

Occurrence and Geographic Distribution of Wheat Fusarium Head Blight and Fusarium Root Rot in Jendouba's Areas of Tunisia

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ABSTRACT

Mycological analysis of infested wheat plants (*Triticum durum* L.) collected from 68 fields in the low valley of Medjerda (Bousselem, Brahim, Bir lakhther, Souk essebet, Jendouba Northern, Oued Meliz and Ghardimaou) was performed in 2006. Eight hundred and fifty three isolates were obtained from the feet and root; 1580 isolates were identified from the head. On the collar, we identified *Fusarium solani* (52.5%), *F. culmorum* (27%), *F. graminearum* (11%) and *F. oxysporum* (9.5%). These species were present in all prospected areas except for *F. graminearum* which was not found in Bousselem. As for head blight, in addition of these four species which were isolated at a 28, 13, 16 and 30% frequency, respectively, we detected *F. equiseti* (9%) in Oued Mliz and *F. poae* (4%) with an important frequency in Souk Essebet area. The technique adopted to efficiently isolate *Fusarium* species was very satisfactory. Our study shows a frequent occurrence in single fields of several species of *Fusarium*.

Keywords: durum wheat, *Fusarium* spp., isolation frequency, survey

INTRODUCTION

Fusarium feet rot (FFR) and Fusarium head blight (FHB) are economically important diseases occurring across a broad range of environmental and temperature conditions, especially in areas with low to intermediate rainfall such as North Africa. In Tunisia, a 44% yield loss was reported in 1974 (Ghodbane *et al.* 1974). These diseases are also responsible for lower grain quality (Wiese 1987). Disease etiology is often complex and varies regionally (Smiley and Patterson 1996). Recent surveys conducted in Tunisia showed that *Fusarium culmorum* was the principal pathogen (Gargouri *et al.* 2001; Boughalleb *et al.* 2006a).

During the wheat harvest, shriveled infected grains that are light enough to be eliminated and cause direct yield loss (Parry *et al.* 1995). The kernels that are less severely infected are harvested but are lighter than uninfected kernels and thus affect the "1000-kernel weight". Fusarium-damaged kernels may also have lower technical and nutritional qualities (Dexter *et al.* 1997).

The greatest concern posed by Fusarium head blight is that certain species of fungi involved in the disease may produce mycotoxins in the infected grains. These mycotoxins, mainly trichothecenes, are harmful to humans and animals. Since 1990, the disease seems to have re-emerged in North America causing severe economic losses (Windels 2000). The problem remains endemic in the European area and in Mediterranean countries. In Tunisia, Fusarium head blight was reported in some areas in Bizerte and Beja, other Northern areas (Boughalleb *et al.* 2006a). The pathogens involved in the disease and the extent of the damage to the cereal crop are influenced by several factors such as: monoculture, seed quality (Boughalleb *et al.* 2006b) and the incidence and severity of Fusarium head blight is mainly influenced by the inoculum and the environmental conditions during the growing season (Parry *et al.* 1995). Several studies have shown that up to 17 species of *Fusarium* can be isolated from cereal grains. In Europe, Fusarium head blight

is caused by at least four species of *Fusarium*: *F. graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae* and by *Microdochium nivale* (Parry *et al.* 1995). In Tunisia, three species were isolated from wheat grain, *F. culmorum*, *F. graminearum* and *Microdochium nivale* (Boughalleb *et al.* 2006a).

The aim of this study was to identify the *Fusarium* spp. that naturally infects Tunisian wheat. This data will provide more knowledge of *Fusarium* species and will allow the occurrence and frequency of *Fusarium* spp. affecting wheat in the northern areas of Tunisia to be modeled.

MATERIAL AND METHODS

Sample collection

Durum wheat (*Triticum durum* L.) plants were collected from different areas in Jendouba province. The sites were chosen on the basis of fields where symptoms of head blight and root rot were historically observed by farmers and growers advisers in 2004-2005 to be sure that we would isolate *Fusarium* species. Surveys were done in April, March and May of 2005-2006 from 68 wheat fields located in Bousselem, Brahim, Elbir lakhther, Souk Essebet, Jendouba Northern, Oued Mliz and Ghardimaou areas at a rate of 10 plants per field (Table 1). The samples were packed in paper bags and immediately sent to the laboratory. Prior to analysis, the

Table 1 Geographical origin and numbers of samples analyzed from collection made in Jendouba's areas of Tunisia in cropping year 2004-2005.

Geographical origin	№ of fields	№ of samples
Bousselem	10	10
Brahim	10	10
Elbir lakhther	10	10
Souk Essebet	10	10
Jendouba Northern	10	10
Oued M'liz	10	10
Ghardimaou	8	8

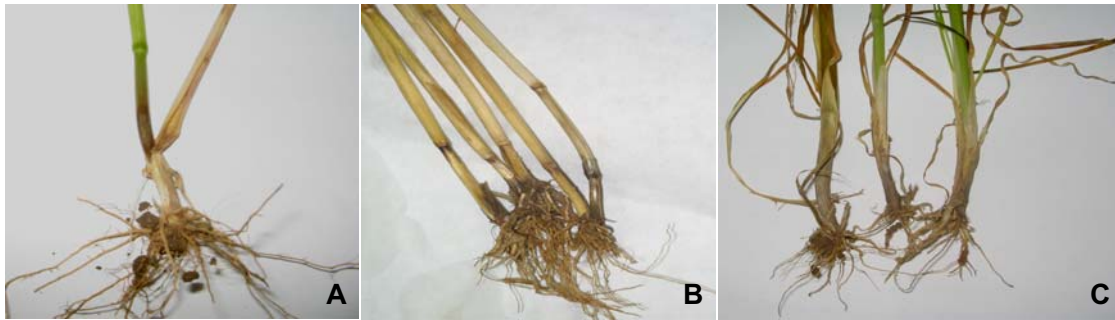


Fig. 1 Typical symptoms on feet and roots. (A) Brown to violet lesions on feet; (B) Migration of symptoms from feet to stems; (C) Black lesions on roots.

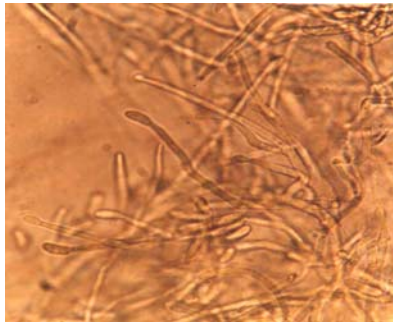


Fig. 2 Monophialidae and microconidia of *Fusarium solani*.

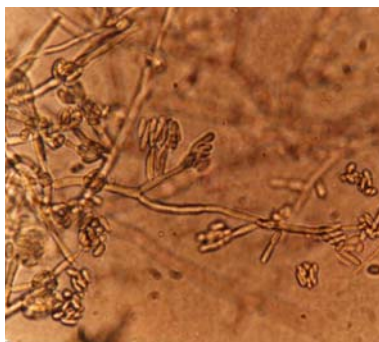


Fig. 3 Sporodochia and microconidia of *Fusarium oxysporum*.

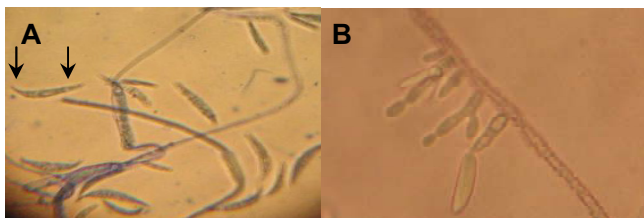


Fig. 4 *Fusarium graminearum*. (A) Macroconidia; (B) Sporodochia.

samples were stored at $5 \pm 1^\circ\text{C}$ until used.

Isolation of *Fusarium* species

Isolation from collar and roots of wheat

The samples taken at the time of the surveys were initially washed with tap water, cut into 5 mm-pieces, surface-disinfected with 0.3% NaHClO, then completely dried on a sterile filter paper (Whatman N°2) under a laminar flow hood. These fragments were plated on 9-cm Petri dishes containing potato dextrose agar (PDA) (Difco, Detroit, MI). Petri dishes were incubated for 5 to 7 days at 25°C . Fungi growing from plated pieces were identified based on colony characteristics as well as spore and culture morphology described by Booth (1977).

Isolation from grains

Approximately 50 ml of grains were randomly sampled from each sample and were surface-sterilized by soaking for 10 min in a solution containing 0.3% NaHClO, then completely dried on a sterile filter paper under a laminar flow hood. One hundred grains were randomly selected from these samples and ten seeds were

plated per PDA plate. Petri dishes were incubated under a combination of long-wave ultraviolet and fluorescent light (12 h light: 12 dark) for 8 to 10 days at 25°C . Isolates obtained were identified according to Booth's criteria (1977).

Microscopic identification of the isolated species

The sporulating isolates of *F. graminearum*, *F. culmorum*, and *F. poae* were readily identified on PDA medium. Typical macroconidia were produced by *F. graminearum*, *F. culmorum* and typical microconidia were produced on swollen-base monophialides by *F. poae* as previously reported by Hocking and Andrews (1987). All the other *Fusarium* species isolated were subsequently transferred to LNA (Low Nutrient Agar) (Nirenberg 1981) and were identified after 6 to 10 days of incubation at 22°C according to Summerell *et al.* (2003).

RESULTS

Identification of *Fusarium* species isolated from wheat feet and roots

Characteristic symptoms were observed on infested feet and roots of wheat, typically appearing brown when caused by *F. roseum* or black to violet lesions due to *M. nivale* (Fig. 1).

From 68 surveyed fields belonging to seven areas of Jendouba province, 680 durum and tender wheat plants were analyzed. *Fusarium* spp. were detected in most samples. Approximately 1020 isolates were recovered including 853 of *Fusarium*. Commonly, up to four different species of *Fusarium* were isolated in a sample collected in a field: 448 isolates of *F. solani* (Fig. 2), 81 of *F. oxysporum* (Fig. 3), 94 of *F. graminearum* (Fig. 4), and 230 isolates of *F. culmorum* (Fig. 5). In Table 2, we summarize the relative frequencies of the main *Fusarium* species obtained from the different growing areas.

Identification of *Fusarium* isolated from wheat head blight

From 3400 fungi isolates, 1580 were identified as *Fusarium* spp. including 438 isolates of *F. solani*, 472 isolates of *F. oxysporum*, 206 isolates of *F. culmorum*, 255 isolates of *F. graminearum*, 144 isolates of *F. equiseti* (Fig. 6) and 65 isolates of *F. poae* (Fig. 7). In Table 3 we report the relative frequencies of the main *Fusarium* species obtained from wheat head blight collected from different growing areas.

Geographical distribution of *Fusarium* spp.

There was no obvious geographical specialization among *Fusarium* species for any particular area. *Fusarium culmorum*, *F. solani* and *F. graminearum* were as common in contaminated wheat feet and roots as they were in head blight. However, *F. culmorum* was predominant on feet and roots in Bousselem and *F. graminearum* in Gardimaou (Fig. 8). From grains, it appears that *F. culmorum* was the most prominent in Bousselem, Elbir Lakhther and Gardimaou (Fig. 9).

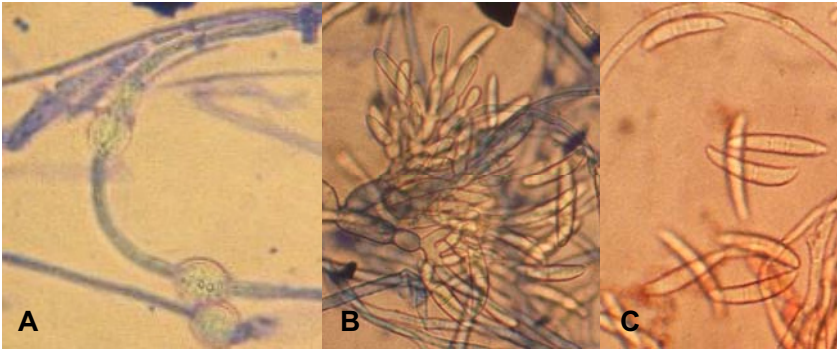


Fig. 5 *Fusarium culmorum*. (A) Chlamydoconidia; (B) Sporodochia; (C) Macroconidia (Magn. $\times 40$)

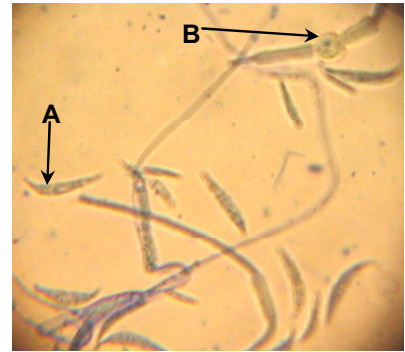


Fig. 6 *Fusarium equiseti*. (A) Macroconidia; (B) Chlamydoconidia.

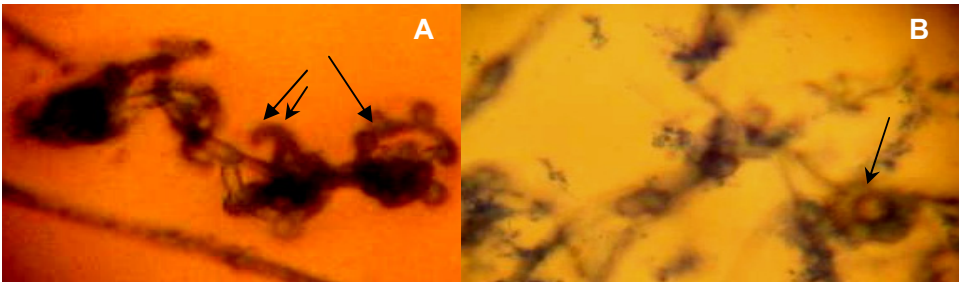


Fig. 7 *Fusarium poae*. (A) Sporodochia; (B) Chlamydoconidia.

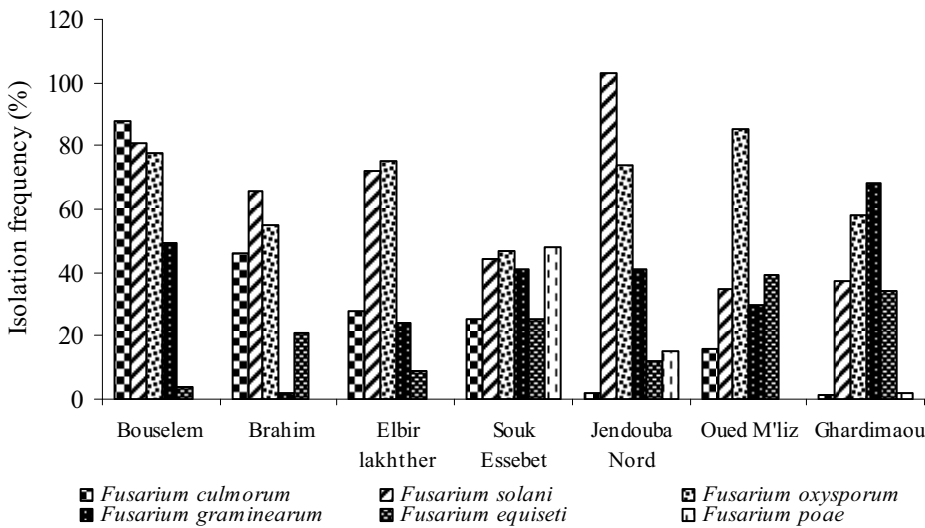


Fig. 8 Frequency of the *Fusarium* species isolated from wheat feet and root samples in Jendouba's areas of Tunisia in the 2004-2005 cropping year.

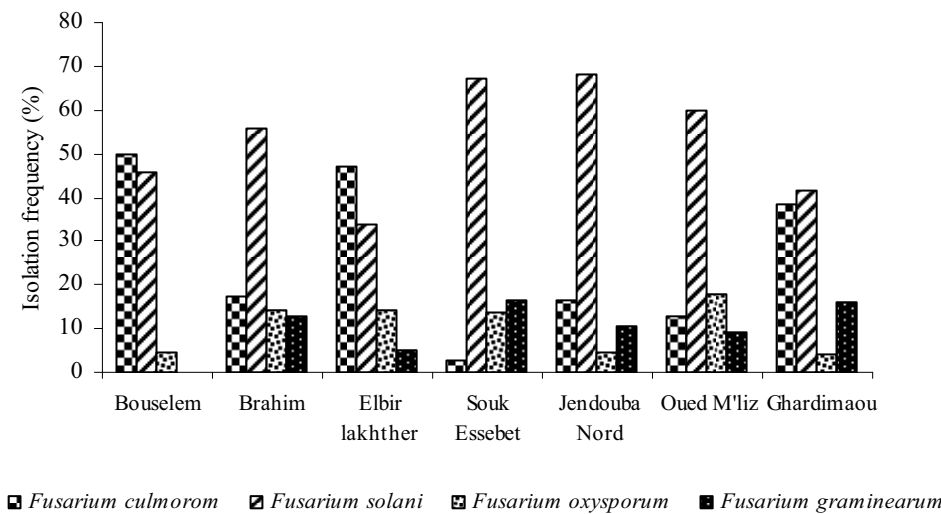


Fig. 9 Geographical distribution of *Fusarium* species isolated from wheat grain samples in Jendouba's areas of Tunisia in the 2004-2005 cropping year.

DISCUSSION

Fusarium spp. were identified in all surveyed areas with different levels of isolation frequency from one area to another

which could be related to climatic variations. Indeed, the more we moved towards the central areas (Souk essebet and northern Jendouba), the less we isolated *F. culmorum* and the greater presence of *F. solani*, which became preva-

Table 2 Frequency of *Fusarium* species isolated from wheat feet and root samples in Jendouba's areas of Tunisia in cropping year 2004-2005.

<i>Fusarium</i> species	Isolation frequency (%)
<i>F. culmorum</i>	27
<i>F. solani</i>	52.5
<i>F. graminearum</i>	11
<i>F. oxysporum</i>	9.5

Table 3 Frequency of the *Fusarium* species isolated from wheat grain samples in Jendouba's areas of Tunisia in cropping year 2004-2005.

<i>Fusarium</i> species	Isolation frequency (%)
<i>F. culmorum</i>	13
<i>F. solani</i>	28
<i>F. oxysporum</i>	30
<i>F. graminearum</i>	16
<i>F. equiseti</i>	9
<i>F. poae</i>	4

lent in the northern area of Jendouba. These results confirm those of Gargouri *et al.* (2001) which showed the sensitivity of the semi-arid Pacific to these pathogenic strains. *F. graminearum* was not identified in the area of Bousselem which is the same result found by Mrabet (1998) and contradicted our previous findings (Boughalleb *et al.* 2006a). This contradiction could be due to climatic conditions that are different from year to year.

In the higher wetland (Bousselem), with strong relative humidity and especially during a rather soft winter temperature, *F. culmorum* predominated. Moreover, as we moved towards the higher mid-arid zone, *F. graminearum* and *F. solani* became dominant in the areas of Oued Meliz and Ghardimaou. These results are in agreement with those of Mahjoub (1978) who found that *F. culmorum* is the most frequent strain in North Western areas and when moving towards the center where the winter temperature is milder, *F. graminearum* becomes prevalent. In the same sense, Mrabet (1998), Gargouri *et al.* (2001) and Boughalleb *et al.* (2006a) showed the heterogeneous geographical distribution of these species according to the bioclimatic stage.

The identification from wheat head blight revealed, for the first time in Tunisia, the presence of *F. poae* and *F. equiseti* on grains. These species were not found on feet and root samples and could have several explanations: sampling, which is done randomly, samples size, the date of sampling. Furthermore, climatic conditions and wind could be favorable to the aerial dissemination of these species and the infection of the ears. *F. poae* was widespread in the area of Essebet Souk and was found in all surveyed fields. The geographical distribution of *F. roseum*, identified in many areas in Tunisia, has been reported in many countries around the world. Schneider (1964, cited by Gargouri *et al.* 2001) mentioned that *F. culmorum* requires more moisture than *F. graminearum*, which is very frequent in the areas with cold and dry climates of Russia (Booth 1977). In Morocco, *F. culmorum* was present in 25% of the fields (Lya-man 1988).

CONCLUSION

Wheat *Fusarium* head blight and *Fusarium* feet and root rot diseases are important problems occurring across a broad range of environmental and temperature conditions. These diseases affect small grain cereal crops by quantitative losses and by qualitative depreciation of the harvested grains. The study of the geographical distribution of fungi responsible of these diseases showed the presence of four species from roots *F. culmorum*, *F. graminearum*, *F. solani* and *F. oxysporum*, dominated by *F. solani*, *F. culmorum* and *F. graminearum*. From grains, we also isolated two other species, *F. equiseti* and *F. poae* although *F. oxysporum* and *F. graminearum* were predominant.

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