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RESEARCH PAPERS

Impacts of previous crops on Fusarium foot and root rot, and on yields of durum wheat in North West Tunisia

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Summary. The impacts of ten previous crop rotations (cereals, legumes and fallow) on Fusarium foot and root rot of durum wheat were investigated for three cropping seasons in a trial established in 2004 in Northwest Tunisia. Fungi isolated from the roots and stem bases were identified using morphological and molecular methods, and were primarily *Fusarium culmorum* and *F. pseudograminearum*. Under low rainfall conditions, the previous crop affected *F. pseudograminearum* incidence on durum wheat roots but not *F. culmorum*. Compared to continuous cropping of durum wheat, barley as a previous crop increased disease incidence more than fivefold, while legumes and fallow tended to reduce incidence. Barley as a previous crop increased wheat disease severity by 47%, compared to other rotations. Grain yield was negatively correlated with the incidence of *F. culmorum* infection, both in roots and stem bases, and fitted an exponential model (R^2 = -0.61 for roots and -0.77 for stem bases, P<0.0001). *Fusarium pseudograminearum* was also negatively correlated with yield and fitted an exponential model (R^2 = -0.53 on roots and -0.71 on stem bases, P < 0.0001) but was not correlated with severity.

Key words: rotation crops, cereals, *Fusarium culmorum*, *F. pseudograminearum*.

Introduction

Fusarium foot and root rot is one of the important diseases of cereals throughout the world and has been reported since the 1970's in Tunisia (Ghodbane *et al*., 1974; Gargouri *et al*., 2001). Different cereals can be infected and durum wheat, which makes up than 50% of the total cereals area cultivated in Tunisia (Slama *et al*., 2005; Anonymous, 2013), is the most susceptible (Wallwork *et al*., 2004). Up to 26% yield losses have been recorded on durum wheat in Tunisia (Chekali *et* al., 2013). The disease is caused by a complex of *Fusarium* species. The most important are *F. culmorum* (W.G. Sm.) Sacc, and *F. pseudograminearum* (O'Donnell & T. Aoki; group I) (= teleomorph *Gibberella coronicola*) (Smiley *et al*., 1996; Paulitz, 2006). These two pathogens produce lesions on the coleoptiles, roots, and sub crown internodes of host plants, and cause browning of the stem bases at or near the soil surface, from soil- or residue-borne inoculum. Damage to cereals is often unnoticed until whiteheads appear in crops shortly before maturity, or until shriveled grain is noticed during harvest (Papendick and Cook, 1974; Burgess *et al*., 2001).

Infection is favoured by wet conditions shortly after seeding, and disease severity increases and yield reductions become significant when infected plants are subjected to water stress and/or high temperatures late in the growing season (Papendick and Cook, 1974; Cook, 1980; Cariddi and Catalano, 1990; Paulitz *et al*., 2002; Smiley *et al*., 1996, 2005; Doohan *et al*. 2003).

Common in-crop disease control methods such as applying fungicides to seed or crops are currently not

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available for foot and root rot, and cultivar resistance is limited. Therefore, management of this disease relies on introduction of non-hosts in the cropping sequences and other cultural techniques (Burgess *et al*., 2001; Kirkegaard *et al*., 2004; Lamprecht *et al*., 2006; Evans *et al*., 2010). Many studies have shown the benefits of non-host crops and fallow in managing foot and root rot in several countries, including the United Kingdom (Bateman and Kwasana, 1999; Bateman and Murray, 2002) and Australia (Sturz and Bernier, 1989; Felton *et al*., 1998; Kirkegaard *et al*., 2004; Lamprecht *et al*., 2006; Evans *et al*., 2010). However, little research has focused on Fusarium foot and root rot in Tunisia, and none has focused on disease control strategies. Hence, it is important to understand and quantify the effects of hosts and non-hosts on inoculum levels of these *Fusarium* spp. in the Tunisian farming systems. The objective of this study was to determine the effects of previous crops on foot and rot development and interactions of the disease with durum wheat yield.

Materials and methods

Site description

Multi-year experiments were conducted during the 2007/08, 2008/09 and 2009/10 growing seasons, in a field trial performed to study the behaviour of durum wheat subjected to different previous rotation crops since 2004. The trial was at the Experimental Station of the National Agronomic Research Institute of Tunisia (INRAT) at Kef. This region is in northwest Tunisia, with an elevation of 780 m, and is characterized by Mediterranean climate, with cold humid winters and very hot dry summers. Most rain normally occurs from December to February each year, and becomes irregular from March to May, with average annual rainfall of 468 mm.

Experimental design and management

Experiments were set out in randomized complete block designs (RCBD). For each experiment, three blocks were divided into 10 plots corresponding to the most common rotation crops used by Tunisian durum wheat farmers (Table 1). The plot area was 90 m^2 (30 m × 30 m); and spacings were 17 cm between plant rows and 1.5 m between plots. The year and crop rotation were considered as main factors. Appropriate crop operations were applied. Seeds of barley, durum and bread wheat were treated with 2.5% triticonazole then sown at a rate of 150 kg ha^{-1} for wheat, and 120 kg ha^{-1} for barley, at dates between 13 and 16 November in 2007 and 2009 and between 11 and 15 November in 2008. The legumes chickpea, medic, lentil, and faba bean, used as a green manures, were sown at 100, 27, 100 and 140 kg ha^{-1}, respectively, after the first rains of autumn, except for spring chickpea. Before sowing, soil of all treatments, except for fallow, was ploughed at 20 cm depth and harrowed twice (10–15 cm depth). At planting, di-ammonium phosphate (100 kg ha^{-1}) was applied, and ammonium nitrate (100 kg ha^{-1}) was ap-

Table 1. Crop rotation applied to durum wheat at the experimental station of INRAT at Kef.

Cereal species	2007–08	2008–09	2009–10
	Rotation tested type		
Durum wheat	DW	DW	DW
	DW / WCP	WCP / DW	DW / WCP
	DW / Med	Med / DW	DW / Med
	DW /SCP	SCP / DW	DW /SCP
	DW /F	F / DW	DW / F
	DW /L	L / DW	DW /L
	DW / FB	FB / DW	DW / FB
	DW /B	B / DW	DW / B
	DW/B/L	L / DW / B	B / L / DW
	DW / BW / GM	GM / DW/ BW	BW / GM / DW

DW, Durum wheat; BW, Bread wheat; B, Barley; L, Lentil; WCP, Winter chickpea; SCP, Spring chickpea; FB, Faba bean; Med, Medic; GM, Green manure (faba-bean); F, Fallow.

plied at tillering and stem elongation. Weeds were controlled with mesosulfuron (30 g kg^{-1}) + iodosulfuron (30 g kg^{-1}) + mefenpyrdiethyl (90 g kg^{-1}). The harvest of durum wheat was in mid-June.

Isolation and identification of fungi

Twenty mature plants of durum wheat were removed from each plot using a zigzag sampling method, and then thoroughly washed under running water. Two millimeter sections of the stem bases and roots were surface disinfested for 2 min in bleach (NaOCl) at 6% concentration and for 10 sec in alcohol at 70%, rinsed with sterile distilled water, dried, and placed on ¼ strength PDA + 100 mg L^{-1} streptomycin sulfate. Cultures were incubated for 5–6 days under a photoperiod of 12 h light / 12 h dark at 25°C. *Fusarium* spp. hyphae were transferred onto Carnation leaf agar (CLA) medium to favour conidia production. *Fusarium culmorum* and *F. pseudograminearum* were identified morphologically according to Leslie and Summerell (2006). Identities were confirmed by molecular techniques using the Möller *et al*. (1992) protocol for DNA extraction and the Schilling *et al.* (1996) and Aoki and O'Donnell (1999) protocols and primers for *F. culmorum* and *F. pseudograminearum*, for PCR amplification and identification.

Disease incidence and severity measurements

The incidence of infections were determined by the frequency of isolation of each *Fusarium* species, and the incidence of the plant inflorescences senescing prematurely and showing white coloration (whiteheads). Plants were assessed at the anthesis stage (end of April). Their numbers of infected heads were estimated visually in each treatment three times and expressed as percent of total heads in each plot.

Disease severity was assessed at the end of April, using a scale of 0 to 3, with 0 = no symptoms, 1 = light browning at the first inter-node (0–25%), 2 = browning extending to second inter-node (clear brown) (25–50%), and 3 = browning extension to the second inter-node (dark brown) (75–100%).

Yield parameter measurements

Biomass (kg), grain yield (kg ha^{-1}) and thousand kernel weight (TKW; g) of durum wheat subjected to different crop rotations were estimated during each year at the end of the experiment.

Statistical analyses

Data were analyzed as a multi factorial analysis of variance (ANOVA), with three replicates, with rotation and year as main factors, using SAS JMP software Version 4.0 published by SAS. Means comparison was performed using Student LSD tests of at $P<0.05$. Due to the ordinal nature of the severity data, they were analyzed using the nonparametric test of Kruskal-Wallis. Pearson's correlation was analyzed by STATISTICA software Version 0.13. Nonlinear regression models were fitted to the yield vs disease incidence data, using Sigma Plot 13.0. The best fits were exponential decay models, with two parameters.

Results

Identification of the pathogens

During the three cropping seasons 2007–08, 2008–09, and 2009–10, morphological identification of isolates recovered from 3600 roots and 1800 stem base sections of durum wheat samples mainly revealed the presence of *F. culmorum* (222 isolates from roots and 111 from stem bases) and *F. pseudograminearum* (132 from roots and 84 from stem bases). Other fungal species were recovered at much lower frequencies, such as *Bipolaris sorokiniana, Microdochium nivale*, *F. equiseti*, *F. solani* and *F. oxysporum.* Molecular identification using the specific primers OPT18 R, OPT18 F amplified approx. 470 bp of *F. culmorum* fragment (Schilling *et al*., 1996) and Fp1-1, Fp 1-2 amplified 520 bp of *F. pseudograminearum* fragments (Aoki and O'Donnell, 1999) (Figure 1).

Disease incidence

The incidence of infection both of *F. culmorum* and *F. pseudograminearum* on durum wheat roots and stem bases were highly significantly affected ($P<0.0001$) by climatic conditions of the three cropping seasons 2007/08, 2008/09, and 2009/10, data of each year were analyzed separately. The effects of the previous crops were statistically significant ($P<0.0001$) only on the incidence of *F. pseudograminearum* recovered from the roots of durum wheat col-

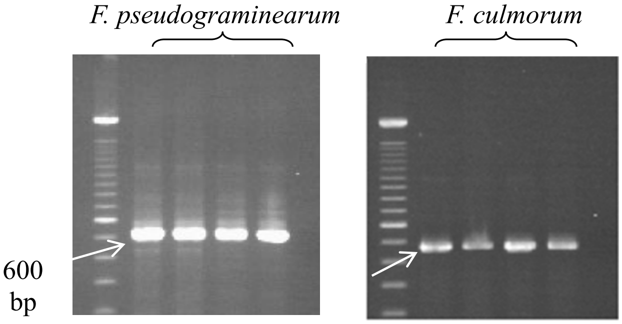

Figure 1. Agarose gel showing PCR products amplified with *Fusarium pseudograminearum* (Fp1-1, Fp1-2) and *F. culmorum* (OPT18R, OPT18F) specific primers: 600-bp ladder as the DNA size marker.

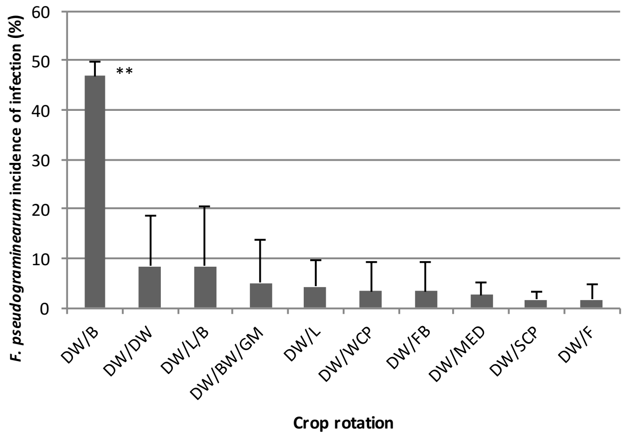

Figure 2. Impact of previous crops on *Fusarium pseudograminearum* incidence of infection in durum wheat roots of plants grown at in the Experimental Station of INRAT at Kef during the 2009–10 cropping season. Error bars are 2 × standard deviation. **: highly significant difference (P<0.001) compared to mono-cropping of durum wheat according to the Student test. DW: durum wheat; BW: bread wheat; B: barley; F: fallow; FB: faba bean; L: lentil; WCP: winter chickpea; SCP: spring chickpea; MED: medic; GM: green manure (faba bean).

Table 2. Analysis of variance of the incidence of infection by *Fusarium culmorum* (Fc) and *F. pseudograminearum* (Fpg) on roots and stem bases of durum wheat for 10 previous rotation crops during 2007/08, 2008/09 and 2009/10 cropping seasons.

Sources of variation	df	Incidence of infection			
		Roots		Stem bases	
		Fc	Fpg	Fc	Fpg
		P > F	P > F	P > F	P > F
Year	2	0.0001	0.0001	0.0001	0.005
Previous crop	9	0.261	0.0001	0.233	0.210
Year × previous crop	2	0.214	0.0001	0.653	0.550

DF: Degree of freedom.

lected during the 2009/10 cropping season (Table 2). During this year, barley culture as the previous crop increased the incidence of *F. pseudograminearum* more than fivefold when compared to durum wheat mono-cropping. However, when lentil was introduced in the rotation before barley, this increase did not occur. There were no significant effects with the nine other previous rotation crops; incidences of pathogens only tended to be less when chickpea and fallow preceded durum wheat (Figure 2). Percentage of whiteheads recorded at the end of heading stages was very low, and did not exceed 6%. Statistical analyses did not reveal any significant differences in the percentage of white heads of durum wheat between the years, nor among the different previous crops.

Disease severity

The previous crops significantly affected the disease severity. Compared to durum wheat mono-cropping, barley culture used as a previous crop increased disease severity (P<0.05). The P value of the crop rotation effect, using χ^2, was 0.05. However, no rotation decreased the disease severity compared to the durum wheat in monoculture (Figure 3).

Crop yields related to Fusarium foot and root rot disease

The grain yields, biomass and TKW of durum wheat were highly significantly (P<0.0001) affected by the yearly climatic conditions. These yield parameters were greater in 2008, with rainfall of 578 mm, compared to 2009 and 2007, with only 470 and 281 mm rainfall, respectively (Table 3).

According to Pearson's correlation test, the reduction in grain yield was negatively correlated with the

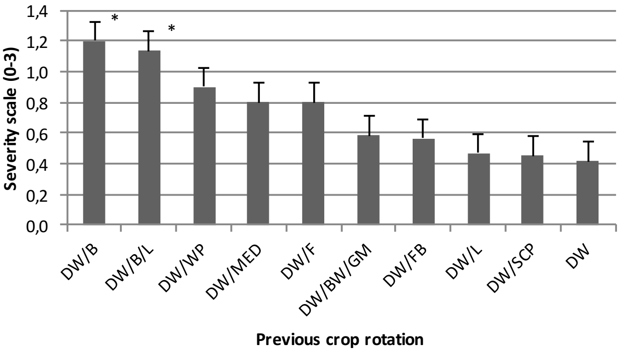

Figure 3. Impact of previous crops on durum wheat disease severity of plants grown at in the Experimental Station of INRAT in Kef during the 2009–10 cropping season. Vertical bars: 2 × standard deviation. *: significant difference (P<0.05) compared to mono-cropping of durum wheat according to the Student test. DW: durum wheat; BW: bread wheat; B: barley; FB: faba bean; L: lentil; WCP: winter chickpea; SCP: spring chickpea; MED: medic; GM: green manure (faba bean); F: fallow.

Table 3. Grain yield, biomass and thousand kernel weight (TKW) of durum wheat during 2007/08, 2008/09 and 2009/10 cropping seasons at the experimental station of INRAT at Kef.

Cropping year Yield parameters	2007–08	2008–09	2009–10
Grain yield (kg ha^{-1})	200	5203	800
Biomass (kg)	31	184	49
TKW (g)	23	50	41

incidence of infection by *F. culmorum*, both on durum wheat roots (R^2 = - 0.56, P=0.0013) and stem bases (R^2 = -0.61, P=0.0003). Similarly, *F. pseudograminearum* incidence on the roots (R^2 = - 0.30, P=0.10) and the stem bases (R^2 = - 0.52, P=0.003) of this cereal were negatively correlated with grain yields. The relationships between grain yields and incidence of infection of roots and stems by *F. culmorum* fit exponential decay models, with R^2 of 0.61 for stem bases and 0.77 for roots (P>0.0001; Figure 4). The relationships between grain yield and incidence of infection of roots and stems by *F. pseudogramineaum* fit exponential decay models, with R^2 of 0.53 for roots and 0.71 for stem bases (P>0.0001; Figure 5). No correlations were detected between the severity of the disease (amount of discolouration of stem bases) and all measured yield parameters.

Discussion

This study aimed to evaluate the impacts of different previous crops on the development of Fusarium crown and root rot of wheat. Different parameters were evaluated, including incidence of recovery of the dominant fungal pathogens from roots and stem bases, the percentage of white heads before maturity, disease severity based on discolouration on stem bases, and yield parameters.

Identification of *Fusarium* species isolated from the stems and the roots revealed that *F. culmorum* and *F. pseudograminearum* were the dominant pathogens. This was not surprising given that *F. culmorum* has been identified as an important fungus causing wheat diseases in Tunisia (Gargouri *et al*., 2007; Fakhfakh, 2012).

The incidences of infection by the two *Fusarium* species were significantly greater in the two dry cropping seasons 2007/08 and 2009/10 (12% for *F. culmorum* and 2.5 % for *F. pseudograminearum*), than in 2008/09. Distribution of rainfall and accumulated temperatures during each cropping season has likely to have influenced incidence of infection by the two fungi. These results are consistent with those of Poole *et al*. (2013), who demonstrated, during a survey in dry areas of the United States of America, that the yearly weather conditions influenced development of crown rot, and indicated that *F. culmorum* and *F. pseudograminearum* are susceptible to short-term environmental changes. In Tunisia, low cereal yields are often attributed to an insufficient rainfall distribution throughout the season (Ben Amar and Ben Abdellah, 1996). This effect would be exacerbated by effects of this disease.

This study showed that all previous crops did not affect the different disease parameters evaluated, except for barley which significantly increased the frequency of isolation of *F. pseudograminearum* from wheat roots during the cropping season 2008/2009. This was most likely due to low infection observed in this study, and the uneven distribution of the inoculum in the soil. Overall, all disease measures including frequency of recovery of the dominant pathogens, percentage of browning on the stems and percentage of white heads, were low. The low infec-

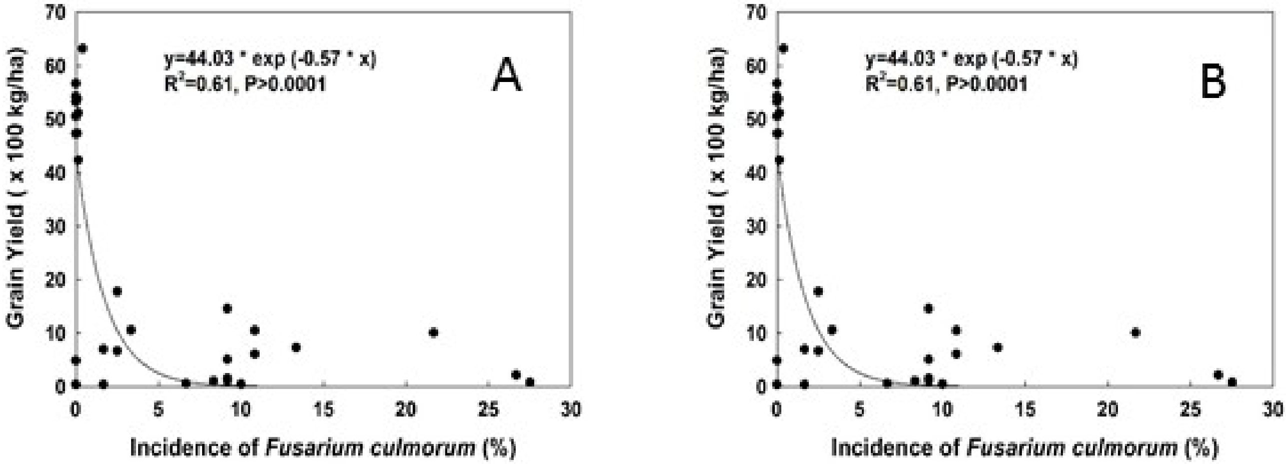

Figure 4. Mean of *Fusarium culmorum* (*Fc*) incidence of infection on roots (A) and stem bases (B) as a function of grain yield reduction for durum wheat cultivated in INRAT station at Kef over three experimental cropping seasons (2007–08, 2008–09 and 2009–10). Models were fitted with an exponential decay model with two parameters.

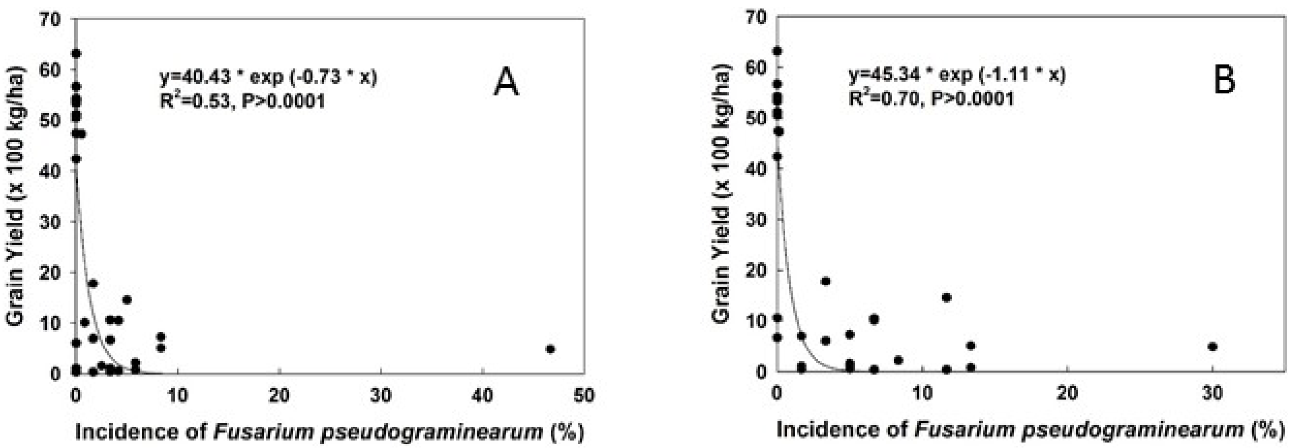

Figure 5. Mean of *Fusarium pseudograminearum* (*Fpg*) incidence of infection on roots and stem bases as a function of grain yield reduction for durum wheat cultivated in INRAT station at Kef over three experimental cropping seasons (2007/08, 2008/09 and 2009/10). Models were fitted with an exponential decay model with two parameters.

tion could either be due to unfavorable weather conditions for disease development, or low inoculum levels in the soil. Tillage is the main practice in Tunisia, and this results in a deep burial of crop residues and their rapid degradation, which may reduce the inoculum levels. Our results support those of Summerell *et al*. (1990), who explained that when infested stubble is maintained on the ground, pathogen penetration mainly occurs through the crown and basal parts of the host stems, while when the culms are incorporated into the soil or burned, penetration occurs through the lower parts of below ground host tissues.

Although not statistically significant, fallow used in the previous rotations tended to reduce the incidence of infection of *F. pseudograminearum* on durum wheat roots. This supports the results of Evans *et al*. (2010) and confirms that tillage reduced the inoculum level in the soil by burying and promoting the rapid degradation of infected plant residues. This also agrees with the results of Windels and Wiersma (1992), Bailey *et al*. (2000, 2001), and Paulitz *et al*. (2002), who reported lower levels of *Fusarium* spp. inoculum in the roots of wheat when the number of tillage operations increased.

Food legumes such as chickpea or faba bean have also tended to reduce disease incidence on the following wheat crops. Similar results were obtained in Australia (Felton *et al*., 1998; Kirkegaard *et al*., 2004; Evans *et al*., 2010) and in the USA (Smiley *et al*., 1996). This reduction can be attributed to the non-host character of these crops, or their high cellulose content which stimulates microbial activity and disadvantages *Fusarium* spp. development (Rasmussen *et al*., 2002).The additional soil moisture left after chickpea, related to its low consumption of water (Krouma, 2010), may decrease disease severity which is generally high when the plants are subject to dry conditions (Chekali *et al*., 2011).

Other studies have indicated that in the case of *F. culmorum,* the previous crop had little effect on subsequent disease, probably because this species persists for more than one season as chlamydospores and in crop residue in dry summer climates (Leslie and Summerell, 2006; Davis *et al*., 2009). These assumptions could be validated through future work under Tunisian conditions.

As mentioned above, previous barley significantly increased the incidence of infection by *F. pseudograminearum* recorded on durum wheat roots in 2009/10. These trends were also observed in the case of *F. culmorum* recovered from wheat stems and roots, however these changes were not statistically significant. These observations agree with the findings of Evans *et al*. (2010) in Australia. Therefore, it is likely that the superficial nature of barley root systems, as described by Moule (1971), favoured contact, development and inoculum conservation of *F. pseudograminearum* in the soil. The slow degradation of plant tissues in the dry conditions of Tunisia could also assist in maintaining the fungal inoculum in crop residues, thus promoting a strong infection on durum wheat crops. These two hypotheses can be considered and developed in future research.

In our study, negative correlations were observed between durum wheat grain yields and *Fusarium* spp. incidence. This is consistent with the results of Hollaway *et al.* (2013). The reduction in disease incidence observed on durum wheat after the previous fallow, faba bean and chickpea cropping sequences coincides with significantly greater yields (respectively, 3000, 2300 and 2100 kg ha^{-1}) compared to durum wheat (1900 kg ha^{-1}) and barley (16 q ha^{-1}) as the previous crops. These results confirm the benefits of using legumes as preceding crops in cereal systems, to increase grain yields and reduce Fusarium foot and root rot. However, use the fallow and non-host crops alone cannot be the only ways to reduce the inoculum of both pathogens in soil.

Our results indicated that dry years increased the effect of Fusarium foot and root rot. Break crops, such as legumes or fallow, can be good management strategies for reducing Fusarium foot and crown rot incidence. However, barley is likely to be unsuitable as a previous crop for durum wheat. The benefits of legumes as previous crops to increasing cereal yields are well known, particularly for their additional nitrogen contributions. Knowing that the cultivated area of legumes in Tunisia covers less than 10% compared to cereals covering 40% of total cultivated land, our results may be a tangible evidence for increasing legume crop rotation benefits rotation for cereal farming systems in Tunisia.

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