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Propagation of Wheat Fusarium Wilt in Morocco

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Abstract: ***The cereal branch is one of the main agricultural production sectors in Morocco, including wheat, which represents the first speculation, given the size of the areas it covers. This culture faces many constraints that negatively affect its productivity. Among these constraints, we find fungal diseases including Fusarium wilt, which affects yields and sanitary quality of seeds. In order to promote the protection and the study of the phytosanitary status of wheat against Fusarium wilt, the Doukkala, Chaouia, Haouz, and Tadla regions are prospected to collect seed samples, while the Gharb-Chrarda-Bni-Hssen, Meknes-Tafilalet,and Fez-Boulemane regions were targeted to collect fresh samples at the heading stage, and that during the year 2013/2014. Thus the calculation of infestation levels, macro, microscopic and molecular identification by "PCR" was performed. The results obtained show that all the prospected regions were contaminated. For seed samples, durum wheat had higher levels of infections than soft wheat, which was not negligible. For durum wheat in the Doukkala, Haouz and Tadla regions, more than half of the samples had an average rate of Fusarium wilt infection, while 50% of soft wheat seeds had low levels of infection. In Chaouia, 50% of durum wheat samples were heavily infected, against 31% only for soft wheat. For fresh samples, the results asserted that the most infected plots were those from Gharb, of which 40% of the studied plots were highly infected, while the Meknes and Fez regions recorded the lowest infection rates.PCR molecular identification of seed samples isolates revealed the presence of two species responsible for Fusarium wilt, Microdochium nivalemajus and Fusarium avenaceum, with a dominance of Microdochium nivalemajus of over 90%. Regarding the fresh samples, the species detected by PCR areMicrodochium nivalemajus with 42% of dominance, followed by Fusarium culmorum: 40% and finally Fusarium graminearum: 18% for the region Gharb-Chrarda-Bni-Hssen. In the Meknès-Tafilalet and Fès-Boulemane regions, the only species that caused the infection is Fusarium avenaceum. We conclude from these results that the yield and sanitary quality of the crop incur significant risk due to Fusarium wilt, which damages the wheat crop by the presence of mycotoxins produced by Fusarium species in the grains.***

Keywords: Seeds, Durum wheat, Soft wheat, Fresh samples, Heading stage, Fusarium wilt, *Fusariumculmorum*, *Microdochiumnivale majus*, *Fusarium graminearum*, *Fusariumavenaceum*,andMycotoxins.

1. Introduction

Many fungal species, including those with anamorph *Fusarium*, are responsible for various wheat diseases at different stages of its vegetative development. In fact, these pathogens can cause damping-off, crown rot or scalding of the ear (Wiese 1987, Agrios 1997).

Ear scalding takes place in humid regions, contamination is caused by aerial spores or those ejected by water that is deposited directly on the spikelets at the time of flowering (Bergstrom, 1993, Ireta and Gilchrist 1994, McMullen and*al*., 1997). Fusarium head blight (FHB) can be caused by various species of *Fusarium* and *Microdochium* (Parry et *al*. 1995). In North-West Europe, the main pathogens of FHB are*F. culmorum, F. graminearum, F. avenaceum*,and*F. poae*, (Bottalico and Perrone, 2002, Ioos and*al*., 2004). This disease is manifested by the early drying of some spikelets. The grains resulting from contaminated spikelets are invaded by the parasite and take on white or pink coloring. Infections cause very significant declines in yield. (Cook, 1981, Martin and Johnson, 1982). In France, strong attacks are reported when a rainy period occurs during flowering and during the period of filling of the grain.

Damping-off manifested by root rot (also known as common rot) is due to contaminated seed or plant debris containing the inoculum and generally occurs in areas where epi disease predominates (Cook, 1980).). This disease can be caused by complex fungi including *Fusarium* or *Microdochium* species. (Wiese 1987, Tinline 1988, Windels and Holen 1989, Smiley and Patterson 1996).

Crown rot (or foot rot) is mainly caused by *Fusarium*anamorphous fungi present in soil or on cereal stubble (Cook, 1968, 1980, Smiley and Patterson, 1996). According to Wiese 1987 species,*F. culmorum*and *F. avenaceum*are responsible for crown rot.

In addition to yield losses caused by Fusarium wilt, *Fusarium* spp. contaminate grain with mycotoxins, which are secondary metabolites produced by certain microscopic fungi (Prandini et *al*., 2007). Among the main fusarium toxins (produced by *Fusarium*), we find Trichothecenes which are inhibitors of eukaryotic protein synthesis (Cumagun et *al*., 2004) and of the activation of plant defense genes (Wagacha and Muthomi, 2007). As a result, Trichothecenes are responsible for adverse effects on human and animal health in the case of consumption of contaminated food and can thus cause a great variability of symptoms such as alterations of the liver, kidneys, central nervous system, hormonal imbalances or a reduction of immune defenses (Prandini et *al*., 2007).

Subspecies of the genus *Microdochium* areconsidered incapable to produce mycotoxins (Logriecoand *al*., 1991). Fusarium wilt is a serious disease in arid and semi-arid regions that can cause high yield losses (Cook 1980). The climatic conditions of Morocco favor the development of this disease. According to the work done by Ghodbane et *al*. (1974), wheat yield losses due to Fusarium wilt were about 44% in 1974. However, little work has been done on the evolution of this disease and on the identification and geographical distribution of species of fungi responsible for the disease in Morocco.

The objective of this study is to evaluate the contamination levels of the collected samples and to identify the pathogens responsible for Fusarium wilt in Morocco.

2. Material and methods

1) Fusarium wilt blight mapping

The mapping is the easiest way to represent the distribution of infestation levels in a given space. This distribution allows us to have a global vision of the epidemic and to identify infested spatial structures, exploiting the innate ability of the human eye and brain to identify patterns. (Gosme, 2007).

2) Field prospecting

Field prospecting is about a spatial analysis of a natural outbreak of Fusarium wilt. The aim of the field survey is to obtain a representative image of the foci of the disease in the 7 regions surveyed: Doukkala, Haouz, Chaouia, Tadla - Azilal Gharb-Chrarda-Bni-Hssen, Meknes-Tafilalet,and Fez-Boulemane during the agricultural season 2013/2014.

The plots and the souk being prospected were determined according to a systematic approach by choosing the first stop at random and setting a distance of 10 to 20 km between two successive stops. This unit was not modified during the collection of fresh samples in order to preserve the randomness of the sampling. While for seed samples the distance between stops was relative to the availability of samples. At each stop, the coordinates of the plot were noted by a GPS (GARMIN, GPSmap 60CSx).

The stops were made in areas planted with cereals, represented on agricultural maps provided by the PDAs (Provincial Directions of Agriculture) of each region. The number of stops per region was determined according to the importance of the cereal areas of the region.

The collection of soft and hard wheat seeds was carried out on the plots of farmers and the souk of Doukkala, Haouz, Chaouia and Tadla regions. For fresh samples, the heading stage was the target stage for sampling in the Gharb-Chrarda-Bni-Hssen, Meknès-Tafilalet and Fès-Boulemane regions, so that the four organs, root, collar, glume and seed may be analyzed.

For the cultivation of the fungus responsible for Fusarium wilt, we used PDA ″Potato, Dextrose, Agar″ (250 g of potato, 20 g of agar powder, 5 g of glucose and 1 liter of water distilled sterile), to which we added chloramphenicol and streptomycin to prevent bacterial growth. The plants taken from each plot (samples) were prepared so that the four organs of the plant (roots, collars, glumes,and grains) be separated.

Then these organs were rinsed for 5 minutes with water. The roots and the collars of the plants were cut into small pieces. The roots, collars, glumes,and grains were shaken in a plastic bag to be mix randomly. Subsequently, they were placed separately in beakers to be disinfected and put in the culture medium.

3) Fungus isolation

The different parts of the studied plants were disinfected with sodium hypochlorite diluted 4 ° (a mixture of 1/3 of sodium hypochlorite and 2/3 of sterile distilled water) for 2 minutes, then rinsed twice with sterile distilled water for 1 minute. The samples were then dried on sterile filter paper under the hood. For the seed samples, 50 grains were randomly deposited on Petri dishes containing PDA medium at the rate of 10 elements per dish. Against 20 units of each organ (root, collar, glume,and grain) issued from all the fresh samples that were deposited on Petri dishes containing PDA medium, at a rate of 5 elements per box, with 4 repetitions. Petri dishes containing all the samples were incubated at 23 ° C, in the dark, for a period ranging from 7 to 10 days. The identification of the fungus requires a purification of the isolates from the fungus. For this, using a cookie cutter, a homogeneous part of the mushroom mycelium was taken from the original box and was deposited on a new box, that to better observe its development.

4) Morphological characterization of Fusarium wilt disease pathogen

After incubation (7 to 10 days), detection of the pathogen was made basing on morphological criteria and using the identification keys: [Fusarium species, an illustrated manual for identification (Nelson et *al* 1983) and The Fusarium laboratory manual (Leslie and Summerell, 2006)] which describe the morphological characters (macro and microscopic) of the studiedfungi's.

a) Macroscopic characterization

The macroscopic identification criteria are:

- The growth rate of the strain;
- An aspect of the mycelium;
- The color of the aerial part of the colony;
- The color of the part of the colony in contact with the medium and eventually the coloration of the culture medium;
- The presence and the coloration of sporodochia (production structures of macroconidia).

b) Microscopic characterization

The reference medium used for the microscopic description is the PDA medium. It allows a large production of conidia of homogeneous form, facilitating the identification of the mushroom.

The observation of the fungus was performed under a microscope (G x 100). The microscopic identification criteria used are:

- Presence of typical multicellular, hyaline, fusiform macroconidia that include a foot cell located at the base of the conidiophores;
- Presence or absence of globular or oval microconidia;
- Presence or absence of chlamydospores.

Infection percentage calculation

The percentages of infection of all the studied samples were calculated according to the following formula:

% Identification= (Number of fragments infected / Tested fragments total number)*100

Molecular characterization of isolated species responsible for Fusarium wilt by the PCR polymerase chain reaction. Molecular detection was performed on all isolates obtained from the treated samples.

c) DNA extraction

In order to extract the genomic DNA from each of the studied populations, the mycelia of the colonies were isolated for confirmation of the identity of the *Fusarium* species.

The extraction protocol adopted is that recommended by Doyle and Doyle (2007), whichsteps are:

- Take 1 cm^2 of the mycelium and deposit it in an Eppendorf tube;
- Add 500 µl of extraction buffer, grind, and vortex;
- Incubate for 30 min at 65 ° C in a water-bath;
- Centrifuge briefly at 13 000 rpm for 5 min;
- Recuperate the supernatant (400 µl);
- Add to the supernatant an equivalent volume (i.e. 400 µl) of chloroform / isoamyl alcohol (24: 1);
- Agitate gently for 5 min then centrifuge for 5 min at 14,000 rpm;
- Recuperate 350 µl of the supernatant and precipitate with 350 µl of isopropanol, centrifuge for 10 min at 14 000 rpm;
- Throw the supernatant, add 500 µl of 70º of ethanol, vortex,and centrifuge for 5 min at 14,000 rpm;
- Dry the pellet in an oven at 60 ° C for a duration of 30 to 45 min;
- Immerse the pellet with 50 µl of the TE buffer.

d) Polymerase Chain Reaction (PCR)

Used primers

For amplification of rDNA, we used the primers described in the table below. The ITS region is the target region for primers.

Table 1: Primers used for the PCR technique

Species	primer	Sequence	PM (bp)	Reference
F. graminearum	FgrMGB-F FgrMGB-R	GGCGCTTCTCGTGAACACA TGGCTAAACAGCACGAATGC	94	(GHAHDERIJANI, 2008)
F. avenaceum	AV-F AV-R	CAAGCATTGTCGCCACTCTC GTTTGGCTCTACCGGGACTG	920	(STOYAN and *al.*, 2003)
F. culmorum	C51-F C51-R	ATGGTGAACTCGTCGTGGC CCCTTCTTACGCCAATCTCG	570	(GÖRTZ, 2009)
F. poae	F poae-F F poae-R	AAATCGGCGTATAGGGTTGAGATA GCTCACACAGAGTAACCGAAACCT	220	(MARIA, 2011)
M. nivale majus	15M-F 15M-R	TGCAACGTGCCAGAAGCT AATCGGCGCTGTCTACTAAAGC	750	(STOYAN and *al.*, 2003)
M. nivale nivale	J2-F JNIV-R	GGTGTTCAAGTATAATGGGCTTCC GGTCACGAGGCAGAGTTCG	310	(STOYAN and *al.*, 2003)

Reaction mixture

The composition of the reaction mixture used to confirm the species of *Fusarium* spp. is detailed in the following table:

Table 2: Reaction mixture composition

	Mix*1 (µl)	Initial concentration	final concentration
DNA	1	-	-
Amorce Forward	0,25	10 µM	0,2 µM
Amorce Reverse	0,25	10 µM	0,2 µM
TP 10x	2	10×	10×
$MgCl_2$	0,6	50 mM	1,5 mM
DNTP	0,2	10 mM	0,1 mM
H_2O	16,45	-	-
Taq polymerase	0,25	5U/µl	1,25U/µl
Volume to be distributed	20	-	-
Reaction volume	21	-	-

PCR Program

The reaction of the amplification was carried out at the level of the thermocycler according to the following program:

- The first cycle at 94 ° C for 5 min to denature the DNA;
- A series of 35 cycles, each of which is as follows;
  - 94 ° C for 30 s;
  - 60 ° C for 45 s;
  - 72 ° C for 1 min.
- The last cycle at 72 ° C for 10 minutes to complete the elongation and complete the reaction.

e) Amplified DNA revelation

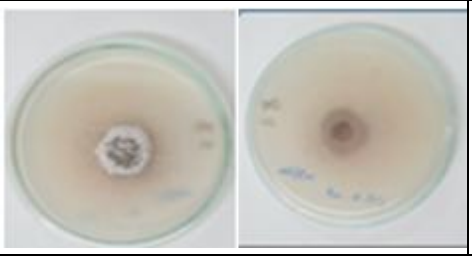
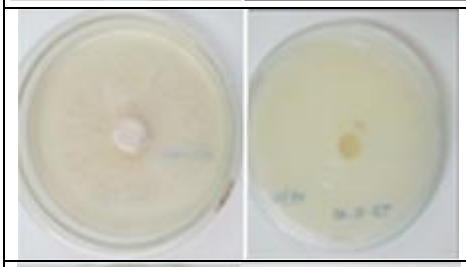
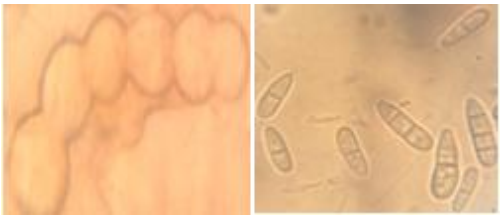
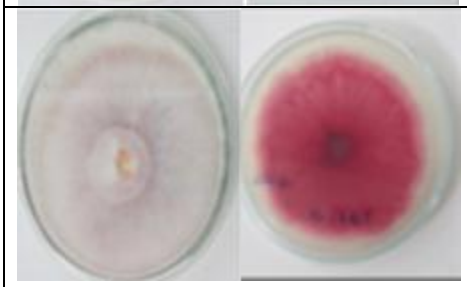
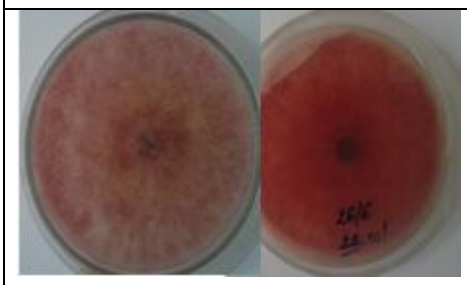
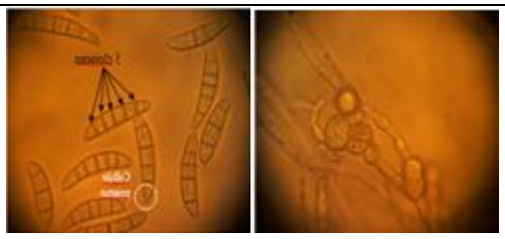
The amplified DNA was visualized on a 2% agarose gel by electrophoresis in Tris-Borate EDTA buffer (0.5x) (TBE). The gel wells have been charged with 10 µl of the amplifiermixed with 2 **µl** of theloading buffer 6x (amplified DNA).

3. Results

1) Macroscopic and microscopic aspects of the fungus

The cultivation of seed and fresh wheat samples in the PDA environment revealed five aspects of the fungus causing Fusarium wilt, which are salmon, white, pink, red and yellowish pink. (Table 3)

Table 3: Macroscopic and microscopic description of isolated aspects

Macroscopic aspect	Microscopic aspect	Comments
		• Colony is salmon-colored, mycelial growth is relatively slow, aerial mycelium has a low density. • Under the microscope, • macroconidia are small, with 1 to 3 septes, had a curved form. Chlamydospores have been observed.
		• Colony is white-colored, the aerial mycelium has a medium density. • Under the microscope, macroconidia are small, with 1 to 3 steps, had a curved form. Chlamydospores have been observed.
		• Dense aerial mycelium, the colonies are downy, initially white to yellowish or pink then red-brownish. • Very long Macroconidia, thin, with apical cells elongated and folded.
		• Dense Aerial mycelium, yellowish pink and edged with red. • Big, short, fusiform, curved and septate (5 septa on average) Macroconidia, with short and pointed apical cells. • Intermediate or terminal subglobulinouschlamydospores, formed by the mycelium.
		• Dense red-purple aerial mycelium. • Macroconidia clearly septate, fusiform, curved, slightly curved at basal and apical cells. • Presence of phyalides.

2) Epidemic mapping

Maps showing the distribution of the epidemic were made based on isolation results, geographical coordinates of each plot, and levels of infection of all samples. These maps illustrate the severely infected localities of the disease, thus presenting an important source of inoculum. Thus a major risk of contamination for the other zones where the infection is weak or absent.

a) Mapping of Fusarium wilt affecting durum wheat seeds

The map showing the foci of the disease has allowed to better locate the areas where the infection is very strong, strong, medium or low.

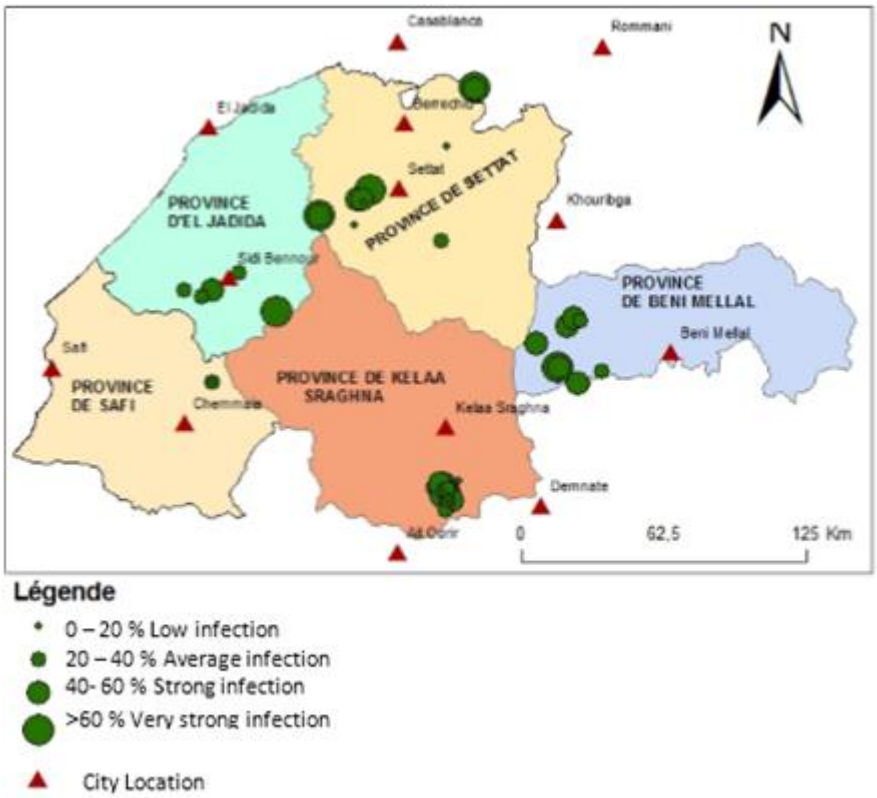


Figure 1: Location map of Fusarium wilt focals affecting durum wheat in the Doukkala, Haouz, Chaouia and Tadla regions.

b) Durum wheat samples grouping by level of infection

The graph below represents the distribution of infected samples in the four studied regions according to the level of infection.

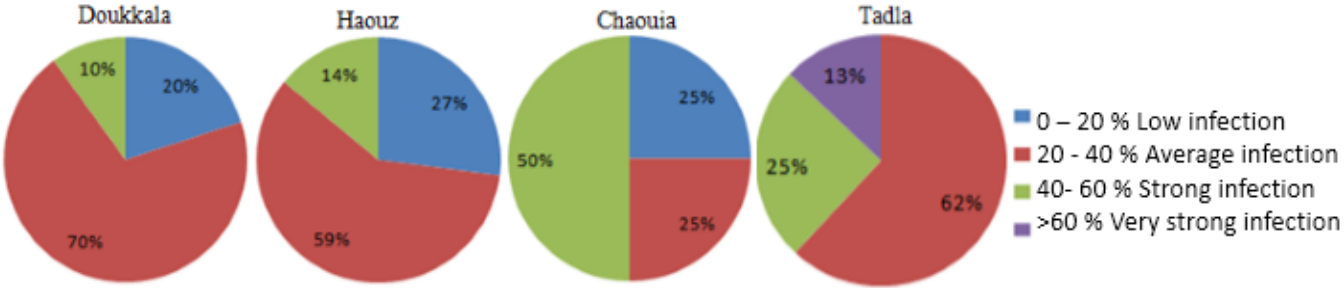


Figure 2: Repartition of infection percentages of durum wheat samples by level of infection

In the Doukkala region, 70% of the seeds were mediumly infected, 20% of the samples had a low level of infection, while the remaining 10% were heavily infected. In the Haouz region, more than half of the samples were mediumly infected, 27% were weakly infected, and a high infection was visible in the remaining 14% of the samples. In Chaouia, the percentages of the high, medium and low infection levels wererespectively 50%, 25%,and 25%. Tadla was the only region where a very high infection rate was recorded, scoring 13%, the percentages of the medium and high infection levels were 62% and 25%.

c) Mapping of Fusarium wilt affecting soft wheat seeds

The map showed the presence of a strong infection varying between 40% and 60% in all regions.This is represented by the circles having significant diameters in the map below:

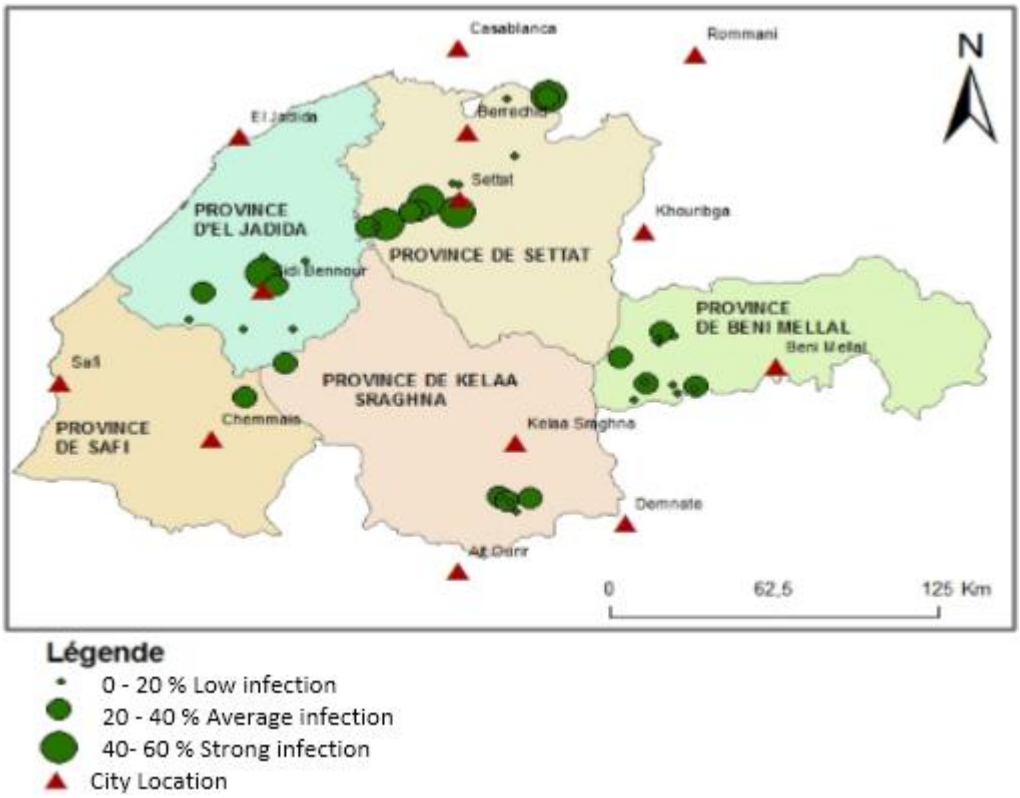


Figure 3: Location map of Fusarium wilt focals affecting soft wheat in the Doukkala, Haouz, Chaouia and Tadla regions.

d) Soft wheat samples grouping by level of infection

The graph below represents the distribution of infected samples in the four studied regions according to the level of infection.

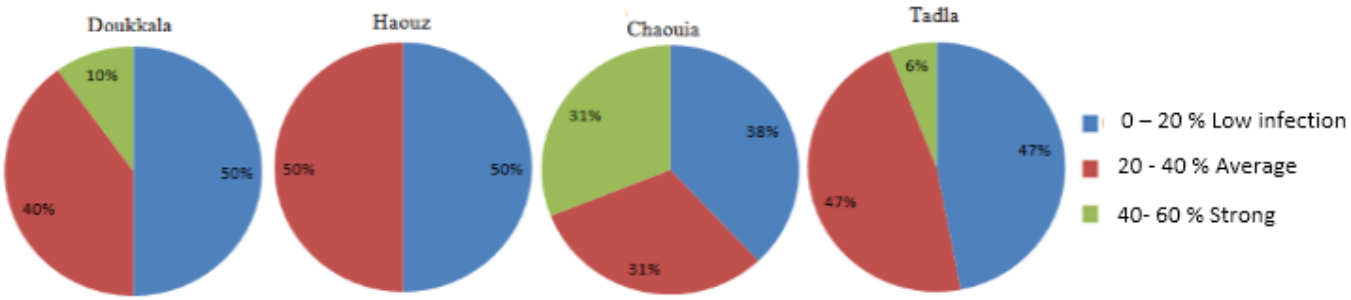


Figure 4: Repartition of infection percentages of soft wheat samples by level of infection.

For the Doukkala and Haouz regions, 50% of thestudied samples represented a low level of infection, while in Chaouia and Tadla, the percentages of low infection were respectively 38% and 47%.

The percentages corresponding to an average infection were respectively 40%, 50%, 31% and 47% for the Doukkala, Haouz, Chaouia and Tadla regions.

In contrast, a high infection was recorded in only three regions; Doukkala Tadla and Chaouia, recording the respective percentages of 10%, 6%,and 31%.

e) Mapping of Fusarium wilt affecting fresh wheat seedssamples in Gharb-Chrarda-Bni-Hssen, Meknès-Tafilalet, and Fès-Boulemane regions

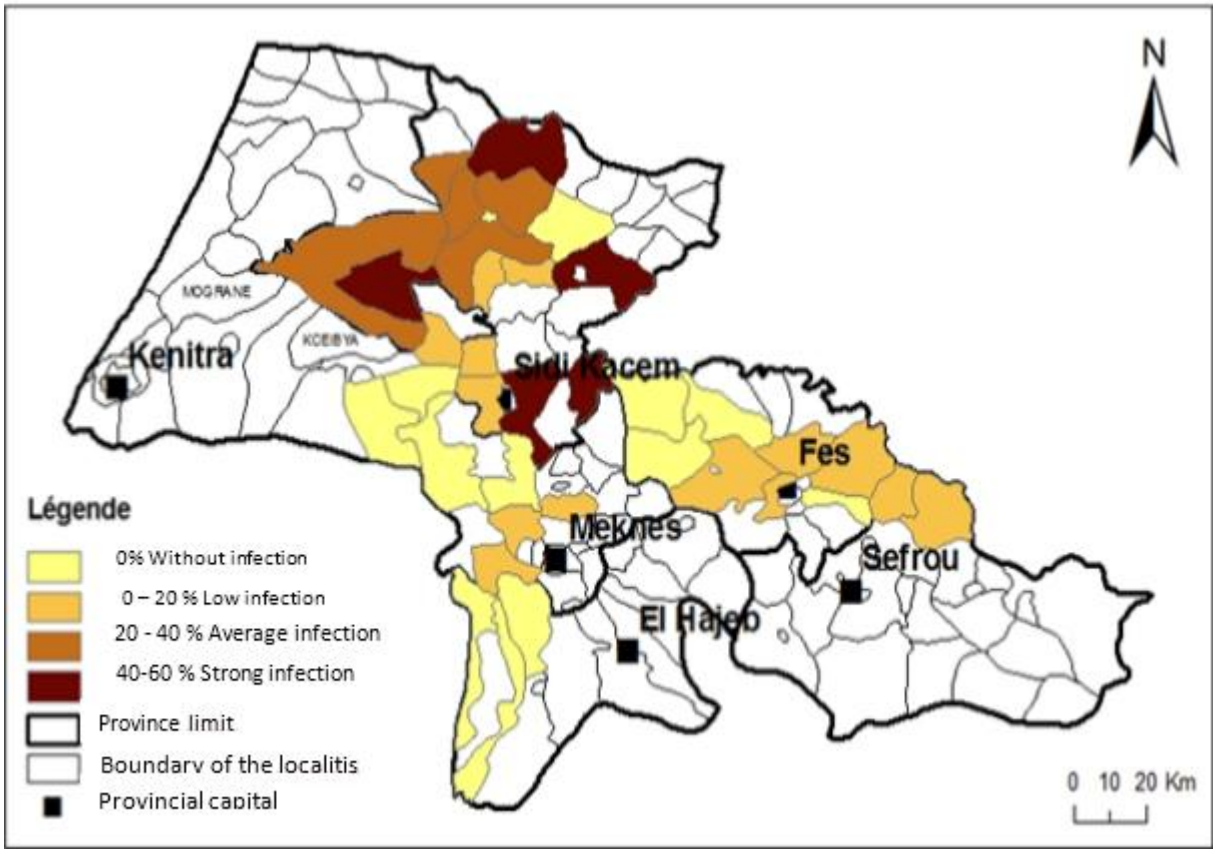


Figure 5: Location map of Fusarium wiltfocals, for fresh samples in Gharb-Chrarda-Bni-Hssen, Meknès-Tafilalet, and Fès-Boulemane regions

This map shows the localities reporting the highest infection levelsof the disease, thus presenting a significant source of inoculum and a major risk of contamination for other regions.

The estimated infection in these localities is between 40 to 60%. On the map, they are represented in dark brown.It is Masmouda, Lamrabih (Jorf el melha), Al haouafate, Selfat,and Bab tiouka.

The map also shows localities affected by a medium infection. Of which percentages of infection are between 20 to 40%. These localities are colored in brown. It is Sidi ahmed ben aissa, Moulay abdelkader, Bni oual, Sidi azouz, Nouirate, Sefsaf, Sidi al-Kamel, Dar elgueddari, Dar laaslouji.

Localities affected with a low level of infection are shown on the map in light brown. The percentage of infection of these zones is between 0 and 20%. It is Sidi m'hamedchelh, Ouled nouel, Ait ouallal, Dar oumsoltane, Mghassiyine, Oulad h'cine, Zirara, Chbanate.

This study also reveals that the samples collected from some localities did not show any symptoms of the disease. Therefore, the percentage of infection in these zones is 0%. They are illustrated on the map by the yellow coloring. It is Ain dfali, Oulad benhammadi, Azghar, Sebtjahjouh, Ras Jerri, Ait yaazem, Ain jemâa, Ain karma, Sidi hrazem, Mikkes, Laajajra, Sebtloudaya.

f) Fresh wheat samples grouping by level of infection

In the Gharb region, 40% of plots were heavily infected with Fusarium wilt. 26%, 20% were respectively moderately and weakly infected, while only 13% of the plots did not show any signs of infection.

In the Meknes region, 61% of the plots reported a low infection by the disease and 13% of plots were moderately infected while 26% were healthy.

In the Fez region, the vast majority of the plots were healthy (60%), the low and medium infected plots were around 33 and 7%.

It should be noted that no plot had a high infection rate in the last two regions (Figure 6).

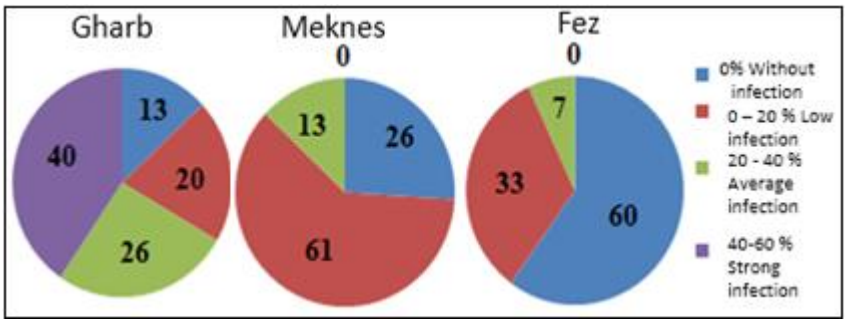


Figure 6: Repartition of infection percentages of fresh wheat samples by level of infectionin Gharb, Fez and Meknes regions

g) Correspondence between the level of infection and infected organs

The following graph describes the correspondence between the infection level of the plots and the infected vegetative organs. For weakly and moderately infected plots, roots and collars were the most affected parts by the fungus. In plots infected highly, we had significant infection rates in the glumes and grains.

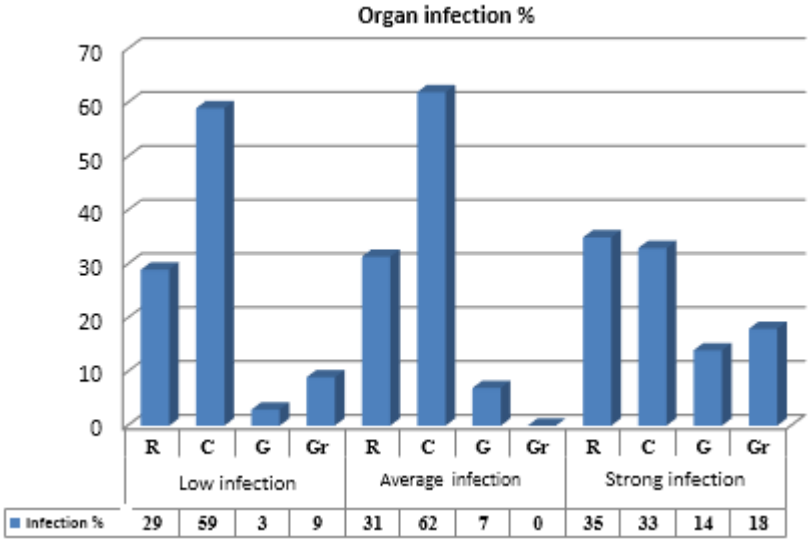


Figure 7: Correspondence between the level of infection and the infected organ. R:Root, C:Collar, G: Glume, Gr: Grain.

3) Molecular characterization of the main Fusarium species by PCR

To confirm the results obtained by microscopic identification, PCR was performed. Fourth species of fungi responsible for Fusarium wilt have been determined. These are *F. culmorum F. avenaceum, F. graminearum,* and *Microdochium nivale majus*.

a) Molecular confirmation of F. culmorum

For the confirmation of presence of *F. culmorum* species, the pair of C51-F / C51-R primers was used on all samples. We then seek to find a DNA fragment measuring 570 base pairs (bp), corresponding to the species *F. culmorum* as indicated in Table 1 of the chapter 'Materials and methods.

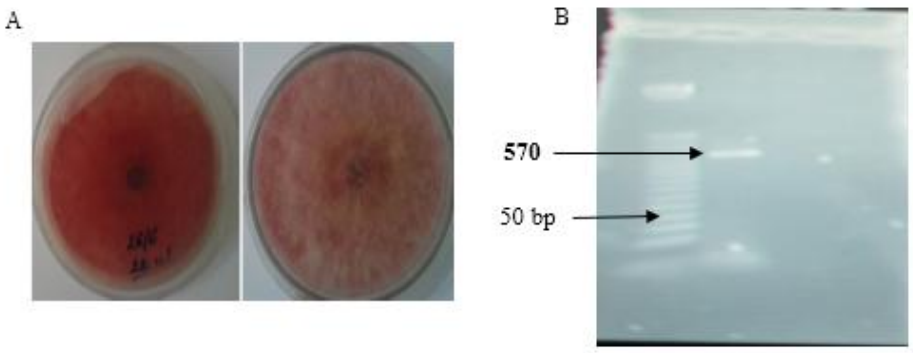


Figure 8: A- Macroscopic appearance of *F. culmorum* / B-profile amplification of *F. culmorum.*

Figure 8 indicates the presence of the desired DNA fragment and subsequently confirmation of *F. culmorum* species.

b) Molecular confirmation of F. avenaceum

The figure below (FIG. 9) shows the amplification profile of a DNA sample of a positive sample corresponding to *F. avenaceum*, amplified using the AV-F / AV-R primers. The size of the DNA fragment of *F. avenaceum* is 920 (bp).

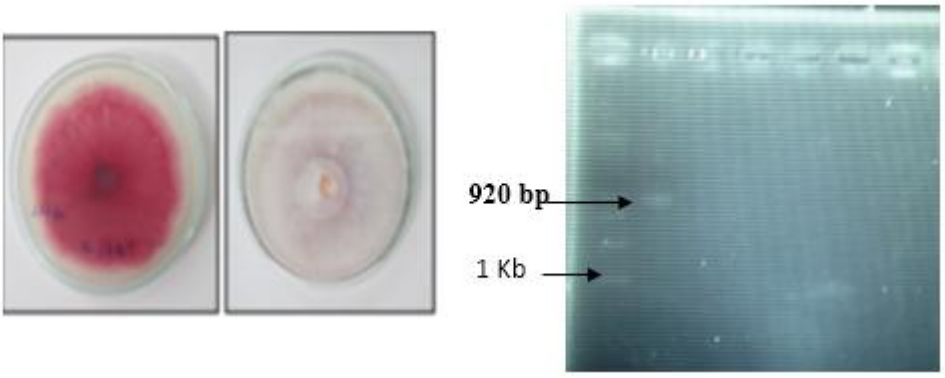


Figure 9: A- Macroscopic appearance of *F. avenaceum* / B-profile amplification of *F. avenaceum*

c) Molecular confirmation of Microdochium nivale majus

For *Microdochium nivale majus*, the pair of 15M-F / 15M-R primer was used for all samples. The size of the DNA fragment of *Microdochium nivale majus* is 750 (Bp). The DNA amplification profile below illustrates the positive fraction measuring 750 bp corresponding to the *Microdochium nivale majus* subspecies. (Figure 10).

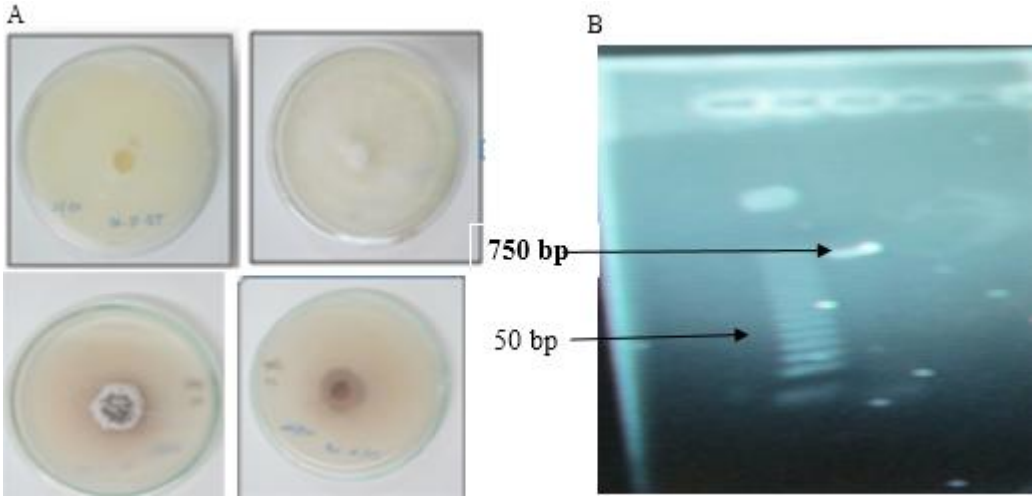


Figure 10: A- Macroscopic appearance of *M. nivale majus* / B-profile amplification of *.M. nivale majus. D.* Molecular confirmation of *F.graminearum*

The figure below (FIG. 11) shows the amplification profile of an extract of DNA of a positive sample corresponding to the *F. graminearum* species, amplified using the FgrMGB-F / FgrMGB-R primers. The size of the *F. graminearum* DNA fragment is 94 (bp).

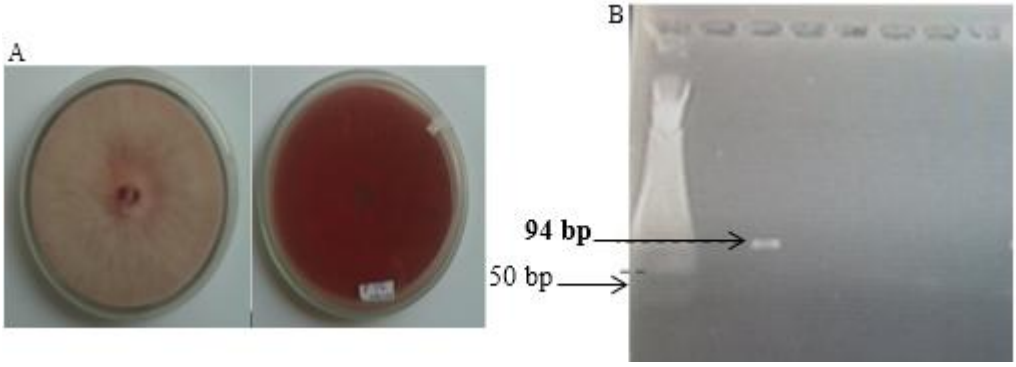


Figure 11: A- Macroscopic appearance of *F. gramineraum* / B-profile amplification of. *F. gramineraum*

4. Discussion

Four fungal species responsible for Fusarium wilt were isolated from the entire analyzed samples, infection by disease varies by type of wheat and by region.

Durum seeds showed higher percentages of infection than soft wheat. The infestation of hard wheat seeds reached 41% in Tadla, 37% in Chaouia 30% in Haouz and 29% in Doukkala. For soft wheat seeds, the infestation did not exceed 28% in Chaouia, 24% in Doukkala and 19% in Tadla and 17% for Haouz.

These results are in agreement with previous work carried out on soft wheat (inoculated with Fusarium culmorum) which showed less accumulation of mycotoxins in soft wheat grains, and this by inhibition of their biosynthesis through biochemical antioxidant compounds such as phenolic compounds (Ponts and*al*., 2006; Ponts and*al*., 2007), of which composition evolves quantitatively and qualitatively during grain development in response to installation of Fusarium spp. (Boutigny, 2007). Moreover, durum wheat is less resistant to Fusarium wilt.

The fungi isolated from the seed samples and inducing an infection with Fusarium wilt are*M. nivale majus* and *F. avenaceum.* It should also be noted that the presence of *M. nivale majus* is dominant (present in more than 90% of all regions).

Table4: Frequency of presence of fungi isolated from seed samples.

Regions	*F. culmorum*	*F. avenaecum*	*F. graminearum*	*M. nivalé majus*
Doukkala	0%	0%	0%	100%
Haouz	0%	8%	0%	92%
Chaouia	0%	5%	0%	95%
Tadla	0%	5%	0%	95%

These results confirm those published by Glynn et al. in 2005, which consider *M. nivale* as the predominant species responsible for Fusarium wilt in Europe. The latter one causes root rot, Damping-off (Smiley and Patterson 1996) or scalding of ears, and so significant seed infection, causing poor germination, thus causing significant yield losses (Humphreys and*al*., 1995, Parry and*al*., 1995).

It is also important to note that *M. nivale majus* is unable to produce mycotoxins (Logrieco and *al*., 1991), so this subspecies does not present any danger to the consumer, whether animal or human.

The species *F. avenaceum* (present with low percentages, not exceeding 8% in all regions) is considered responsible for collar rot and Fusarium head blight (Ioos and*al*., 2004). In addition, it is capable of synthesizing ionophore inhibiting enzyme molecules such as cholesterol-acetyl transferase in humans (Meca and*al*., 2010).

The fresh samples from the Gharb-Chrarda-Bni-Hssen region showed the highest level of Fusarium wilt infection for this class of samples. Indeed, the percentage of infection by the disease exceeds 40%. We also recorded the presence of the fungus in all the organs of the analyzed plants (roots, collars, glumes,and seeds).

The Fez-Boulemane and Meknès-Tafilalet regions had registered the lowest percentages of infection. The majority of plots in these areas did not show symptoms of the disease. roots and snares are the main vegetative parts at which infection has been recorded.

The diffusion of the disease in the Gharb-Chrarda-Bni-Hssen region can be explained by the climatic conditions of the area, the climate around the flowering, the previous crop, the soil tillage,and the varietal sensitivity. All these factors play a role in the spread of the disease (Obst et Bechtel, 2000; Schaafsma et *al*., 2001; Barrier-Guillot et *al*., 2006; Lemmens, 2007;Gourdain et *al*., 2009).

Fungal species isolated from fresh samples are*M. nivale majus, F. culmorum, F. avenaceeum*,and*F. graminearum*.

Table 5: Frequency of presence of fungi isolated from fresh samples.

Regions	*F. culmorum*	*F. avenaceum*	*F. graminearum*	*M. nivale majus*
Gharb-Chrarda-Bni-Hssen	40%	0%	18%	42%
Fez-Boulemane	0%	100%	0%	0%
Meknes-Tafilalet	0%	100%	0%	0%

M. nivale majus (isolated from roots, collars, glumes,and seeds) and *F. culmorum* (isolated mainly from roots and cllars) are responsible for 82% of the Gharb-Chrarda-Bni-Hssen infection, while *F. graminearum* (isolated from the glumes and grains of wheat) is present with a frequency of 18%. These results are in agreement with those reported in northern Tunisia in 2006, where *Fusarium* species causing the collar rot and the scalding of ears are *Microdochium nivale Fusarium culmorum* and *Fusarium graminearum* (Boughalleb and*al*. , 2006).

In Fez-Boulemane and Meknes-Tafilalet, the low infestation by the disease is caused by F. avenaceum, responsible for collar rot.

The detection of these species of *Fusarium* in our national territory constitutes a risk for the cultivation of wheat (yield reduction and synthesis of mycotoxins produced by *F. graminearum F. culmorum* and *F. avenaceum*).

F. culmorum is the major species isolated from wheat in Germany while it is fourth in France in 2002 causing the epidemiology (Ioos and*al*., 2004). it is considered toxigenic since it produces mycotoxins such as zearalenone (ZEA) which has an estrogenic effect (Haglerand*al*., 2001).

F. avenaecum synthesizes enniantines, which are enzyme inhibitory ionophore molecules such as cholesterol-acetyltransferase in humans (Meca and *al*., 2010).

F. graminearum is classified as the fourth most important pathogen fungus in the world. This result is explained by the

mycotoxigenic potential of *F. graminearum*, which causes, in addition, to yield reductions, decreases in the quality of grain. The grains are stunted and contaminated with mycotoxins (Osborne and Stein 2007). This species is today the dominant on wheat and barley in most European countries and in North America (Parikka and*al*., 2012). The main mycotoxins produced by *F. graminearum* are trichothecenes B, including deoxynivalenol (DON), nivalenol (NIV) and their acetylated derivatives (O'donnellan*d*al., 2008). The latter's are considered Immunosuppressive (Bennett and Klich 2003).

Even if the percentage of infestation caused by *F. graminearum* is weak compared to that of *F. avenaceum* and *F. culmorum* for 2013/2014, this may vary with climate change. And given that climate studies all agree on an increase in global average temperature (Intergovernmental Panel on Climate Change, IPCC, 2007), this would increase the importance of *F. graminearum* at the expense of species like *F. culmorum* or *F. avenaceum* that preferentially develop in cold temperate zones (Parikka and*al.*, 2012).

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