainfall between planting and tillering was highest in 2006–2007 (Fig. 1). Between tillering and stem elongation, the

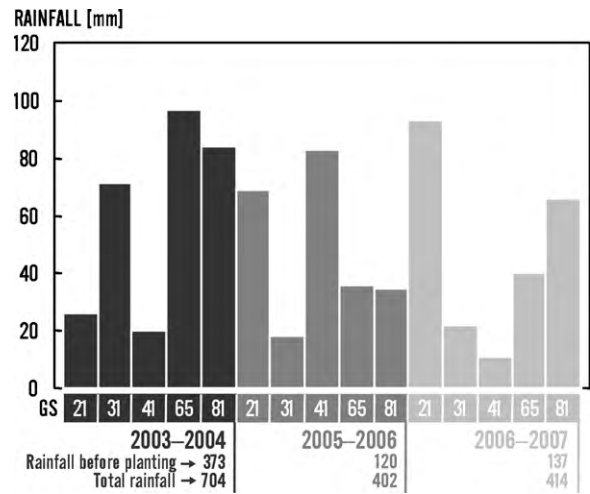


Fig. 1. Accumulated rainfall between the Zadoks growth stage (GS)(Zadoks et al., 1974) indicated and the preceding one. Growth stage 21 represents the amount between planting and this GS.

greatest rainfall was recorded in 2003–2004 (71 mm), while rainfall was scarce in the other two years (around 20 mm) (Fig. 1). Between

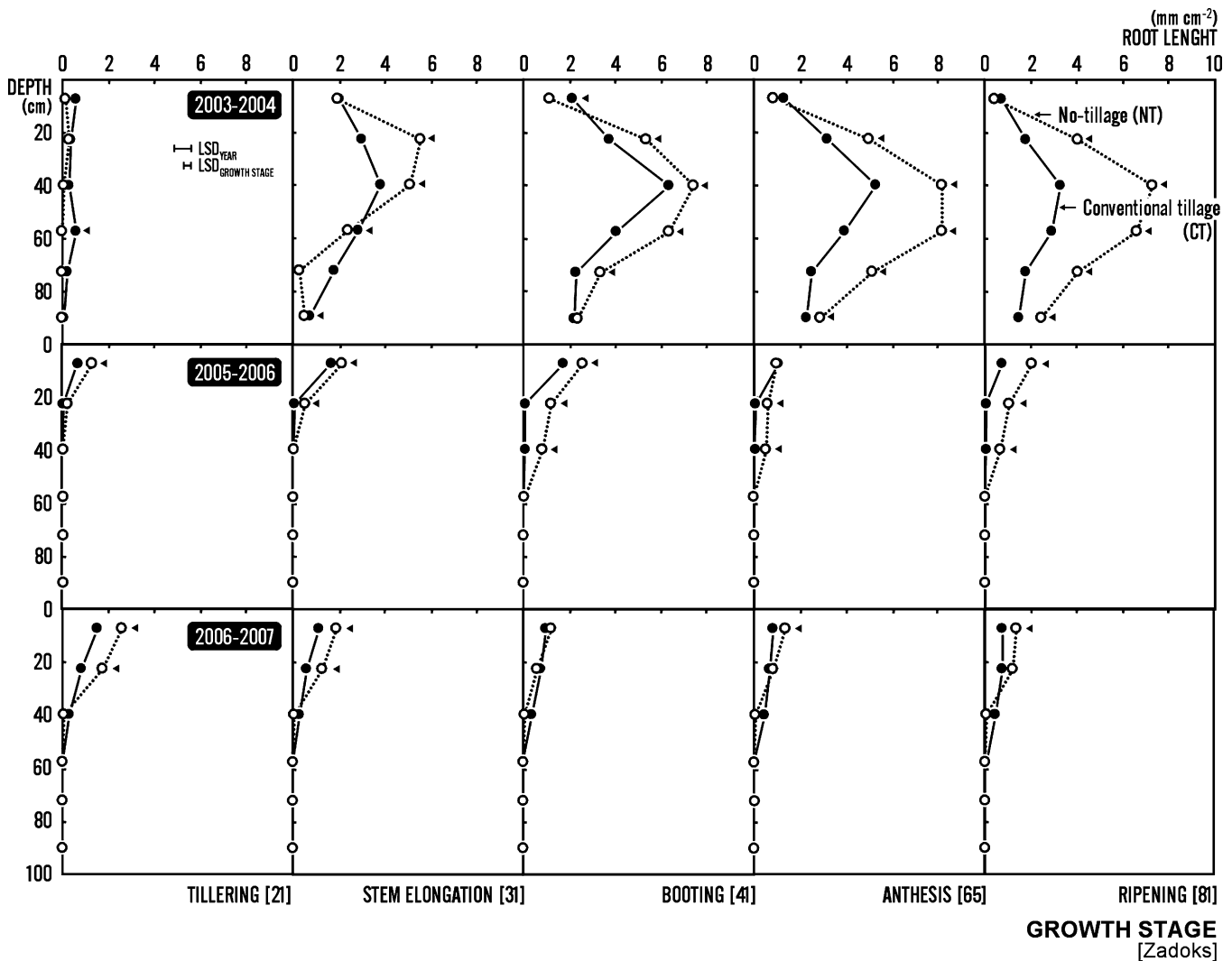


Fig. 2. Wheat root length as influenced by year and tillage system for different soil depths and growth stages (Zadoks et al., 1974). The triangle ( $\Delta$ ) represents significant difference between tillage systems. Bar represent LSD for comparison:  $LSD_{YEAR}$ , different levels of year;  $LSD_{GROWTH\_STAGE}$ , the same levels of year and tillage system.

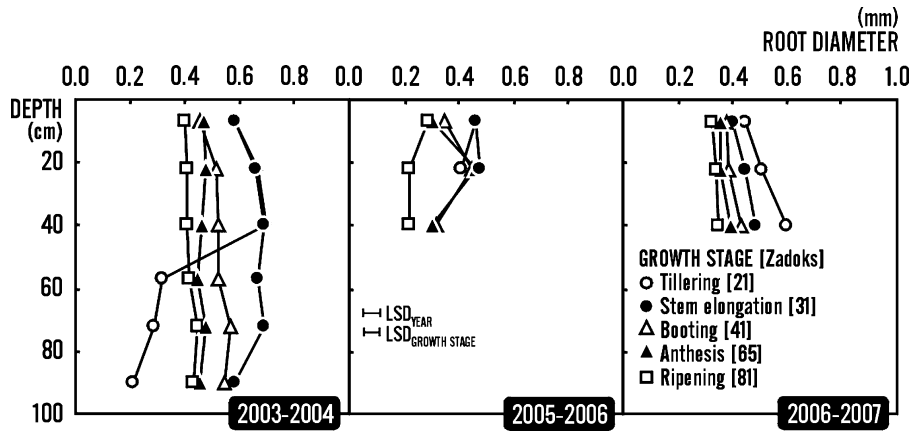


Fig. 3. Wheat root diameter as influenced by year for different soil depths and growth stages (Zadoks et al., 1974). Bars represent LSD for comparison:  $LSD_{YEAR}$ , different levels of year;  $LSD_{GROWTH\_STAGE}$ , the same level of year and soil depth.

stem elongation and booting, the rainfall recorded was high in 2005–2006 (83 mm) and very low for the other years (Fig. 1). From the booting stage until ripening, rainfall was high in 2003–2004 and low in the other two years (Fig. 1).

### 3.2. Root growth and grain yield

The year exerted a strong influence on RL across the soil profile, with RL increasing with soil water content at planting and growing season rainfall (Figs. 1 and 2). The RL showed the highest values in 2003–2004 (Fig. 2). This year also exhibited greater variation across the 0–100 cm profile than in the other years. However, greater RL values were obtained for the beginning of the tillering stage in 2006–2007 (Fig. 2). In 2003–2004, the pattern observed for mean RL with the minirhizotron was an increase in RL from the surface until a maximum depth of between 40 and 60 cm, followed by a subsequent decrease (Fig. 2). In the other two years studied, the RL growth pattern was a decrease in RL as the soil depth increases (Fig. 2). In both years, RL was poor, with no roots found at depths greater than 50 cm (Fig. 2). In the year 2003–2004, the crop received 71 mm rainfall during tillering; while in other years, rainfall was around 20 mm.

The RLs were higher under NT than under CT for most of the growth stages and depths (Fig. 2). This was more evident in the wettest year (2003–2004). In this year, the increase in values under NT was observed from stem elongation onwards, at depths below 15 cm. Differences were not apparent in the uppermost (0–15 cm) horizons except for in the booting stage where RL under CT was higher than under NT (Fig. 2). In the dry years, differences in RL as a function of tillage system in favour of NT were observed primarily in the first 0–50 cm in 2005–2006 and in the first 0–30 cm in 2006–2007 (Fig. 2). The bulk density values did not show differences between tillage systems, with a mean value for the years of 0.92, 0.98, 1.06 and 1.09  $Mg\ m^{-3}$  for depths of 0–15, 15–30, 30–60 and 60–90 cm.

The maximum RL in 2003–2004 was produced at anthesis under NT and at booting under CT (Fig. 2). However, in 2005–2006, the maximum was produced at booting under both tillage systems preceded by rainy period of 83 mm (Figs. 1 and 2). In 2006–2007, the maximum RL was observed at tillering for both tillage systems preceded by 93 mm rainfall (Figs. 1 and 2).

In the rainiest year (2003–2004), RD values were higher than in the other two years at all depths and varied throughout the soil profile from tillering to booting, but without a clear pattern (Fig. 3). In general, the greatest diameters were observed during tillering and/or stem elongation. Tillage system treatments exerted no influence on RD in any of the study years.

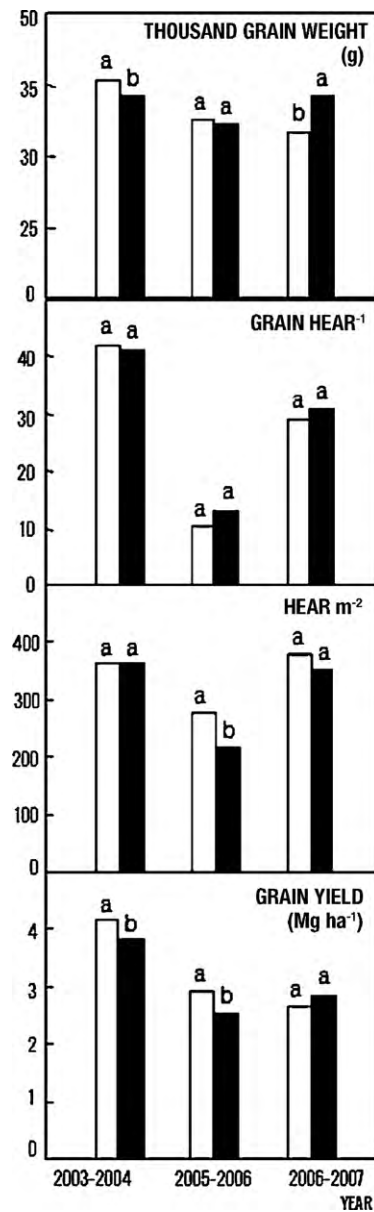


Fig. 4. Grain yield and yield components. Year  $\times$  tillage effects on grain yield and yield components. Within years means followed by the same letter are not significantly different at  $P < 0.05$  according to LSD.

Grain yield was significantly higher under NT than under CT for the years 2003–2004 and 2005–2006 (Fig. 4), precisely the years with the greatest significant differences in RL for the different growth stages and depths (Fig. 2). Grain yield increased with the increase in RL at anthesis [grain yield ( $\text{kg ha}^{-1}$ ) =  $0.31\text{RL} (\text{mm cm}^{-2}) + 2.64$ ;  $r = 0.96$ ,  $n = 6$ ,  $P < 0.001$ ]. Thousand grain weight was higher for NT in the year 2003–2004, with the opposite occurring in the year 2006–2007. Head  $\text{m}^{-2}$  was only greater under NT for the year 2005–2006.

#### 4. Discussion

Although rainfall before sowing was very similar in the years 2005–2006 and 2006–2007, the rainfall distribution was different for both years. This led to significant differences only in the soil water content at sowing with respect to tillage system in the year 2005–2006. López-Bellido et al. (2007a) argued that the typical extremely hot summers can destroy any possible soil water content difference due to an increased soil area exposed to the atmosphere by the formation of large cracks.

In the wettest year (2003–2004), the higher RL values are in accordance with Fordham (1969), who reported that the root system is longer when water is not a limiting factor. The typical pattern is a decrease in RL as soil depth increases. The only optimal year in terms of required rainfall for wheat development was 2003–2004. The reduced RL in the upper horizons of a Vertisol can be attributed to the soil hardness which prevents the hole from being drilled with precision for the installation of the minirhizotron tube, and to the formation of small cracks on the soil surface during the growth season. This leads to poor contact between the tube and heavy clay soil in these horizons, allowing light to enter, thus reducing RL (Dubach and Russelle, 1995; Liedgens and Richner, 2001; Merrill et al., 2002). In the other two years studied, the RL growth pattern was consistent with that normally obtained by the soil core method, due to the very dry years, which deprived the deepest horizons of water, hindering the development of the root system.

The key growth stage for the development of an optimal root system was tillering, being strongly dependent on the rainfall. Even though other later growth stages received little rainfall in 2003–2004, the root system continued to develop adequately. According to Dickin and Wright (2008), prolific tiller production facilitates compensation and may be associated with increased nodal root initiation. On the contrary, in 2005–2006, for example, the high rainfall recorded between stem elongation and booting (83 mm) did not induce the development of an optimal RL. This can be attributed to the variations in the number of tillers formed during tillering, which was lower in this last year and therefore the root system could not increase its density. This occurrence is reflected in the lower number of head  $\text{m}^{-2}$  for this year (Fig. 4).

In 2003–2004, the higher RL under CT in the upper horizon during the booting stage could be due to the existence of greater soil compaction in the first 15 cm under NT. According to Huwe (2003), under NT, compaction may occur at the surface soil, while under CT this occurs at a greater depth, beneath the ploughed layer. When roots encounter hard soil, extension is restricted and apices have much shorter elongation zones and are distorted (Watt et al., 2006). Nonetheless, the bulk density values obtained do not differ between tillage systems for any of the years. Jordán et al. (2010) reported that long-term studies, have shown that bulk density under reduced tillage long-term experiments was comparable with conventional tillage. In addition, Deen and Katakai (2003) compared the results from long-term conventional tillage practices, and reported that different management practices had no significant

influence on soil bulk density at different soil layers between 0 and 60 cm. The increase in RL under NT vs CT obtained in the dry year of our experiment was also reported by Merrill et al. (1996), even under low rainfall conditions, during wheat booting and anthesis. The increase in water use under NT with respect to CT during the dry years reported by López-Bellido et al. (2007b) in rainfed Vertisols under Mediterranean conditions may be due to this greater root development under NT.

In the year 2003–2004, when there was no strong water stress, the maximum wheat RL was observed at anthesis, while in the two dry years, the maximum RLs were always preceded by rainy periods. Campbell et al. (1977) showed that the root system of wheat increases up until anthesis, although this may vary as a function of soil moisture levels. In the two dry years of our study an increase in RL was observed after anthesis only under NT (Fig. 2). Herrera et al. (2007) reported that the percentage of roots grown after anthesis ranges from 1% to 22% of the total roots grown by physiological maturity, demonstrating that the root growth of spring wheat can be high after anthesis.

The smaller root diameters in the dry years can be due to the greater resistance to penetration. Aggarwal et al. (2006) showed a greater RD in the first 30 cm than in the rest of the profile in dry years. However, it did not occur in this study for the last two dry years. Tillage system did not affect RD, which could be justified by the absence of significant differences in the bulk density between both tillage systems, as previously mentioned. Nevertheless, there is discrepancy in preceding studies. Sidiras et al. (2001) reported thicker barley roots under CT, in contrast to Braim et al. (1992); while Qin et al. (2004) found no effect of tillage on the diameter of wheat roots.

The RL-grain yield relationship obtained was also shown by Izumi et al. (2004) and Chakraborty et al. (2008). The significantly positive correlation between RL and yield involve not only the amount but the spatial enlargement of a root system, considered important for water and nutrient acquisition and closely related to productivity (Izumi et al., 2004). Merrill et al. (1996) found greater grain yield under NT, just as in the first two years of this experiment, attributing it to a greater root density.

#### 5. Conclusions

In a Vertisol under rainfed Mediterranean conditions, root system development and productivity were both greater under NT than CT. It has been shown that a key factor in the development of a good wheat root system is the rainfall recorded during the tillering stage, regardless of both soil water content at planting and rainfall before or after of this growth stage. This fact could be of interest in connection with the nitrogen fertilizer response as a function of the rainfall-root system development at tillering. The RD did not vary with respect to tillage system. The RD decreases when the year is dry and from the stem elongation stage onward. These results suggest the need for further studies to determine whether the greater RL under NT is due to less compaction of the soil with respect to CT.

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