Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Paper

Impacts of previous crops on inoculum of *Fusarium culmorum* in soil, and development of foot and root rot of durum wheat in Tunisia

Eya KHEMIR[1,2,*], Samira CHEKALI[3], Antonio MORETTI[4], Mohamed Salah GHARBI[1], Mohamed Bechir ALLAGUI[1], Samia GARGOURI[1]

[1] *National Agronomic Institute of Research of Tunisia, University of Carthage, Tunis, Tunisia*
[2] *National Agronomic Institute of Tunisia, University of Carthage, Tunis, Tunisia*
[3] *Regional Pole of Agricultural Research and Development of North-West, Kef, Tunisia*
[4] *Institute of Sciences of Food Production, Research National Council (ISPA-CNR), Bari, Italy*
*Corresponding author: eya-khemir@hotmail.fr

Summary. Fusarium foot and root rot (FFRR) of cereals, caused by *Fusarium culmorum* and other *Fusarium* spp., is one of the most important soil- and residue-borne diseases in Tunisia. Management of the disease relies primarily on cultural practices such as crop rotation. Impacts of previous crops on the population of *F. culmorum* in the soil, and the incidence and severity of FFRR in durum wheat, were evaluated under Tunisian farming systems. A field trial showed that break crops of faba bean and fenugreek reduced the amount of *F. culmorum* DNA in soil, by 58% (faba bean) and 65% (fenugreek), and decreased numbers of *F. culmorum* propagules per g of soil by 83% (faba bean) and 85% (fenugreek). Farm demonstration trials also showed that faba bean and vetch used as previous crops reduced *F. culmorum* inoculum in the soil. Non-cereal crops also reduced the incidence of *F. culmorum* present in durum wheat roots and stem bases. The greatest grain yields and thousand kernel weights were recorded when faba bean and vetch were used as previous crops, but were less where durum wheat was previously grown. There were strong correlations between inoculum level of *F. culmorum* in the soil and incidence of FFRR in the following year. Results obtained in the field trial were supported by those collected from three demonstration farm trials during two cropping seasons. This study demonstrated for the first time in Tunisia and the Mediterranean region that break crops are effective for reducing *F. culmorum* inoculum in the soil and decreasing the pathogen in wheat roots and stem bases. Inoculum levels in soil can predict the expression of the disease in the following year in Tunisian farming conditions. These results are likely to be useful for developing and implementing guidelines for the management of FFRR of durum wheat.

Keywords. Faba bean, fenugreek, legumes, rotation, vetch.

OPEN ACCESS

Citation: E. Khemir, S. Chekali, A. Moretti, M.S. Gharbi, M.B. Allagui, S. Gargouri (2020) Impacts of previous crops on inoculum of *Fusarium culmorum* in soil, and development of foot and root rot of durum wheat in Tunisia. *Phytopathologia Mediterranea* 59(1): 187-201. doi: 10.14601/Phyto-10827

Accepted: March 5, 2020

Published: April 30, 2020

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Andy Tekauz, Cereal Research Centre, Winnipeg, MB, Canada.

INTRODUCTION

In Tunisia, cereals and their by-products are the main sources of dietary calories, and are the common base of all diets and the historical base of the

Mediterranean diet. The average *per capita* consumption of cereals in Tunisia is 184 kg per year (Slama *et al*., 2005; ONAGRI, 2016). Cereals are grown on approx. 1 million hectares of the agricultural land, with the number of the farmers involved estimated to be approx. 250,000. About 63% of farmers are smallholders, each with land areas of less than 10 ha (Bachta, 2011; Anonymous 2, 2018). Fusarium foot and root rot (FFRR) has been recognized as one of the most important diseases of cereals in Tunisia since the 1970s (Ghodbane *et al*., 1974), and this disease is responsible for significant economic losses, especially in arid and semi-arid regions (Van Wyk *et al*., 1987; Bateman, 1993; Hollaway *et al*., 2013). Yield losses of up to 26% in durum wheat and 18% in barley have been recorded (Chekali *et al*., 2013).

FFRR is caused by a complex of fungal pathogens. The two most reported are *Fusarium pseudograminearum* (O'Donnell & Aoki) (syn. *F. graminearum* group 1, *Giberella coronicola*) and *F. culmorum* (W. G. Sm.). *F. pseudograminearum* is the dominant species in several countries, including Australia (Burgess *et al*., 1975; Akinsanmi *et al*., 2004; Smiley *et al*., 2005), United States of America (Cook, 1980; Smiley and Patterson, 1996; Smiley *et al*., 2005) and New Zealand (Cromey *et al*., 2006), while *F. culmorum* is the dominant pathogen in Tunisia and the Mediterranean region (Cassini, 1981; Balmas,1994; Rossi *et al*., 1995; Mergoum *et al*., 2000; Gargouri *et al*., 2001).

FFRR is difficult to manage since *F. culmorum* can survive as hyphae in stubble residues of cereals (Cook, 1968; Bateman and Murray, 2001; Burgess, 2011; Khemir *et al*., 2018) and other grasses (Wallwork *et al*., 2004), and has the ability to persist as chlamydospores in soil (Sitton and Cook, 1981). This survival ability has major implications for designing strategies for effective disease management. Control of FFRR relies on agronomic management strategies, since in-crop fungicides are largely inefficient, and host resistance is limited (Burgess *et al*., 2001; Pereyra and Dill-Macky, 2004; Wisniewska and Kowalczyk, 2005).

The market situation in Tunisia has influenced the diversity of crops that are grown, where continuous wheat cropping and short crop rotations have become popular during the last 30 years. These cropping systems promote the increase of soil-borne diseases and resulting yield losses. In addition, durum wheat, which is highly susceptible to FFRR (Burgess *et al*., 2001), is the dominant crop, representing approx. 60 % of the cereal growing area (Slama *et al*., 2005; Gharbi and Felah, 2013). In the context of climate change, this disease could become more important, especially as the Mediterranean region has been qualified as a "hot spot for climate change" (Giorgi, 2006; Vicente-Serrano, 2006; Anonymous 1, 2019). It is important, therefore, to consider the best cultural practices for FFRR control. Crop rotations with non-hosts such as legumes has been reported to efficiently control the disease in many countries including the United Kingdom (Bateman and Kwasana, 1999; Bateman and Murray, 2001), Australia (Felton *et al*., 1998; Kirkegaard *et al*., 2004; Evans *et al*., 2010) and South Africa (Lamprecht *et al*., 2006).

Very little research has been conducted in the Mediterranean basin, including Tunisia (Chekali *et al*., 2016), to evaluate the impacts of break crops on development of FFRR. Fenugreek, an annual legume that grows well in Mediterranean climates (Duke *et al*., 1981), and which has demonstrated strong insecticidal, nematicidal and antifungal activity (Pemonge *et al*., 1997; Zia *et al*., 2001; Evidente *et al*., 2007; Haouala *et al*., 2008; Omezzine *et al*., 2014), has not been evaluated for reducing *F. culmorum* levels in soil, and its impacts on the disease. Very few studies have focused on monitoring *F. culmorum* soil inoculum under different cropping systems (McKenzie and Taylor, 1983; Evans et *al*., 2010).

The present study aimed to assess: i) the potential benefits of previous crops for reducing *F. culmorum* populations in the soil; ii) the impacts of previous crops on the incidence and severity of FFRR on stems and roots of durum wheat; and iii) the relationships between preplanting inoculum of *F. culmorum* and FFRR disease expression and grain yields.

MATERIALS AND METHODS

Site characteristics and cultural practices

To understand the effect of previous crops on populations of *F. culmorum* in the soil and incidence of FFRR in durum wheat, a field trial and three demonstration trials were established during the cropping seasons 2012/13, 2013/14 and 2014/15, in Northwest Tunisia.

Field trial

The field trial was carried out in a farm field located at Bou Salem (N36 53.012 E9 31.251). The area has a typical Mediterranean climate (Köppen, 1936), with hot summers and cold, wet winters. The monthly rainfall during the three cropping seasons at Bou Salem is summarized in Table 1, where the 10-yr annual average rainfall is approx. 500 mm.

The trial was established in 2012/13 as a randomized complete block design (RCBD) with three crop-

Table 1. Monthly rainfall (mm) recorded during the 2012/2013, 2013/14 and 2014/15 cropping seasons at Bou Salem, Tunisia.

Cropping season	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Total
2012/13	62	67	22	27	50	64	22	28	4	0	10	31	387
2013/14	14	45	118	44	81	23	82	24	47	4	0	0	482
2014/15	1	36	33	103	118	128	79	2	18	1	0	21	540
10 years means (2006/2015)	35	58	50	61	73	60	69	46	29	8	1	9	499

Source: National Institute of Meteorology.

Table 2. Monthly rainfall (mm) recorded during the 2012/2013, 2013/14 and 2014/15 cropping seasons at Fernana, Tunisia.

Cropping season	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Total
2012/13	89	106	22	138	149	221	25	28	23	0	0	1	802
2013/14	23	18	298	82	91	46	109	70	32	9	4	2	784
2014/15	45	95	35	138	115	120	65	40	40	7	2	1	703
15 years means (2001/2015)	31	78	81	126	128	120	82	83	32	12	3	5	781

Source: National Institute of Meteorology.

ping sequences and four replications. An area of 7,500 m^2 was divided into four blocks. Each block was subdivided into three plots (each of 12 × 50 m) which were sown with durum wheat (*Triticum durum*) var. 'Karim', faba bean (*Vicia faba*) var. 'Bachar' or fenugreek (*Trigonella foenum-graecum*) var 'Rihana'. The impacts of previous crops were assessed during the two following cropping seasons of 2013/14 and 2014/15. Durum wheat was sown at the recommended rate of 150 kg ha^{-1}; faba bean was sown at 140 kg ha^{-1} and fenugreek at 35 kg ha^{-1}. All these crops were seeded in early to mid-November of each year. Diammonium phosphate (P) (150 kg ha^{-1}) was applied with seed (Zadoks growth stage (ZGS) 1; Zadoks *et al*. 1974), and ammonium nitrate fertilizer (N) (150 kg ha^{-1}) was applied at tillering (ZGS 21) and at stem elongation (ZGS 32). For uniform suppression of grass weeds in the faba bean and fenugreek plots, clethodim (Select super®) was applied at 1 L ha^{-1} post-emergence of grass weeds from the 3-leaf stage of the crop plants.

Demonstration trials

Demonstration trials were established in three farmer's fields (36°37'29.28'N, 8°42'04.56'E; 36°36'47.56'N, 8°34'39.42'E and 36°37'01.10'N. 8°40'35.90'E) at Fernana. This region is also characterized by hot summers and cold, wet winter, but with greater annual rainfall than at the field trial site. The monthly rainfall during the three cropping seasons is summarized in Table 2, and the 15 y average rainfall was approx.780 mm at this location.

In 2012/13, an area of 3,000 m^2 at each farm was divided into three plots, which were each sown with faba bean (*Vicia faba*) var. 'Bachar', durum wheat (*Triticum durum*) var. 'Karim' or a local variety of vetch (*Vicia sativa*). The plots were 10 m wide and 100 m long. The effects of previous crops were evaluated during the two subsequent cropping seasons (2013/14 and 2014/15). These trials were direct seeded (without tillage) to retain uniform and high inoculum levels of *F. culmorum* at the beginning of the study. The three previous crops were applied according to common farmer practices. Durum wheat was seeded at 150 kg ha^{-1}, faba bean at 130 kg ha^{-1} and vetch at 100 kg ha^{-1}. Diammonium phosphate (P) (150 kg ha^{-1}) was applied with seed (Zadoks growth stage (ZGS) 1; Zadoks *et al*. 1974), and ammonium nitrate fertilizer (N) (150 kg ha^{-1}) was applied at tillering (ZGS 21) and at stem elongation (ZGS 32).

To control annual grass weeds, glyphosate (3 L ha^{-1}) was applied prior to sowing of durum wheat and mésosulfuron-méthyl (Mesomax®) + iodosulfuron + méfenpyr (Amilcar®) (1 L ha^{-1}) was applied in January 2014 and in January 2015. The faba bean plots received simazine (1.5 L ha^{-1}) and clethodim (Select Super ®) (1 L ha^{-1}) at post-emergence of the grassy weeds.

Fusarium culmorum *inoculum density*

Soil sampling

Soil samples were collected in July 2013, following the crop sequence treatments from the 12 plots of

the field trial at Bou Salem, and the three plots from each of the demonstration trials at Fernana. One hundred soil cores, including any plant residus, were taken within each plot to a depth of 100 mm, using a 15-mm diam. Accucore corer (Spurr Soil Probes), and were then bulked to provide a single sample for each plot. The samples were air-dried for several days. Dry soil (500 g) for each plot was sent to the South Australian Research and Development Institute (SARDI), Adelaide, Australia, for assays of *F. culmorum* DNA ('Predicta® B' Soil Testing Service). The remaining soil from each plot was used for soil dilution plating (below).

Soil dilution plating

This was carried out to estimate populations of *F. culmorum* (Cook, 1980), following the procedure of Snyder and Nash (1968), with modifications as described by Bateman and Coskun (1995), Bateman *et al*., (1998) and Bateman and Murray (2001). Briefly, 10 g of soil from each plot were added to 100 mL of 0.1 % water agar and mixed thoroughly for 30 sec. Further dilution series were made using 1 mL aliquots from the starting suspension. An aliquot of 1 mL of the final dilution (1/100, based on premilary tests) was transferred to each of five Petri dishes containing Peptone-PCNB agar medium. This medium contained 15 g Difco peptone, 20g agar, 1 g KH_2PO_4, 0.5g $MgSO_4\,7H_2O$, 1 g pentachloronitrobenzene (PCNB), 100 mg L^{-1} streptomycin sulfate and 80 mg L^{-1} neomycin. Aliquots were dispersed by circular agitation of the Petri dishes (Steinkellner and Langer, 2004). The Petri dishes were incubated at 20°C for 5–7 d with a 16 h light/8 h dark regime. *Fusarium culmorum* was identified morphologically according to Leslie and Summerell (2006).

To confirm fungal identifications, some colonies were transferred to ¼ strength potato dextrose agar (PDA) and then to carnation leaf agar (CLA). Amounts of *F. culmorum* in the soil samples were expressed as the numbers of colony forming units per gram of air-dried soil (CFU g^{-1} soil).

CFU g^{-1} was calculated using the formula:

$$\text{CFU g}^{-1} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Volume of culture plate}}$$

With dilution factor = 10^2, and volume of culture plate = 1 mL.

Quantitative real-time polymerase chain reaction (qPCR)

The amount of *F. culmorum* DNA present in soil from each plot was evaluated, after applying the crop sequence treatments (July 2013). Soil samples were submitted to the SARDI Root Disease Testing Service. This includes a suite of tests for the quantification of *F. culmorum* DNA in soil. QPCR assays using rDNA (TaqMan) probe sequences specific to *F. culmorum* were made for the total pathogen DNA extracted from the soil. Levels of *F. culmorum* DNA in soil can be related to disease risk in the following crop year (Hogg *et al*., 2007). Extraction protocols and analyses of DNA from soil were done according to Ophel-Keller *et al*. (2008). Inoculum concentrations of DNA per gram of soil were expressed in pictograms.

Pathogen isolation and disease incidence

During the 2013/14 and 2014/15 seasons, 50 plants of durum wheat were collected randomly along a W-shaped transcript, from each of the 12 plots of the field trial, and from the nine plots of the three demonstration trials, at the durum wheat anthesis stage (ZGS 85). The incidence of FFRR was calculated as the frequency of isolation of *F. culmorum*, separately from roots or stem bases.

Stem base sections and root sections (2–3 mm length) were surface-sterilized with 70% ethanol for 15 s, followed by 3% sodium hypochlorite for 1min, and then rinsed three times with distilled water, and were then air-dried on sterile filter paper. Five sections from each sample were then plated onto ¼ strength PDA containing 20 mL L^{-1} streptomycin sulfate (100 mg L^{-1}) and 12 mL L^{-1} neomycin sulfate (80 mg L^{-1}) in Petri dishes. The plates were incubated up to 7 days at 25°C in a 12 h light/12 h dark regime. Hyphae of *Fusarium* spp. were subsequently transferred onto CLA to promote conidium production. *Fusarium culmorum* was identified using morphological criteria, according to Leslie and Summerell (2006).

Disease severity

FFRR disease severity was assessed only during the 2014/15 cropping season in the three farm demonstration trials, at the durum wheat anthesis stage (ZGS 85). Severity of *F. culmorum* was evaluated by measuring surface of stem bases showing browning. Fifty durum wheat plants were randomly removed from each plot and scored using a scale 0 to 3, where: 0 = no browning (no visible symptoms); 1 = 0.1 to 1.9 cm extent of browning; 2 = 2 to 3.9 cm of browning; and 3 = > 4 cm browning.

Ratings were converted to severity indices using the following formula:

$$\text{Severity index} = \frac{((0) \times (n0) + (1) \times (n1) + (2) \times (n2) + (3) \times (n3))}{N}$$

Where: N = number of evaluated plants; n_0 = number of plants in class 0; n_1 = number of plants in class 1; n_2 = number of plants in class 2; and n_3 = number of plants in class 3.

Yield parameters

Grain yields of durum wheat, subjected to different crop rotations, were assessed from the three demonstration trials in the 2013/14 and 2014/15 cropping seasons. The yields (kg ha^{-1}) were based on harvesting all of the grain from each plot.

Statistical analyses

Field trial

The trial was arranged in a randomized complete block design (RCBD) with four replicates. All data were subjected to analysis of variance (ANOVA) using 'SPSS Statistics version 20' software published by 'IBM Crop 2011'. The year and previous crop were considered as main factors. Means comparisons were performed using the Student's LSD test (at $P = 0.05$ or $P = 0.01$).

Regression analyses used Pearson's correlation test, to identify possible associations between the incidence of *F. culmorum* isolated from stem bases and roots (2013/14 cropping season data) and the amounts of *F. culmorum* in the soil (DNA and CFU) left after each treatment, measured at plant maturity in July 2013.

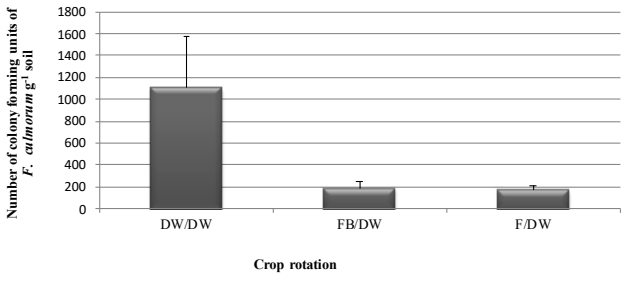

Figure 1. Mean numbers of colony forming units of *Fusarium culmorum* g^{-1} of soil, estimated by dilution plating, sampled from the experimental trial at Bou Salem, Tunisia, after different crop rotations. Error bars are 2 × standard deviations. DW = durum wheat, FB = faba bean, F = fenugreek.

Demonstration trials

Regression analyses were carried out (as above), to identify possible correlations between *F. culmorum* DNA content in soil (determined by qPCR), the number of CFU of *F. culmorum* per gram of soil (determined by soil dilution plating) and the incidence of *F. culmorum* isolations from stem bases and roots of durum wheat (2013/14 cropping season data). In addition, regression analysis was used to identify possible association between FFRR expression and grain yields from subsequent durum wheat crops.

RESULTS

Fusarium culmorum *inoculum in the soil*

Soil dilution plating and morphological identification revealed several *Fusarium* species from the soil samples. These included *F. acuminatum*, *F. avenaceum*, *F. culmorum*, *F. compactum*, *F. equiseti*, *F. oxysporum* and *F. solani*. However, *F. culmorum* was by far the dominant pathogenic species in the samples. The DNA analyses conducted for detection of the two recognized pathogenic species, *F. culmorum* and *F. pseudograminearum*, showed the presence of only *F. culmorum* in the samples.

Field trial

Soil dilution plating revealed the presence of *F. culmorum* in all 12 plots of the field trial at Bou Salem. ANOVA of data from the trial revealed that the number of CFU of *F. culmorum* in the upper 10 cm soil layer was significantly ($P = 0.002$) affected by the different break crops. Faba bean and fenugreek used as previous crops, *vs.* durum wheat monoculture, reduced the number of *F. culmorum* propagules in soil by 83% for faba bean and 85% for fenugreek (Figure 1). The number of propagules ranged from 133 to 1,733 CFU g-1 of soil, with a mean of 1100 for durum wheat, 183 for faba bean and 166 for fenugreek. DNA analyses confirmed the presence of *F. culmorum* in soil from the 12 plots of the field trial. The amounts of DNA of the pathogen were significantly ($P = 0.004$) different among previous crop treatments, ranging from 27 to 212 pg DNA g^{-1} of soil. Overall, the amounts of *F. culmorum* DNA in the soil were decreased by 58% following faba bean and by 65 %, following fenugreek. The greatest amounts of *F. culmorum* DNA occurred in the treatment where durum wheat was cultivated in monoculture, and here was no statistically

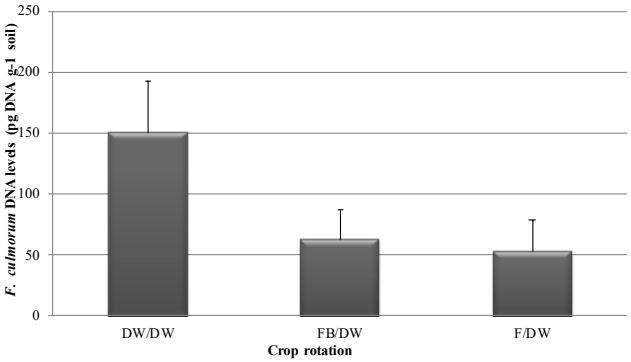

Figure 2. Amounts of *Fusarium culmorum* DNA in soil (pg DNA $^{-1}$g of soil) 1 year after different crop rotations from the experimental field trial at Bou Salem, Tunisia. Error bars are 2 × standard deviations. DW: durum wheat, FB = faba bean, F = fenugreek.

significant difference in DNA amounts between the two legume treatments (Figure 2).

Soil dilution plating and qPCR both showed greater amounts of *F. culmorum* in soil following a previous durum wheat crop, compared to previous faba bean or fenugreek crops. Pearson's correlation test showed that *F. culmorum* DNA concentration (pg DNA g^{-1} soil) was correlated (r = 0.92; $P < 0.001$) with the number of propagules of this fungus (CFU g^{-1} soil) detected in the soil 1 year after treatment.

Demonstration trials

Based on soil dilution plating, propagules of *F. culmorum* in the soil were more abundant after wheat (average of 866 propagules g^{-1} of soil) compared to vetch (358 propagules g^{-1} of soil) or faba bean (289 propagules g^{-1} of soil) (Table 3). Thus, faba bean and vetch, used as previous crops, reduced the number of *F. culmorum* propagules in soil by 66 % after faba bean and 58 % after vetch, compared to durum wheat monoculture. In addition, the results indicated that *F. culmorum* propagules were less abundant after faba bean as a previous crop compared to vetch.

The DNA analyses showed greater amounts of *F. culmorum* DNA in soil from Farm 1 and 2 compared to Farm 3 (Table 3). Based on real-time qPCR, the amount of DNA of *F. culmorum* was less following a legume compared to wheat at Farms 1 and 2. However, at Farm 3, where the DNA amounts were low in all cases, there were no differences in *F. culmorum* DNA between the different previous crops. Based on the SARDI risk assessment protocol, DNA amounts for all plots at Farm 3 were classified as leading to low risk of subsequent crop damage.

Overall, for the 12 plots of the field trial and the six plots of the demonstration trials at Farms 1 and 2, legumes used as previous crops reduced the inoculum of *F. culmorum*, as detected by qPCR and by soil dilution plating.

Disease assessments

A total of 4,200 stems and roots were examined to determine the impacts of crop sequence on soil-borne diseases of cereals in the field and demonstration trials. Visual assessments revealed evidence of take-all, eyespot and FFRR. Only the results for previous crop effects on FFRR development are presented here.

Field trial

Incidence of infection by *F. culmorum* in durum wheat was greater in durum wheat stem bases than in roots. Statistical analyses showed that there was a difference ($P = 0.047$) between the two growing seasons (2013/14 and 2014/15) for incidence of *F. culmorum* recovered from durum wheat stem bases. No significant difference was detected in the incidence of *F. culmorum* recovered from wheat roots during these two cropping seasons (Figure 3).

ANOVA of data from the field trial revealed that there was a highly significant ($P < 0.001$) effect of previous crop on the incidence of FFRR infection of stem bases and roots of durum wheat. Fenugreek or faba

Table 3. Number of colony forming units (CFU g^{-1} of soil), levels of DNA (pg DNA g^{-1} soil) of *Fusarium culmorum* 1 year after treatment at three demonstration trial sites, and risk of resultant crop losses (Predicta® B test).

Previous crop rotation	Site 1			Site 2			Site 3		
	CFU g^{-1}	pg DNA g^{-1}	Risk	CFU g^{-1}	pg DNA g^{-1}	Risk	CFU g^{-1}	pg DNA g^{-1}	Risk
Durum wheat/durum wheat	1071	104	medium	928	328	high	600	14	low
Durum wheat/vetch	142	41	low	500	150	medium	433	44	low
Durum wheat/faba bean	0	14	low	500	163	medium	366	50	low

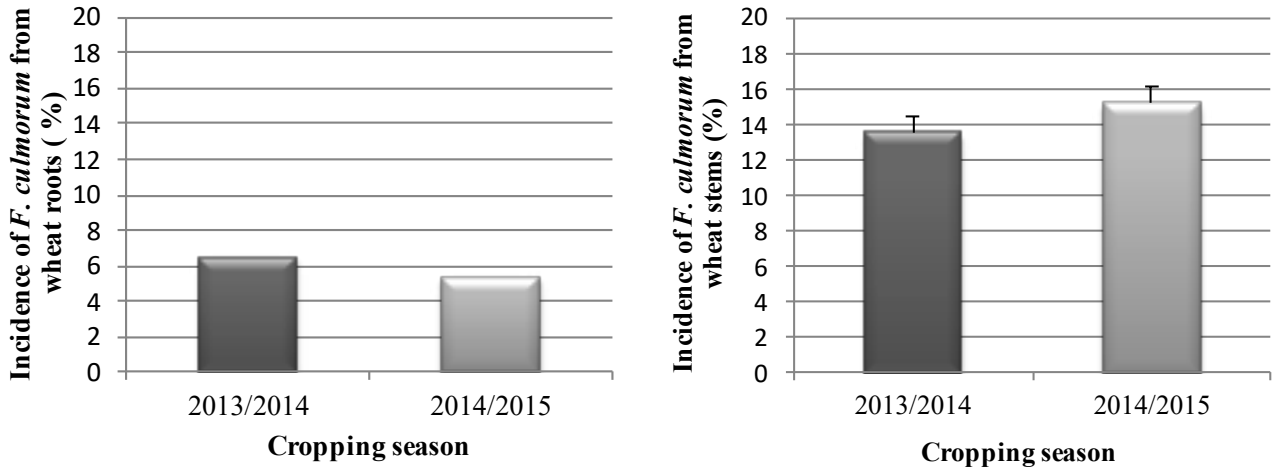

Figure 3. Mean incidence of *Fusarium culmorum* isolations from durum wheat roots (A) and stem bases (B) during two cropping seasons, at the Bou Salem,Tunisia, field trial. Error bars are 2 × standard deviations.

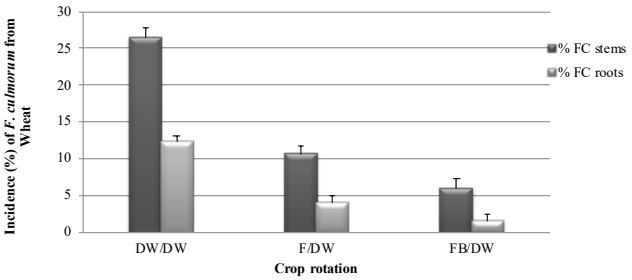

Figure 4. Mean incidence of *Fusarium culmorum* infection on wheat stem bases and roots as affected by different previous crops (DW = durum wheat; F = fenugreek; FB = faba bean), at the Bou Salem, Tunisia, field trial in the 2013/14 and 2014/15 cropping seasons. Error bars are 2 × standard deviations.

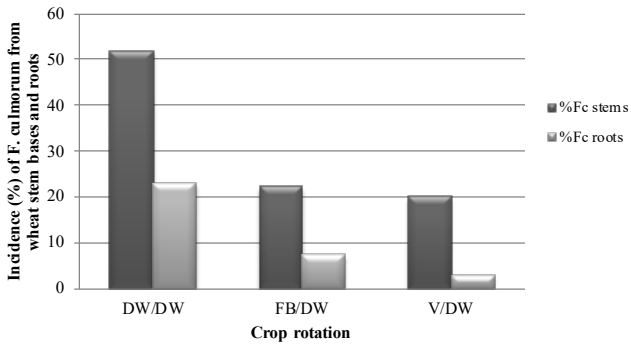

Figure 5. Mean incidence of *Fusarium culmorum* (Fc) from durum wheat stem bases and roots from three demonstration trials, after previous crops of durum wheat faba bean and vetch.

bean used as the previous crop decreased the incidence of *F. culmorum* affected durum wheat roots more than 3× for fenugreek and 9× for faba bean, compared to durum wheat. Similarly, fenugreek and faba bean decreased the incidence of *F. culmorum* in durum wheat stem bases, by more than 3× for fenugreek and 5× for faba bean. Data analyses showed that the incidence of infection by *F. culmorum* on durum wheat stem bases and roots was least ($P < 0.001$) when faba bean was the previous crop (Figure 4).

Demonstration trials

Data from the three demonstration trials showed that the presence of *F. culmorum* in durum wheat roots and stem bases when they were sampled during the 2013/14 and 2014/15 cropping seasons. Assessments of development of FFRR showed that stem bases were much more likely to be infected by *F. culmorum* than roots. Faba bean and vetch, used as previous crops, resulted in a less isolation incidence of *F. culmorum* than for durum wheat monoculture. Compared to durum wheat, the mean reduction in *F. culmorum* incidence after faba bean was 59% in stem bases and 68% in roots, and after vetch was 62% in stem bases and 87% in roots (Figure 5).

FFRR severity was evaluated only in spring 2015 (2014/15 cropping season). Compared to durum wheat monocropping, vetch used as the previous crop reduced disease severity by 80% and faba bean as the previous crop reduced severity by 50 % (Figure 6).

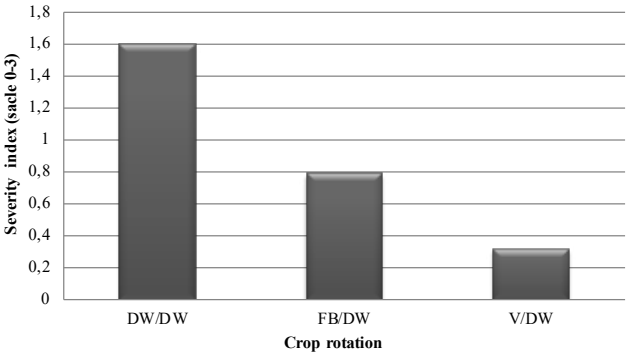

Figure 6. Mean durum wheat foot and root rot severity indices for wheat grown after durum wheat (DW), faba bean (FB) or vetch (V) at the Fernana demonstration trials during the 2014/2015 cropping season.

Relationships between Fusarium culmorum inoculum density in soil and development of FFRR

Field trial

According to Pearson's correlation test, the amounts of *F. culmorum* DNA detected in soil samples 1 year after treatment were positively correlated with the incidence of *F. culmorum*, durum wheat roots (R^2 = 0.56, P = 0.005) and in stem bases (R^2 = 0.80, P = 0.001) for wheat grown in the following year (Figure 7).

The association between pre-planting inoculum concentrations, indicated by *F. culmorum* CFU g^{-1} of soil, and disease expression, indicated by incidence of *F. culmorum* in wheat roots, was significantly positive (R^2 = 0.58, P = 0.004) (Figure 7A). This was also true for this relationship in stem bases (R^2 = 0.83, P = 0.000) (Figure 7B).

Demonstration trials

Inoculum levels of *F. culmorum* detected in the soil after each treatment, as determined by soil dilution plating, were positively correlated with incidence of *F. culmorum* in wheat roots (R^2 = 0.66) and stem bases R^2 = 0.64 (Figure 8, A and B). In addition, a positive relationship was observed between the amounts of *F. culmorum* DNA and the incidence of *F. culmorum* in wheat roots (R^2 = 0.65) and stem bases (R^2 = 0.7) after each treatment (Figure 8, A and B).

Grain yields

The greatest durum wheat grain yield (3,833 kg ha^{-1}) was recorded when vetch was the previous crop, followed by faba bean (3,583 kg ha^{-1}) and durum wheat (3,239 kg ha^{-1}) (Figure 9). Regression analyses showed a negative correlation between grain yields (kg ha^{-1}) and *F. culmorum* incidence on durum wheat stem bases (Figure 10) (r = -0.74), and grain yields FFRR severity indices (r = - 0.56) (Figure 11).

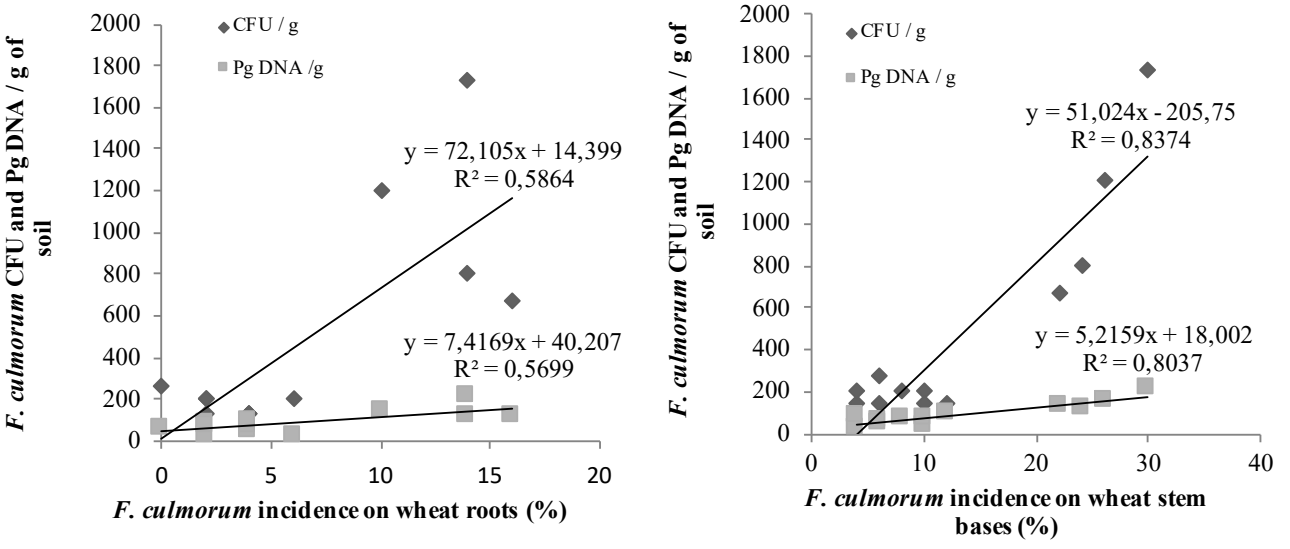

Figure 7. Correlations (Pearson's correlation test) between *Fusarium culmorum* on wheat roots (A) and stem bases (B) and amounts of *F. culmorum* inoculum left in the soil after 1 year at the Bou Salem experimental trial, as analyzed by soil dilution plating and quantitative real-time polymerase chain reaction (qPCR).

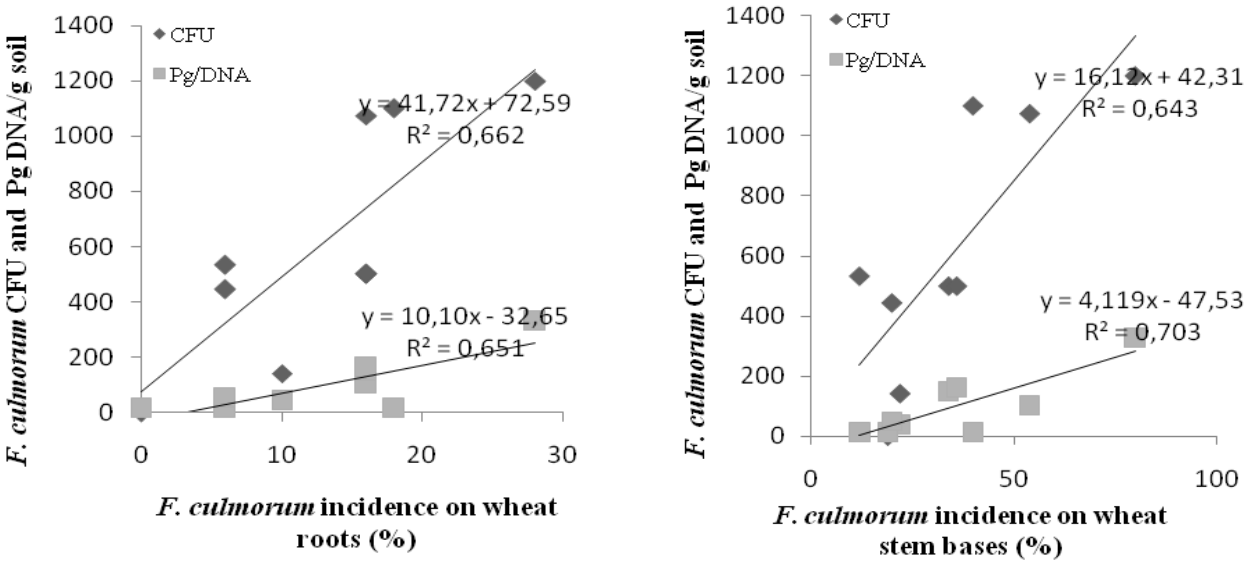

Figure 8. Correlations between *Fusarium culmorum* incidence (%) on wheat roots (A) and stem bases (B) and inoculum levels of *F. culmorum* left in the soil after 1 year, for three demonstration trials at Fernana, Tunisia.

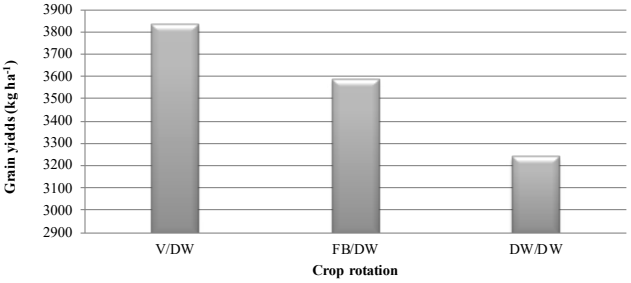

Figure 9. Mean grain yields (kg ha^{-1}) of durum wheat harvested after different rotation crops (DW = durum wheat, FB = faba bean, V = vetch), from three demonstration trials at Fernana, Tunisia, during the two cropping seasons 2013/14 and 2014/15.

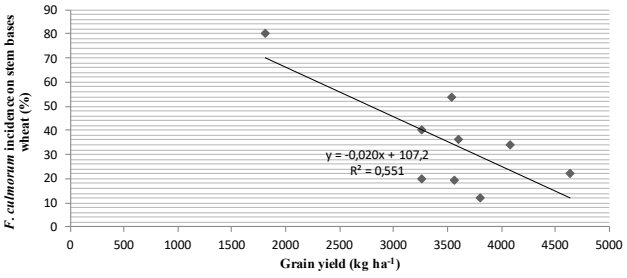

Figure 10. Correlation between *Fusarium culmorum* incidence (%) on wheat stem bases and grain yields (kg ha^{-1}) of durum wheat harvested from three demonstration trials at Fernana, Tunisia.

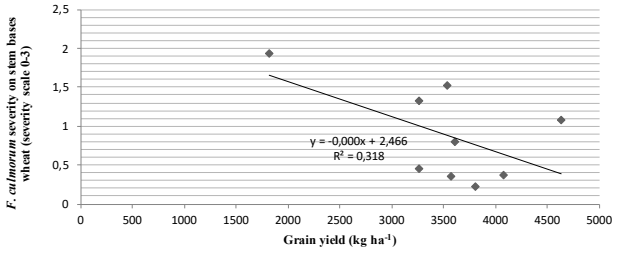

Figure 11. Correlation between disease severity index (scale 0-3) and grain yield (kg ha^{-1}) of durum wheat harvested from three demonstration trials at Fernana, Tunisia.

DISCUSSION

In this study effects of previous crops on inoculum levels of *F. culmorum* in soil, development of FFRR, and yield parameters of durum wheat were evaluated in Tunisian farming conditions. Data were collected from an experimental trial, and from demonstration trials established in three farmer's fields.

Inoculum levels of *F. culmorum* in the soil were quantified using two methods, soil dilution plating and qPCR. Both methods showed the dominance of *F. culmorum* in the upper 10 cm soil layers of the trial sites. *Fusarium pseudograminearum*, which has been isolated in other studies from Tunisia (Gargouri *et al*., 2001, 2007; Chekali *et al*., 2016), was not detected in the present study. However, this is not surprising since it has been demonstrated that *F. pseudograminearum* is restricted to arid zones in Tunisia (Gargouri *et al*., 2001), whereas in more humid regions, such as Fernana and Bou Salem where this study was conducted, *F. culmorum* is the dominant species. These observations have also been confirmed in other regions of the world, including

the Pacific Northwest of United States of America, and in Australia (Backhouse *et al*., 2004; Poole *et al*., 2012).

The results presented here demonstrated that break crops, including faba bean, vetch and fenugreek, reduced the number of propagules of *F. culmorum* in the soil. These data are confirming previous results which showed that populations of *F. culmorum* in the soil are affected by the previous crops (Snyder and Nash, 1968; McKenzie and Taylor, 1983). Steinkellner and Langer (2004) also demonstrated that the number of *Fusarium* CFU in soil was affected by previous crops.

Overall, the results from soil dilution plating were confirmed by qPCR, except for those from Farm 3 in the demonstration trials. At this farm, the amounts of *F. culmorum* DNA detected were very low, and this may have affected the qPCR analyses. The correlations observed between the two techniques suggest that both are effective for quantifying inoculum levels of *F. culmorum* in field soil. To our knowledge, this is the first report examining correlations between soil dilution and qPCR data for *F. culmorum* and durum wheat. In addition, this study was the first to measure the levels of *F. culmorum* inoculum in Tunisian soils, and may have been the first to make these assessments in the Mediterranean region.

The amount of *F. culmorum* DNA in the soil was generally reduced when legumes were used as previous crops before durum wheat. This is consistent with the results of Evans *et al*. (2010) in south-east Australia, who demonstrated that DNA of *F. culmorum* in soil was much less after field pea (115 pg DNA g^{-1}) and greater after durum wheat (974 pg DNA g^{-1}) or barley (1,196 pg DNA g^{-1}).

The numbers of propagules of *F. culmorum* detected in the present study (0 to 1,974 CFU g^{-1} of soil) was low compared to other studies. In Austria, Steinkllner and Langer, (2004) reported that inoculum of *F. culmorum* and *F. pseudograminearum* in the upper 10 cm layer, ranged between 0 to 8,750 CFU g^{-1}. Similarly, the amounts of *F. culmorum* DNA recorded in the present study were less than those found in Australia (Evans *et al*., 2010; Halloway *et al*., 2013).

Development of soil-borne diseases depends on the concentration of the inoculum in the soil (Cook, 1981). Cook (1968) demonstrated that 100 propagules of *F. culmorum* per gram of soil were sufficient to cause damage even when host plants are not under stress. In addition, McKay *et al*. (2008) suggested guidelines that related DNA amounts of *F. culmorum* in the soil before sowing to the risk of FFRR and yield losses in bread and durum wheat. They indicated that pre-planting DNA soil amounts less than 100 pg of fungal DNA g^{-1} of soil for bread wheat and less than 25 pg for durum wheat, would lead to minimal risk of yield losses due to crown rot caused by *Fusarium* spp.

The low amounts of *F. culmorum* inoculum in the soil may be explained by several factors. Differences in climatic conditions (Doohan *et al*., 2003; Xu *et al*., 2018), tillage methods (Steinkellner and Langer, 2004) and various cultural practices (Klem *et al*., 2007; Muller *et al*., 2010; Blandino *et al*., 2012; Chekali *et al*., 2016; Hemissi *et al*., 2018) are known to affect *Fusarium* populations in the soil. However, the low levels of inoculum observed in the present study were likely related to soil tillage. Low- or no-till are not common practices in Tunisia. Even in the demonstration trials reported here, direct seeding was applied for the first time. Steinkellner and Langer, (2004) found that the total number of CFU of *Fusarium* spp. was affected by tillage treatment. This agrees with previous studies by Toledo-Souza *et al*. (2008), who demonstrated that *Fusarium* inoculum was greater in a no-tillage system than a conventional one. Paulitz (2006) also showed that FFRR can cause significant damage when no-tillage practices and stubble retention are used. Recently, in Tunisia, Khemir *et al*. (2018) showed that stubble residue retention on soil surfaces increased *F. culmorum* inoculum levels in plant residues. The impact of no-tillage was also previously reported by Evans *et al*. (2010), who demonstrated that tillage reduced inoculum levels in soil by burying and promoting rapid degradation of infested plant residues. Furthermore, type and quantity of crop residues left on the soil surface, influence microorganisms and microbial processes in soil (Kandeler *et al*., 1999). Yi *et al*. (2002) showed that decomposition of crop residues was associated with a decline in CFU of *Fusarium* species.

The present study showed that frequency of isolation of *F. culmorum* from wheat roots and stem bases was affected by the previous crop in both trials, and was reduced by up to 70%. However, the results showed that in general the incidence of the pathogen was greater in stem bases than in roots. Knudsen *et al*. (1995) reported that FFRR was a consequence of initial stem base infections, while Beccari *et al*. (2011) and Covarelli *et al*. (2012) found that the stem bases of durum wheat were more heavily infected by *F. culmorum* compared to the roots. Knight and Sutherland (2013; 2017) reported that *F. culmorum* caused minor necrosis of the primary roots of cereals (durum wheat, bread wheat and barley), but caused serious cortical rot in leaf sheath tissues. These results emphasize the importance of crop residues left on the soil at the level of plant crowns for infection by the *Fusarium* species and the development of FFRR.

The use of break crops, including faba bean, fenugreek and vetch, decreased the incidence and sever-

ity of FFRR and increased grain yield in the subsequent durum wheat crop. These results are consistent with previous studies (Felton *et al*., 1998; Montanari *et al*., 2006; Zhou and Everts, 2007; Evans *et al*., 2010; Chekali *et al*., 2016). Other studies also demonstrated that preceding crops are key factors increasing the risk of *Fusarium* diseases on cereals (Klem *et al*., 2007; Muller *et al*., 2010; Blandino *et al*., 2012; Chekali *et al*., 2016). Rotations with chickpea (Felton *et al*., 1998; Chekali *et al*., 2016), faba bean (Kirkegaard *et al*., 2004), pea (Smiley *et al*., 1996; Evans *et al*., 2010) or lentil (Chekali *et al*., 2016) have been reported to reduce the incidence of FFRR. Chekali *et al*. (2016), showed, in northwest of Tunisia, that faba bean and chickpea used as previous crops could contribute to decreased incidence of infection of roots and stem bases of durum wheat by *F. culmorum*, and increase grain yields compared to durum wheat as a previous crop.

Rasmussen *et al*. (2002) hypothesized that this reduction in *F. culmorum* could be due to the non-host character of legumes, or to their high cellulose contents which increases microbial activity in the soil and leads to decrease of *Fusarium* spp. survival. In contrast, a high level of infection of wheat by *F. culmorum* has been reported when monoculture is practiced (Blecharczyk *et al*., 2006; Kurowsk et Adamiak, 2007; Kraska et Mielniczuk, 2012). In the present study, the Mediterranean forage legume fenugreek was reduced soil inoculum levels of *F. culmorum* by more than 60%, and decreased *F. culmorum* incidence on durum wheat stem bases, compared inoculum and disease incidence in durum wheat monoculture. These results confirm the antifungal activity of fenugreek. Omezzine *et al*. (2014) assessed *in vitro* antifungal activity of aqueous extracts from *T. foenum-graecum*, *against F. oxysporum f.* sp. *radicis-lycopersici and F. oxysporum f.* sp. *lycopersici*, and reported the antifungal and allelopathic potential of extracts from aerial parts of a Tunisian fenugreek cultivar. Other studies have also reported similar activities (Devasena and Menon, 2003; Oddepally and Guruprasad, 2015; Dharajiya *et al*., 2016; Sudan *et al*., 2020). Here, we have demonstrated, for the first time in Tunisia, the beneficial effects of fenugreek as a previous crop to decrease *F. culmorum* populations in soil and the development of FFRR in durum wheat.

Our results showed positive correlation between *F. culmorum* inoculum concentrations in the soil prior to sowing and subsequent FFRR expression. A negative correlation was also observed between FRRR expression and durum wheat grain yield. These results are similar to those of Smiley *et al*. (2005), Hollaway *et al*. (2013) and Chekali *et al*. (2016). Hollaway *et al*. (2013) suggested that pre-planting amounts of *F. culmorum* inoculum and expression of FFRR were positively correlated. Given that control options for FFRR are limited, this information would be valuable for grain producers, to prevent the yield losses caused by these diseases. In Australia, a DNA-based soil testing service (the 'PreDicta® B' system) was developed in 1997, to improve assessment of risks from root diseases and assist grain producers in planning cropping programmes (Ophel-Keller *et al*., 2008). Previous applications of this system have been mainly utilized in Australia and the United States (Paulitz *et al*., 2010; Bithell *et al*., 2012). This analysis tool is currently not available in Tunisia or in other Mediterranean countries. Our results encourage the use of systematic surveys across years and regions to document *Fusarium* inoculum levels in soil and FFRR occurrence and severity, and grain yield of durum wheat. This will assist development of a predictive model useful to farmers in Tunisia and the wider Mediterranean region.

In this study, the demonstration trials which involved farmers may impact on extension workers and other grain production stakeholders, to promote adoption of crop rotation as a tool for reducing soil-borne diseases of wheat in Tunisian cropping systems. Further studies would be useful to develop and implement a predictive model based on *Fusarium* inoculum levels in soil, to assess the risks and estimate the potential yield losses resulting from FFRR. This could be achieved by adjusting cropping sequences and other cultural practices to maximize yields. Our results could be useful for the Mediterranean basin, where FFRR is an endemic disease wherever wheat is cultivated.

ACKNOWLEDGEMENTS

This research was supported by the Ministry of Higher Education and Scientific Research of Tunisia, and was part of the CANA project CSE-2011-025 (Adapting conservation agriculture for rapid adoption by smallholder farmers in North Africa) financed by the Australian Centre for International Agricultural Research (ACIAR) and managed by the International Centre for Agricultural Research in the Dry Areas (ICARDA). We thank the farmers who collaborated with the researchers in this project.

LITERATURE CITED

Akinsanmi O.A., Mitter V., Simpfendorfer S., Backhouse D., Chakraborty S., 2004. Identity and pathogenic-

ity of *Fusarium* spp. isolated from wheat fields in Queensland and northern New South Wales. *Australian Journal of Agricultural Research* 55: 97–107.

Anonymous 1, 2019. Middle East and North Africa (MENA) is the most water-stressed region on earth. ICARDA. Available at: https://www.icarda.org/media/news/middle-east-and-north-africa-mena-most-water-stressed-region-earth.

Anonymous 2, 2018. Tunisie : Plus de 1 million d'hectares de superficies ensemencées en céréales. WEBMANAGERCENTER. Available at https://www.webmanagercenter.com/2018/03/08/417049/tunisie-les-superficies.

Bachta M. S., 2011. La céréaliculture en Tunisie une politique de régulation à repenser. *Les notes d'analyse du CIHEAM* 64: 1-19.

Backhouse D., Burgess L.W., 2002. Climatic analysis of the distribution of *Fusarium graminearum*, *F. pseudograminearum* and *F. culmorum* on cereals in Australia. *Australasian Plant Pathology* 31: 321–327.

Backhouse D., Abubakar A.A., Burgess L.W., Dennis J.I., Hollaway G.J., … Henry F.J., 2004. Survey of *Fusarium* species associated with crown rot of wheat and barley in eastern Australia. *Australian Plant Pathology* 33: 255–261.

Balmas V., 1994. Root rot of wheat in Italy caused by *Fusarium graminearum* Group 1. *Plant Disease* 78: 317.

Bankina B., Bimsteine G., Paulvoska L., Paura L., 2019. Effects of soil tillage and crop rotation on the development of wheat stem base diseases. *Canadian Journal of Plant Pathology* 41: 1-27.

Bateman G. L., 1993. Development of disease symptoms and fungal pathogens on shoot bases in continuous winter wheat. *Plant Pathology* 42: 595–608.

Bateman G. L., Coskun H., 1995. Populations of *Fusarium* spp. in soil growing continuous winter wheat, and effects of long-term application of fertilizers and of straw incorporation. *Mycological Research* 99: 1391–1394.

Batteman G.L., Kwasana H., 1999. Effects of number of winter wheat crops grown successively on fungal communits on wheat roots. *Applied Soil Ecology* 13: 271–282.

Bateman G.L., Murray G., 2001. Seasonal variation in population of *Fusarium* species in wheat-field soil. *Applied Soil Ecology* 18: 117–128.

Bateman G.L., Murray G., Gutteridge R.J., Coskun H., 1998. Effects of method of straw disposal and depth of cultivation on populations of *Fusarium* spp. in soil and on brown foot rot in continuous winter wheat. *Annals of Applied Biology* 132: 35–47.

Beccari G., Covoralli L., Nicholson P., 2011. Infection processes and soft wheat response to root rot and crown rot caused by *Fusarium culmorum*. *Plant Pathology* 60: 671–684.

Bithell S.L., McKay A., Butler R.C., Herdina, Hartley D., Cromey M.G., 2012. Predicting take-all severity in second-year wheat using soil DNA concentrations of *Gaeumannomycesgraminis* var. *tritici* determined with qPCR. *Plant Disease* 96: 443–451.

Blandino M., Haidukowski M., Pascale M., Plizzari L., Scudellari D., Reyeneri A., 2012. Integrated strategies for the control of *Fusarium* head blight and deoxynivalenol contamination in winter wheat. *Field Crops Research* 133: 139–149.

Blecharczyk A., Sierpowski J., Sawinska Z., 2006. Impact of tillage systems on diseases in continuous cropping of winter wheat. *Progress Plant Protection* 46: 677–680.

Burgess L.W., 2011. Foot, crown, and root rots of cereals - insidious diseases of global importance: A Tunisian perspective. 2011. *Tunisian Journal of Plant Protection*, 6 (Guest Editorial).

Burgess L.W., Wearing A.H., Toussoun T.A., 1975. Surveys of Fusaria associated with crown rot of wheat in eastern Australia. *Australian Journal* of *Agricultural Research* 26: 791–799.

Burgess L.W., Backhouse D., Summerell B.A., Swan L.J., 2001. Crown rot of wheat. Pages: 271–294. In: *Fusarium.* Paul E. Nelson Memorial Symposium. (B.A. Summerell, J.F. Leslie, D. Backhouse, W.L. Bryden, and L.W. Burgess, eds.) APS press, The American Phytopathological Society, St. Paul, Minnesota, USA.

Cassini R., 1981. *Fusarium* diseases of wheat and corn in Western Europe. Pages 56–63 in*: Fusarium*: Diseases, Biology, and Taxonomy. (P.E. Nelson, T.A. Toussoun, and R.J. Cook, eds). The Pennsylvania State University Press, University Park, PA, USA.

Chekali S., Gargouri S., Berraies S., Gharbi M.S., Nicol J.M., Nasraoui B., 2013. Impact of *Fusarium* foot and root rot on yield of cereals in Tunisia. *Tunisian Journal of Plant Protection* 8: 75–86.

Chekali S., Gargouri S., Rezgui M., Paulitz T. and Nasraoui B., 2016. Impact of previous crops on *Fusarium* foot and root rot, and on yields of durum wheat in North West Tunisia. *Phytopathologia Mediterranea* 55: 253–261

Cook R.J., 1968. *Fusarium* root and foot rot of cereals in the Pacific Northwest. *Phytopathology* 58: 127–131.

Cook R.J., 1980. *Fusarium* foot rot and its control in the Pacific Northwest. *Plant Disease* 64: 1061–1066.

Cook R.J., 1981. *Fusarium* diseases of wheat and other small grains in North America. Pages: 39–52 In:

Fusarium. Diseases, Biology and Taxonomy. (P.E. Nelson, T.A. Toussoun, and R.J. Cook, eds). Editions of Pennsylvania State University Press, University Park, PA, USA.

Covarelli L., Beccari G., Steed A., Nicholson P., 2012. Colonization of soft wheat following infection of the stem base by *Fusarium culmorum* and translocation of deoxynivalenol to the head. *Plant Pathology* 61: 1121–1129.

Cromey M.G., Parkes R.A., Fraser P.M., 2006. Factors associated with stem base and root diseases of New Zealand wheat and barley crops. *Australian Plant Pathology* 35: 391–400.

Devasena T., Menon V.P., 2003. Fenugreek affects the activity of beta-glucuronidase and mucinase in the colon. *Phytotherapy Research* 17: 1088–1091.

Dharajiya D., Jasani H., Khatrani T., Kapuria M., Pachchigar K., Patel P., 2016. Evaluation of antibacterial and antifungal activity of fenugreek (*Trigonella foenum-graecum*) extracts. *International Journal of Pharmacy and Pharmaceutical Sciences* 8: 212–217.

Doohan F.M., Brennan J., Cooke B.M., 2003. Influence of climatic factors on *Fusarium* species pathogenic to cereals. *European Journal of Plant Pathology* 109: 755–768.

Duke J.A., Reed C.F., Weder P., 1981. *Trigonella foenum graecum* L. In: Handbook of legumes of world economic importance (J.A. Duke, ed.) New York: Plenum Press.

Evans M.L., Hollaway G. J., Dennis J. I., Correll R., Wallwork H., 2010. Crop sequence as tool for managing populations of *Fusarium pseudograminearum* and *Fusarium culmorum* in south-eastern Australia. *Australasian Plant Pathology* 39: 376–382.

Evidente A., Monoca F.A., Andolfi A., Rubiales D., Motta A., 2007. Trioxazonane, a monosubstituted trioxazonane from *Trigonella foenum-graecum* root exudates, inhibits *Orobranche crenata* seed germination. *Phytochemistry* 68: 2487–2492.

Felton W.L., Marcellos H., Alston C., Martin R. J., Backhouse D., … Herridge D.F., 1998. Chickpea in wheat-based cropping systems and crown rot in the following crop. *Australian Journal of Agricultural Research* 49: 401–408.

Gargouri S., Hajlaoui M.R., Guermech A., Marrakchi M., 2001. Identification des espèces fongiques associées à la pourriture du pied du blé et étude de leur répartition selon les étages bioclimatiques en Tunisie. *Bulletin OEPP/EPPO Bull* 31: 499–503.

Gargouri S., Kammoun L., Guermech A., Hajlaoui M.R., 2007. Evaluation de l'incidence, de la sévérité et des pertes de rendement dues à la pourriture du pied du blé en Tunisie. *Annales de l'INRAT* 80: 7-20.

Gharbi M.S., El Felah M., 2013. Les céréales en Tunisie : plus d'un siècle de recherche variétale. *Annales de l'INRAT* 86 : 45–68.

Ghodbane A., Mahjoub M., Djerbi M., Mlaik A., Sharen A.L., 1974. Etude des pertes causées par les pathogènes du blé, *Septoria tritici* et *Fusarium* spp. *Rapport annuel du ministère de l'agriculture. Office des Céréales, Tunisie* 106.

Giorgi F., 2006. Climate change hot-spots'; geophysical Resources Letters, 33: 707–715. GTZ. 2007. Adaptation to climate change: development of a national strategy for agriculture, ecosystems and water resources in Tunisia 2005 to December 2007. 3p

Haouala R., Hawala S., El-Ayeb A., Khanfir R., Boughanmi N., 2008. Aqueous and organic extracts of *Trigonella foenum-graecum* L. inhibit the mycelia growth of fungi. *Journal of Environmental Sciences* (China) 20: 1453–1457.

Hemissi I., Gargouri S., Hlel D., Hachana A., Abdi N., Sifi, B., 2018. Impact of nitrogen fertilization on *Fusarium* foot and root rot and yield of durum wheat. *Tunisian Journal of Plant Protection* 13: 31–38.

Hogg A.C., Johnston R.H., Dyer A.T., 2007. Applying real time quantitative PCR to *Fusarium* crown rot of wheat. *Plant Disease* 91: 1021–1028.

Hollaway G., Evans M., Wallwork H., Dyson C., McKay A., 2013. Yield loss in cereals, caused by *Fusarium culmorum* and *F. pseudograminearum*, is related to fungal DNA in soil prior to planting, rainfall and cereal type. *Plant Dis*ease 97: 977–982. Available at https://doi.org/10.1094/PDIS-09–12-0867-RE.

Kandeler E., Tscherko D., Spiegel H., 1999. Long-term monitoring of microbial biomass, N mineralization and enzyme activities of a Chernozem under different tillage management. *Biology Fertility Soils* 28: 343–351.

Khemir E., Chekali S., Souissi A., Gharbi M.S., 2018. Survival of *Fusarium culmorum*, causal agent of foot and root rot of cereals, on wheat, barley and oat residues in Tunisia. *Annales de L'INRAT* 91: 162–172.

Kirkegaard J.A., Simpfendorfer S., Holland J., Bambach R., Moore K.J., Rebetzke G.J., 2004. Effect of previous crops on crown rot and yield of durum and bread wheat in northern NSW. *Australian Journal of Agricultural Research* 55: 321–334.

Klem K., Vanova M., Hajslova J., Lancova K., Sehnalova M., 2007. A neutral network model for prediction of deoxynivalenol content in wheat grain based on weather data and preceding crop. *Plant Soil Environment* 53: 421–429.

Knight N.L., Sutherland M.W., 2013. Histopathological assessment of wheat seedling tissues infected by

Fusarium pseudograminearum. *Plant Pathology* 62: 679–687.

Knight N.L., Sutherland M.W., 2017. Assessment of *Fusarium pseudograminearum* and *F. culmorum* Biomass in seedlings of potential host cereal species. *Plant Disease* 101: 2116–2122.

Knudsen I.M.B., Hockenhull J., Jensen D.F., 1995. Biocontrol of seedling diseases of barley and wheat caused by *Fusarium culmorum* and *Bipolaris sorokiniana*: effects of selected fungal antagonists on growth and yield components. *Plant Pathology* 44: 467–477.

Köppen W., 1936. The geographic climate system. In: Handbuch der Klimatologie (W. Köppen, R. Geiger, ed.), Gebrüder Borntraeger, Berlin, 1-44 (in German).

Kurowski T., Adamiak E., 2007. Occurrence of stem base diseases of four cereal species grown in long-term monocultures. *Polish Journal Natural Sciences* 22: 574–583.

Kraska P., Mielniczuk E., 2012. The occurrence of fungi on the stem base and roots of spring wheat (*Triticum aestivum* L.) grown in monoculture depending on tillage systems and catch crops. *Acta Agrobotanica* 65: 79–90.

Lamprecht S.C., Marasas W.F.O., Hardy M.B., Calitz, F.J., 2006. Effect of crop rotation on crown rot and the incidence of *Fusarium pseudograminearum*in wheat in the Western Cape, South Africa. *Australian Plant Pathology* 35: 419–426.

Leslie J.F., Summerell B.A., 2006. The *Fusarium* Laboratory Manual. Blackwell Publishing, 388 pp.

McKay A., Roget D., Hannam R., Ophel Keller K., 2008. Root Disease Risk Management Resource Manual. PIRSA Publishing: Adelaide.

McKenzie F., Taylor G.S., 1983. *Fusarium* populations in British soils relative to different cropping practices. *Transactions of the British Mycological Society* 80: 409–413.

Mergoum M., Braun H.J., Nicol J., Bagci A., Ekiz H., … Keser M., 2000. Screening wheat for root rot disease in WANA region. Page 186. In: Abstracts of the 6th International Wheat Conference, 4–9 June, 2000, Budapest, Hungary.

Montanari A., Innocenti G., Toderi G., 2006. Effects of cultural management on the foot and root disease complex of durum wheat. *Plant Pathology* 88: 149–156.

Muller M.E.H., Brenning A., Verch G., Koszinski S., Sommer M., 2010. Multifactorial spatial analysis of mycotoxin contamination of winter wheat at the field and landscape scale. *Agriculture, Ecosystems & Environment* 139: 245–254.

Oddepally R., Guruprasad L., 2015. Isolation, purification, and characterization of a stable defensin-like antifungal peptide from *Trigonella foenum-graecum* (fenugreek) seeds. *Biochemistry* (*Moscow*) 80: 332–342.

Omezzine F., Daami-Remadi M., Ladhari A., Haouala R., 2014. Variation in phytochemical content and antifungal activity of *Trigonella foenum-graecum* L. with plant developmental stage and ploidy level. *South African Journal of Botany* 92: 120–125

ONAGRI, 2016: Observatoire Nationale de l'Agriculture, Annuaire statistique 2016. 190 pp. (in French).

Ophel-Keller K., Mckay A., Hartely D., Herdina P., Curran J., 2008. Development of a routine DNA-based testing service for soil borne diseases in Australia. *Australian Plant Pathology* 37: 243–253.

Paulitz T.C., 2006. Low input no-till cereal production in the Pacific Northwest of the U.S.: The challenges of root diseases. *European Journal of Plant Pathol*ogy 115: 271–281.

Paulitz T.C., Schroeder K. L., Schillinger W. F., 2010. Soil borne pathogens of cereals in an irrigated cropping system: effects of tillage, residue management, and crop rotation. *Plant Disease* 94: 61–68.

Pemonge J., Pascual-Villalobos M.J., Regnault-Roger C., 1997. Effects of material and extracts of *Trigonella foenum-graecum* L. against the stored product pests *Triboleum castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). *Journal of Stored Products Research* 33: 209–217

Pereyra S.A., Dill-Macky R., 2004. Survival and inoculum production of *Gibberella zeae* in wheat residue. *Plant Disease* 88: 724–730.

Poole G.J., Smiley R.W., Paulitz T.C., Walker C.A., Carter A.H., …, Garland-Campbell K., 2012. Identification of quantitative trait loci (QTL) for resistance to *Fusarium* crown rot (*Fusarium pseudograminearum*) in multiple assay environments in the Pacific Northwestern US. *Theoretical and Applied Genet*ics 125: 91–107.

Rasmussen P.H., Knudsen I.M.B., Elmholt S., Jensen D.F., 2002. Relationship between soil cellulotytic activity and suppression of seeding blight of barley in arable soils. *Applied Soil Ecology* 19: 91–96.

Rossi V., Cervi C., Chiusa G., Languasco L., 1995. Fungi associated with foot rots on winter wheat in northwest Italy. *Journal of Phytopathology* 143: 115–119.

Sitton J.W., Cook R.J., 1981. Comparative morphology and survival of chlamydospores of *Fusarium roseum* '*Culmorum*' and '*Graminearum*'. *Phytopathology* 71: 85–90.

Slama A., Ben Salem M., Ben Naceur M., Zid E., 2005. Les céréales en Tunisie: Production, effet de la sécheresse et mécanismes de résistance. *Sècheresse* 16: 225–229 (in French).

Smiley R.W., Gourlie J.A., Easly G.S., Patterson L.M., Whittaker R.G., 2005. Crop damage estimates for crown rot of wheat and barley in the Pacific Northwest. *Plant Disease* 89: 595–604.

Smiley R.W., Patterson L.M., 1996. Pathogenic fungi associated with *Fusarium* foot rot of winter wheat in the semiarid Pacific Northwest. *Plant Disease* 80: 944–949.

Smiley R.W., Patterson L.M., Shelton C.W., 1996. Fungicide seed treatments influence emergence of winter wheat in cold soil. *Journal of Production Agriculture* 9: 559–563.

Snyder W.C., Nash, S.M., 1968. Relative incidence of *Fusarium* pathogens of cereals in rotation plots at Rothamsted. *Transactions of the British Mycological Society* 51: 417–425.

Steinkellner S., Langer I., 2004. Impact of tillage on the incidence of *Fusarium* spp.in soil. *Plant and Soil* 267: 13–22.

Sudan P., Goswami M., Singh J., 2020. Antifungal potential of fenugreek seeds (*Trigonella foenum-graecum*) crude extracts against *Microsporum gypseum*. *International Journal of Research in Pharmaceutical Sciences* 11: 646–649.

Toledo-Souza E.D., Silveira P.M., Lobo J.M., Café Filho A.C., 2008. Soil Density and Soil Pathogens Survival. *Pesquisa Agropecuária Brasileira* 43: 971–978.

Van Wyk P.S., Los O., Rheeder J.P., Marasas W.F.O., 1987. *Fusarium* species associated with crown rot of wheat in the Humansdrop District, Cape Province. *Phytophylactica* 19: 343–344.

Vicente-Serrano S.M., 2006. Spatial and temporal analysis of droughts in the Iberian Peninsula (1910–2000). *Hydrological Sciences Journal* 51: 83–97.

Wallwork H., Butt M., Cheong J.P.E., Williams K.J., 2004. Resistance to crown rot in wheat identified through an improved method for screening adult plants. *Australian Plant Pathology* 33: 1–7.

Wisniewska H., Kowalczyk K., 2005. Resistance of cultivars and breeding lines of spring wheat to *Fusarium culmorum* and powdery mildew. *Journal of Applied Genetics* 46: 35–40.

Xu F., Yang G., Wang J., Song Y., Liu L., Zhao K., Li Y., Han Z., 2018. Spatial distribution of root and crown rot fungi associated with winter wheat in the north China plain and its relationship with climate variables. *Frontiers in Microbiology* 9: 1054.

Yi C., Kaul H.-P., Kübler E., Aufhammer W., 2002. Populations of *Fusarium graminearum* on crop residues as affected by incorporation depth, nitrogen and fungicide application. J*ournal of Plant Diseases and Protection* 109: 252–263.

Zadoks J.C., Chang T.T., Konzak C.F., 1974. A decimal code for the growth stages of cereals. *Weed Research* 14: 415–421.

Zhou W.G., Everts K.L., 2007. Effects of host resistance and inoculum density on the suppression of *Fusarium* wilt of watermelon induced by hairy vetch. *Plant Disease* 1: 92–96.

Zia T., Siddiqui I.A., Nazrul-Hasnain., 2001. Nematicidal activity of *Trigonella foenum-graecum* L. *Phytotherapy Research* 15: 538–540.