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Incidence of Fusarium foot and root rot of cereals under conservation agriculture in north west Tunisia

Samira CHEKALI[1,*], Samia GARGOURI[2], Moncef BEN HAMMOUDA[3], Hatem Cheikh M'HAMED[4], Bouzid NASRAOUI[5]

[1] *Laboratoire de Protection des Végétaux, Institut National de Recherche Agronomique de Tunisie, Pôle Régional de Recherche-Développement Agricole du Nord-Ouest Semi-Aride au Kef, Université de Carthage, BP 221, 7100, Le Kef, Tunisia*
[2] *Laboratoire de Protection des Végétaux, Institut National de la Recherche Agronomique de Tunisie, Université de Carthage, Rue Hédi Karray, 2049, Ariana, Tunisia*
[3] *Laboratoire de Physiologie de la Production des Céréales, Ecole Supérieure d'Agriculture du Kef, Université de Jandouba, 7119 Le Kef, Tunisia*
[4] *Laboratoire de Recherche de Science et Techniques Agronomiques, Institut National de Recherche Agronomique de Tunisie, Université de Carthage, Rue Hédi Karray, 2049, Ariana, Tunisia*
[5] *Laboratoire de Recherche des Bioagresseurs et Protection Intégrée en Agriculture, Institut National Agronomique de Tunisie, Université de Carthage, 43 Avenue Charles Nicolle, 1082, Tunis-Mahrajène, Tunisia*
*Corresponding author: samirachekali@gmail.com

Abstract. Conservation agriculture, based on direct drilling for crop establishment, has emerged in Tunisia since 1999/2000 as an alternative to conventional agriculture based on conventional drilling. The main objective of this approach is to ensure yield stability from crops and replenishment of soil organic matter. Previous research has demonstrated increased risks from pathogens favoured by mulching. The impacts of direct drilling on yields, and Fusarium foot and root rot of durum wheat, oat and barley, were studied over three successive growing seasons (2009/10, 2010/11, 2011/12) in northwest Tunisia. Disease incidence was estimated by the recovery frequency of *Fusarium* spp. isolates from stem bases and roots of plants of the three cereals. In addition, disease severity was assessed by occurrence of whiteheads that senesced prematurely, and the browning intensity on durum wheat stem bases. Grain yields were recorded at the ripening stages. *Fusarium culmorum* and *F. pseudograminearum* were isolated from the three cereals, with *F. culmorum* being the dominant pathogen. Direct drilling increased the incidence (60%) of these pathogens on stem bases and roots during the warmer seasons of 2009/10 and 2010/11, but less infection (37%) compared to conventional drilling was observed in the wetter season of 2011/12. Direct drilling increased the percentage of whiteheads of durum wheat (73%), but did not affect disease severity, which was estimated by the discolouration on stem bases and grain yield.

Keywords. Direct drilling, conventional drilling, Fusarium foot and root rot, cereals, grain yield.

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INTRODUCTION

Conservation agriculture technology is increasingly relevant for addressing the needs of resource-poor farmers, and the challenges of resource degradation, sustainability, food insecurity, poverty alleviation, climate change, labour shortages and high energy costs (Kassam *et al*., 2012). Direct drilling (hereafter abbreviated as DD) for crop establishment is practiced on about 111 million ha worldwide, and the proportional of adoption by farmers is 47% in South America, 38% in the United States and Canada, 12% in Australia,4% in New Zealand, and 4% in the rest of the world, including Europe, Asia and Africa (Derpsch *et al*., 2010). In North Africa, conservation agriculture systems have been promoted, particularly in Morocco and Tunisia. In Morocco, 4,000 ha of DD have been reported, despite long-term research on DD farming initiated in the early 1980s (Mrabet, 2012). In Tunisia, the promotion and development of these approaches was farmer-centered, and the area under DD increased from 27 ha in 1999 to nearly 6,000 ha in 2007 and 8,000 ha in 2008 (FAO, 2011). DD is now applied on 12,000 ha, which cover 0.8 % of cereal agricultural land, an area distributed among 200 farmers and operated by 102 direct drilling machines (ICARDA, 2016).

Under DD, the soil is left undisturbed from harvest to planting, except for narrow strips where the seeds are planted and the fertilizer is applied (Schillinger *et al*., 1999). Despite the benefits, DD may constitute potential risk for disease development since leaving infected plant residues on soil surfaces contributes to increasing pathogen inoculum. This may influence the incidence of diseases caused by pathogens which survive on crop debris. These include the complexes of species causing foot and root rot (Smiley *et al*., 2005). This pathogen complex includes *Fusarium pseudograminearum* (teleomorph *Giberella coronicola*), *F. culmorum*, *F. avenaceum* (teleomorph *Giberella avenacea*), *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*), and *Microdochium nivale* (teleomorph *Monographella nivalis*). Under adopted conventional drilling (CD) in Tunisia, *F. culmorum* followed by *F. pseudograminearum* are the main pathogens isolated from cereals in the semi-arid regions (Gargouri, *et al*. 2001; Boughalleb *et al*. 2008). Both of these fungi survive in crop residues, but with different biologies. *F. culmorum* is can grow at lower temperatures than *F. pseudograminearum* (Doohan *et al*., 2003), and persists as hyphae within stubble residues and as chlamydospores in soil (Burgess, 2011).

These pathogens produce lesions on the coleoptiles, roots, and sub-crown internodes of affected plants, and cause browning of the stem bases. Damage to cereals is often unnoticed until white heads appear shortly before crop maturity, or as shriveled grain that is noted during harvest (Papendick and Cook 1974; Burgess *et al*., 2001). Infection is favoured by wet conditions shortly after sowing of crops, and disease severity increases and yield reductions become significant when infected plants are under water stress and/or exposed to high temperatures late in the growing season (Cook, 1981; Paulitz *et al*., 2002.; Smiley *et al*., 2005; Chekali *et al*., 2011). In Tunisia, losses in grain yields as much as 25% occur during the dry seasons (Chekali *et al*., 2013). Crop rotation is a major control method for these pathogens, reducing disease incidence in legume / fallow / cereals cropping systems (Chekali *et al*., 2016).

Reports of effects of DD on development of cereal foot and root rot diseases have been varied among regions. For DD adopted in Tunisia, there is no information on these diseases. The objective of the present study was to evaluate the impacts of DD on Fusarium foot and root rot development and yields of durum wheat, oat and barley subjected to different cropping sequences over 3 years in northwest Tunisia.

MATERIALS AND METHODS

Site description

A field trial was conducted during the 2009/10, 2010/11 and 2011/12 growing seasons, to compare DD to CD. The trial was located at the Experimental Station of Institut National des Grandes Cultures(INGC) located at Boussalem (36°36'35" N, 8°58'17" E), at an altitude of 124 m. The area has a warm temperate Mediterranean climate. The soil at the trial site contained 54% clay, 31% silt and 15% sand. The winter is the rainy season. According to Köppen(1936), the climate is classified as hot-summer Mediterranean climate with annual mean precipitation of 500–600 mm. This precipitation varied between 400 and 700 mm during the three growing seasons of this study (Table 1).

Experimental design and field lay-out

The trial was set up as a Split Plot Design. Four blocks were divided into two Main Plots, corresponding to CD and DD. These were each divided into five Sub Plots, each of 360 m^2 (30 m × 12 m), of the following rotations scenarios: durum wheat after oat, oat after durum wheat, durum wheat after barley, barley after durum wheat or durum wheat after faba bean. Prior to sowing, the soil was harrowed, and durum wheat var.

Table 1. Monthly rainfall (mm) recorded during the experimental period. The critical period for pathogen infection and disease development is highlighted in gray.

Growing seasons	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Total
2009/10	104.5	38.5	29.0	37.0	44.0	32.5	70.0	41.5	28.0	0.0	0.0	0.0	425.0
2010/11	14.5	64.0	88.0	42.0	55.0	102.0	42.0	420.0	26.5	49.0	4.0	0.0	529.0
2011/12	28.0	228.0	85.0	89.5	50.0	105.5	60.5	74.5	5.5	35.0	7.0	5.0	751.0

Table 2. Minimum and maximum temperatures recorded during the experimental period. The critical period for pathogen infection and disease development is highlighted in gray.

Growing seasons		Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.
2009/10	Max.	27.9	24.2	22.5	14.7	17.3	17.8	16.9	22.5	27.1	30.8	35.0	35.2
	Min.	17.4	12.6	7.5	7.6	5.0	5.1	6.4	11.4	13.0	15.9	20.8	21.1
2010/11	Max.	28.1	26.1	20.0	18.1	17.2	14.5	18.0	23.1	23.8	32.0	37.3	37.4
	Min.	17.4	13.0	10.5	4.9	5.2	4.5	7.8	9.0	13.5	15.7	20.4	19.7
2011/12	Max.	33.6	22.0	20.1	15.1	14.2	11.4	17.4	22.7	26.4	36.5	37.8	37.8
	Min.	18.3	13.2	12.0	7.0	5.3	4.7	8.1	10.6	11.2	19.1	21.7	20.3

'Rezzek', a local line of oat, barley var. 'Rihane'or fababean var. 'Sabeur' were sown. Ammonium nitrate fertilizer was added at different plant growth stages. Weeds, diseases (powdery mildew, Septoria blotch, rust) and insects (aphids) were controlled throughout the trial. Grain harvests were in June each season (Table 3).

Pathogen isolation and disease incidence

Forty mature plants of each of the three cereals were removed from individual plots using a zsampling pattern in each plot, and the plants were then thoroughly washed with tap water. Sections (each 2 mm length) of stem bases and roots were surface sterilized for 2 min in 6% NaClO and then for 10 s in 70% alcohol, followed by rinsing in sterile distilled water. These tissue pieces were then dried, and placed on 25% potato dextrose agar (¼ PDA) containing 100 mg L^{-1} streptomycin sulfate. Resulting fungal cultures were incubated for 5 d (12 h light/12 h dark) at 25°C. *Fusarium* spp. hyphae were transferred on carnation leaf agar (CLA) to favour conidium production, and were morphologically identified according to Leslie and Summerell (2006). *F. culmorum* and *F. pseudograminearum* identifications were confirmed by molecular techniques using the protocol of Möller *et al.* (1992) for DNA extraction. Primers and protocols used for PCR amplification of *F. culmorum* were those of Schilling and Geiger (1996), and for *F. pseudograminearum* those of Aoki and O'Donnell (1999). The incidence of infections was determined by the frequency of isolation of each *Fusarium* sp. from stem bases and roots.

Disease severity

Disease severity was assessed only for durum wheat, by determining prematurely senescing inflorescences (whiteheads) and the brownish discolourations on the stem bases. At the anthesis stage (Feekes 10.51), (late April), whiteheads were estimated visually three times in each treatment, and expressed as percent of total heads per plot. The brown colour that appeared between the first and the second internodes of stems was evaluated using a 0-3 scale (0 = no symptoms, 1 = light browning at the first internode (0-25%), 2 = clear browning extending to the second internode (25-50%), or 3 = dark browning extending to the second internode (75-100%)). Ratings were converted to severity indices using the following formula:

$$\text{Severity index} = \frac{((0) \times (0) + (1) \times (1) + (2) \times (2) + (3) \times (3))}{\text{Total number of observed plants}}$$

Yield estimates

Grain yields from different crop rotations were measured during each growing season.

Table 3. Technical itinerary adopted during the experimental period.

Growing season	2009/10		2010/11		2011/12	
	CD	DD	CD	DD	CD	DD
Soil management (from October to November)						
Harrowing number (0–15 cm deep)	3	0	2	0	2	0
Sowing rates (kg ha^{-1})						
Durum wheat	160	160	160	160	160	160
Oat	150	150	150	150	150	150
Barley	120	120	120	120	120	120
Fababean	120	120	120	120	120	120
Fertilizer applications (kg ha^{-1}) Ammonium nitrate (NH_4NO_3)						
- *Seedling stage (Feekes 1)*						
Durum wheat	120	120	100	100	100	100
Oat	100	100	100	100	100	100
Barley	100	100	100	100	100	100
- *Stem elongation stages (Feekes 7)*						
Durum wheat	100	100	130	130	100	100
Oat	100	100	130	130	100	100
Barley	100	100	130	130	100	100
Pesticide treatments (L ha^{-1})						
Herbicide:						
Before sowing - Glyphosate (as Round up+)	0	2	0	2	0	2
- *Seedling stage (Feekes 1)*						
- Durum wheat: Fénoxaprop-Ethyl, Iodosulfuron-Méthyl Sodium and Méfenpyr-diethyl	1	1	1	1	1	1
- Oat: 2-4-D-Acide, di Métosulam	1	1	1	1	1	1
- Barley: Pinoxadan, Cloquintocet-mexyl	1–0.8	1–0.8	1–0.8	1–0.8	1–0.8	1
- Fababean: Bentazone	1.25	1.25	1.25	1.25	1.25	1.25
Fungicide						
- Epoxiconazole	1	1	1	1	-	-
Insecticide						
- Deltamethrine	1	1	1	1	-	-

Statistical analyses

Data were analyzed using ANOVA to test the significance of main effects with four replicates, growing season, sowing method (DD *vs* CD) and crop sequences as main factors, using SPSS Statistics version 20 software published by IBM Crop 2011. Means comparisons was performed using Student's LSD tests ($P = 0.05$ or 0.01).

RESULTS

Identification of pathogens

During the three growing seasons, morphological identification of isolates recovered from 4,800 stem base fragments and 4,800 root fragments of the three cereals revealed that *F. culmorum* was isolated from 39.3% of stem bases and 16.2% from roots, and *F. pseudogramiearum was isolated from* 2.4% of stem bases and 0.9% of roots. Molecular identification using the specific primers OPT18 R and OPT18 F amplified 470 bp of *F. culmorum* fragments, and Fp1-1 and Fp 1-2 amplified 520 bp of *F. pseudograminearum* fragments.

Disease incidence

Data analyses revealed that growing season conditions significantly affected the incidence of infection by both *F. culmorum* ($P = 0.02$) and *F. pseudograminearum* ($P < 0.01$) on stem bases of the cereals. In addition,

Table 4. ANOVA of the effects of growing season (GS), sowing method (DD *vs* CD) and crop sequences (CS) on incidence of *Fusarium culmorum* (*Fc*) or *F. pseudograminearum* (*Fpg)* infections of durum wheat, oat or barley stem bases and roots during the experimental period.

SV	DF	*Fc*				*Fpg*			
		Stem base		Roots		Stem base		Roots	
		F value	Pr> F	F value	Pr> F	F value	Pr> F	F value	Pr> F
GS	2	3.910	0.024	0.007	0.993	11.53	0.000	2.050	0.136
DD *vs* CD	1	33.740	0.000	15.130	0.000	5.540	0.021	0.070	0.782
CS	4	0.930	0.446	1.220	0.309	2.620	0.042	0.680	0.602
CS × DT	2	0.610	0.545	0.790	0.457	5.977	0.004	0.370	0.964
GS × CS	8	1.500	0.170	0.870	0.543	2.301	0.029	0.480	0.864
DT × CS	4	1.990	0.120	0.630	0.638	3.533	0.011	0.430	0.786

there was a statistically significant incidence difference between the DD and CD treatments for *F. culmorum* on stem bases and roots (both $P < 0.01$) and for *F. pseudograminearum* only on stem bases ($P = 0.02$). However, no statistically significant effects were detected for the crop sequence effects, except for the incidence of *F. pseudograminearum* recovered from the stem bases ($P = 0.04$), and between the different tested factors (Table 4). Hence, combined data analyses were carried out for all of the crop sequences.

DD increased the incidence infection of *F. culmorum* on stem bases of durum wheat, oat and barley combined, compared to CD during the trial period. This effect was greater in the two dryer seasons, at 60%; ($P < 0.01$) in 2009/10, and 58% ($P < 0.01$) in 2010/11, than in the wetter season of 2011/12 (32%: $P < 0.01$). On roots, the incidence of infection by this pathogen (60%) was increased ($P < 0.05$) by DD only in 2010/11 (Figure 1).

Fusarium pseudograminearum was present in isolation only in the two growing seasons 2009/10 and

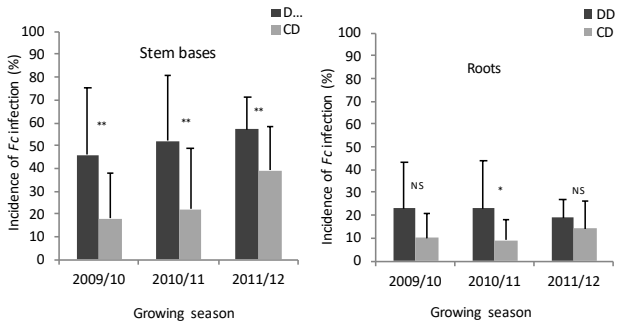

Figure 1. Mean incidence of *Fusarium culmorum* infections on stem bases and roots of durum wheat, oat or barley as affected by different sowing methods (DD *vs* CD) during three growing seasons. Error bars are 2 × standard deviations. NS: No significant difference ($P > 0.05$). *: significant difference ($P < 0.05$). **: highly significant difference ($P < 0.01$) according to Student's Test.

2010/11. Despite the low incidence on stem bases of the three cereals, DD significantly increased incidence of this pathogen (80%; $P < 0.02$) during the 2009/10 growing season, compared to CD (Figure 2).

Disease severity on durum wheat

DD affected occurrence of whiteheads only in the 2010/11 growing season. This drilling method greatly increased the percentage of durum wheat whiteheads (97%; $P < 0.01$; Figure 3), but had no effect on disease severity estimated by discoloration on stem bases.

Durum wheat yields, under DD vs CD and Fusarium foot and root rot

The average of grain yields of durum wheat estimated over the three growing seasons were not significantly different between DD and CD. The yields were estimated 2,700 kg ha^{-1} from DD, and 2,600 kg $ha^{-1}$from CD, in the 2009/10 and 2010/11 growing seasons. However, in the 2011/12 growing season, the durum wheat yields were 4,760 kg ha^{-1} from DD and 4,660 kg $ha^{-1}$from CD. No statistically significant interaction was detected between DD or CD and infection incidence or disease severity, or for occurrence of whiteheads.

DISCUSSION

Conservation agriculture and crop residue management may have impacts on development of soilborne pathogens and the diseases they cause, in medium or long term periods. In this study durum wheat, oat and barley were subjected to different crop rotations and DD or CD crop establishment methods, and assessed for dif-

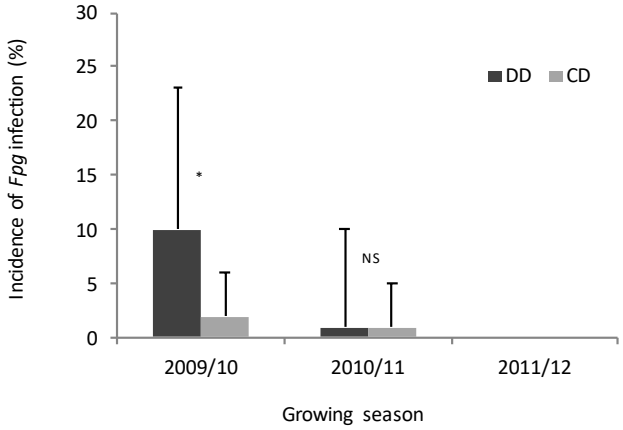

Figure 2. Mean incidence of *Fusarium pseudograminearum* stem base and root infections resulting from different cereal sowing methods (DD *vs* CD) for durum wheat, oat of barley during three growing seasons. Error bars are 2 × standard deviations. NS: No significant difference ($P > 0.05$). *: significant difference ($P < 0.05$).

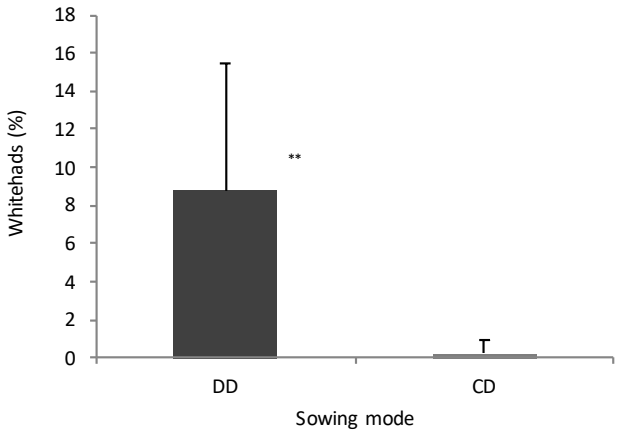

Figure 3. Mean proportions of whiteheads in durum wheat for different sowing methods (DD vs CD) during the 2010/11 growing season. Error bars are 2 × standard deviations. **: highly significant difference ($P < 0.01$) according to Student Test.

ferences in root and crown rot diseases caused by *Fusarium* spp. *Fculmorum* and *F. pseudograminearum* were frequently isolated from stem bases and roots of the three cereals, These results are in agreement with those of Gargouri (2003) and Boughalleb *et al.* (2008), who reported the dominance of *F. culmorum* in Tunisia.

The presence and distribution of each of these species varied in the three experimental years. Both pathogens were isolated as a complex during dry growing seasons (2009/10, 2010/11), while in the wet season (2011/12) only *F. culmorum* was isolated. Similar results were obtained by Lipps and Deep (1991), and Steinkellner and Langer (2004) in Austria. Precipitation and temperature occurring during the experimental period probably influenced the development of each species. Rainfall over the three growing seasons was 425 mm in 2009/10, 529 mm in 2010/11 and 751 mm in 2011/12, with considerable fluctuation during each season. Precipitation over the infection periods (January-February) was almost the same in 2010/11 and 2011/12, but less in 2009/10, and during the disease development period (March-April) was slightly greater in 2009/10 and 2011/12 than in 2010/11 (Table 1). In parallel, the mean recorded temperatures from infection to disease development (January-April) in 2009/10 (11.3–14.3°C) and 2010/11 (10.4–14.5°C) were greater than those in 2011/12 (8.9–14.7°C) (Table 2). The relatively low temperatures in January (9.8°C) and February (8°C) of 2012 (Table 2), in comparison with the other two seasons, probably favoured the development only of *F. culmorum*. These results indicate that this pathogen developed at low temperatures, as reported by Doohan *et al.* (2003), Gargouri (2003), Pitt and Hocking (2009) and Poole (2010). This indicates that *F. culmorum* is more adapted to cold regions than *F. pseudograminearum*, which develops better when it is warm. Some authors have suggested that the relationship between the two *Fusarium* species and DD depends on physical, nutritional and microbiological characteristics of soils. Smiley and Patterson (1996) demonstrated that diseases caused by these pathogens increased with amount of soil surface crop residues that occur, and they are directly correlated with amounts of soil organic nitrogen and carbon. However, under DD, more soil water is conserved than for CD, which could reduce the incidence of cereal foot rot, especially if caused by *F. culmorum* which is enhanced by plant water stress (Papendick and Cook, 1974). On the other hand, *F. pseudograminearum* becomes a serious problem on wheat both in low and high precipitation zones of the Pacific northwest of the United States of America, and was more dependent than *F. culmorum* on crop residues for survival (Paulitz*et al.*, 2002).

The present research showed that after only one year of the experiment, DD increased the incidence of infection by the two fungi, mainly on stem bases of the three different cereals. This effect was consistent with those reported by Windels and Wiersma (1992), Smiley and Patterson (1996) and Bailey *et al.* (2001); and those obtained by Cook (2001), Paulitz*et al.* (2002)and Schroeder and Paulitz (2006). These authors suggested that the main reasons for the increasing range and prevalence of root diseases on wheat and barley in the United States of America and many other cereal-growing areas of the world are the increased frequency of cereals in the crop rotations, and the use of minimum or

no-tillage. Other authors have expressed opposite views. Papendick and Cook (1974) explained that under DD, more water is conserved in soil, leading to reduced incidence of cereal foot rot, especially if caused by *F. culmorum*, which is enhanced by plant water stress. Govaerts*et al*. (2006) suggested that DD may decrease the effects of the disease by improving soil quality, water retention and microbial activity. In our research conditions, we suggest that both high temperature and water stress at flowering time both had major effects, increasing the incidence of the disease.

The severity of disease estimated by whiteheads appearing prematurely on durum wheat was greatly increased (97%) by DD in 2010/11, which is not in agreement with the results of Wildermuth *et al*. (1997). They found fewer whiteheads (4.3%) after DD,and more (19.3%) after reduced drilling and CD (12.2%) and *F. pseudograminearum* infection. Others have suggested that DD reduces the formation of whiteheads by inducing humid crop canopy atmospheres. Results from the present study can be explained by insufficient moisture at soil level, which was probably caused by high evapotranspiration in the high temperature recorded in the region.

DD did not increase disease severity, estimated by the brown discolourations on durum wheat stem bases. In addition, the grain yields were not affected.

CONCLUSIONS

Results of this study have shown that *F. culmorum* followed by *F. pseudograminearum* were the main pathogens recovered from stem bases and roots of the three cereals examined. The results also showed that for the three cereal types, DD increased incidence of *F. culmorum* infection by 58-60% in dry seasons, and by 32% in a wetter season. However, incidence of *F. pseudograminearum* infection increased by 80% in dry seasons under DD. In contrast to several other reports, DD increased disease severity by 97%, as expressed by whiteheads appearing in durum wheat prior to maturity. This may be attributed to the high temperatures and water stress at the experimental site.

There was no difference between DD and CD for disease severity, estimated by the discolouration of cereal stem bases.

In conservation agriculture, for cereal production, inputs are reduced, soil quality improves, and grain yields get close to, or slightly greater than, those from conventional agriculture. This is because there is more available water for the crop growth where conservation agriculture is applied. In the present study, no significant differences in grain yields from the three cereals were observed between DD and CD.

Given the high incidence of *F. culmorum* infections on durum wheat, oat and barley under DD observed in this study, further research is required at more sites over long periods, and in different farming systems. Furthermore, these studies should also consider host variety/species effects on diseases caused by *Fusarium* spp.

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