

## SHORT NOTES

# Survey of the main causal agents of fusarium head blight of durum wheat around Bologna, northern Italy

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**Summary.** Several *Fusarium* species and *Microdochium nivale* are involved in fusarium head blight (FHB), which in Italy has been constantly present on wheat since 1995. This study was carried out from 1995 to 2007 on FHB-infected durum wheat heads collected in the Bologna countryside, Emilia-Romagna, northern Italy. The most frequent *Fusarium* species found were: *Fusarium graminearum* (32.1%), *F. culmorum* (25.2%) and *F. poae* (17.8%), while *F. avenaceum* and *M. nivale* occurred discontinuously. Other *Fusarium* species were also found, but only sporadically. It is important to identify and characterize the main species involved in the FHB syndrome for this will help us to establish control strategies that will contain the disease and the content of mycotoxins in food and animal feed.

**Key words:** identification, *Fusarium* species, FHB, *Triticum durum* Desf.

## Introduction

Fusarium head blight (FHB) is a wheat disease that causes losses in yield from 30 to 70% (Parry *et al.*, 1995; Pancaldi *et al.*, 2005). The aetiology of the disease is complex. The causal agents of FHB are several species of *Fusarium*, and *Microdochium nivale* (Fr.) Samuels & I.C. Hallett, and the species that are most frequently associated with FHB worldwide are: *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* [Schwein.] Petch), *F. culmorum* (W.G. Sm.) Sacc. and *F. poae* (Peck) Wollenw. *F. avenaceum* (Fr.) Sacc. (teleomorph

*Gibberella avenacea* R.J. Cook) and *F. sporotrichioides* Sherb. are mainly found in Europe (Parry *et al.*, 1995; Miedaner *et al.*, 2001; Logrieco *et al.*, 2002; Gale, 2004; Goswami and Kistler, 2004; O'Donnell *et al.*, 2004; Osborne and Stein, 2007). *F. culmorum*, *F. poae*, *F. avenaceum* and *M. nivale* are common in the cooler maritime regions of the world such as northwestern Europe (Nicholson *et al.*, 1998; Nicholson *et al.*, 2003; Zeller *et al.*, 2004; Xu *et al.*, 2005), while *F. graminearum* is mostly predominant in the hotter areas of the USA (Parry *et al.*, 1995; Brennan *et al.*, 2003).

While this aetiology is only partly understood (Xu, 2003), the environment is known to play an important role in FHB development. The distribution and predominance of a *Fusarium* species in a country seems to be determined by climatic factors (temperature, humid conditions, etc.),

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agronomic practices (cropping sequence, soil tillage, sowing on untilled soil, use of nitrogen fertilizers, etc.), as well as by competition from the *Fusarium* species (Parry *et al.*, 1995; Saremi *et al.*, 1999; Doohan *et al.*, 2003). For this reason, none of the *Fusarium* species can be considered of secondary importance; even when clearly visible symptoms of a species are lacking there is a risk that the mycotoxins from it will occur in commercial grains (Salas *et al.*, 1999). A single *Fusarium* strain can produce multiple mycotoxins (Thrane *et al.*, 2004). Some *Fusarium* strains associated with FHB produce trichothecenes (deoxynivalenol-DON, nivale-nol-NIV, T-2 and HT-2) and zearalenones (zearalenone-ZEA) (Bottalico and Perrone, 2002). An accurate identification of a given *Fusarium* species is difficult because they vary so greatly in their morphological characteristics: the colony shape and pigmentation, the growth rate, the presence or absence of macro- and/or microconidia and their shape, the presence or absence of chlamydospores, sclerotia and sporodochia of

many strains depend on the particular culture medium in which the strain is grown (Nelson *et al.*, 1983; Leslie and Summerell, 2006).

*Fusarium* head blight has occurred in Italy on wheat without a break since 1995, though with varying incidence and severity depending on the year, the area and the wheat variety cultivated (Figure 1). Durum wheat cultivars, especially in northern Italy, are at greater risk of seed infection from FHB-associated pathogens (Pancaldi *et al.*, 1996; Pancaldi *et al.*, 1997; Balmas *et al.*, 1998; Balmas *et al.*, 2000; Shah *et al.*, 2005; Rossi *et al.*, 2006). This is an important aspect that closely concerns Emilia-Romagna, where the area under durum wheat cultivation has risen from 29,983 ha in 1999 to 46,467 ha in 2007. In the province of Bologna, the area under wheat cultivation increased from 8,200 ha in 1999 to 14,100 ha in 2007 (ISTAT data, <http://agri.istat.it>).

The purpose of the present work was to identify the *Fusarium* species causing FHB in durum wheat heads collected in some fields around Bologna in the period from 1995 to 2007.



Figure 1. Varying degrees of fusarium head blight (FHB) severity.

## Materials and methods

### Mycological analysis of the wheat heads

Every year from 1995 to 2007, head samples (Figure 2) were collected from several durum wheat cultivars growing in 150 fields around Bologna, and were analyzed for *Fusarium* species. One hundred FHB heads at the late milk stage (Zadoks growth stage GS 77-79, Zadoks *et al.*, 1974) were randomly collected from each field. Ten ears, varying in FHB severity, were selected, and from each ear 10 parts of glume, rachis, sub-glume and palea were sampled. Samples were washed in sterile water, disinfected in a 2% sodium hypochlorite solution for 2 min, rinsed twice in sterile water to eliminate any hypochlorite residue and dried on sterile filter paper, then placed in Petri dishes containing potato dextrose agar (PDA, Difco, Lawrence, Kansas, USA) with 0.3 g L<sup>-1</sup> streptomycin and neomycin sulphate. The Petri dishes were incubated at 22°C in the dark for 7 days. *Fusarium* and *Micro-*



Figure 2. A wheat head affected by fusarium head blight (FHB).

*dochium* colonies, grown from heads taken from different fields, were subcultured by plating dilutions in order to obtain single-spore cultures, to ensure that the different structures belonged to the same fungus. A plug of mycelium from each colony was placed in sterile water, subjected to several dilutions and drops of the last suspension were plated with a loop on Petri dishes containing water agar. After 48 hours of incubation, the germinated spores were transferred to PDA.

Pure cultures of *Fusarium* species and *M. nivale* were grown at 22°C with a 12-h day for 10 days on carnation leaf piece agar (CLA) to produce macroconidia of uniform size and form, which were identified to species level by their colony characteristics and conidial morphology (Nelson *et al.*, 1983; Leslie and Summerell, 2006). The average isolation frequency (number of strains of a given species / total number of isolates) was calculated each year.

### Diagnostic and qualitative PCR

Morphological identification has since 2001 been supplemented with molecular techniques, to ensure that morphological similarities do not mask significant genetic differences. Some *Fusarium* and *Microdochium* strains were selected for morphotypes, and from a representative colony of each morphotype, qualitative PCR was performed on the genomic DNA from strains of *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae* and *M. nivale* to confirm the morphological identification. DNA was extracted from mycelium that was harvested from 7-day-old single-spore cultures grown on PDA, using a CTAB (exadecyl-trimethyl-ammonium bromide) method adapted from Lhodi *et al.* (1994). To detect *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae* and *M. nivale*, the following specific primers were used respectively: Fg16F/R (5'-CTCCGATATGTTGCGTCAA-3'/5'-GGTAGGTATCCGACATGGCAA-3') (Nicholson *et al.*, 1998); Fc01F/R (5'-ATG GTGAACTCGTCGTGGC-3'/5'-CCCTTCTTACGCCAATCTCG-3') (Nicholson *et al.*, 1998); FaU17F/R (5'-CAAGCATTGTCGCCACTCTC-3'/5'-GTTTGGCTCTACCGGGACTC-3') (Turner *et al.*, 1998); Fp82F/R (5'-CAAGCAAAC AGG CTC TTC ACC-3'/5'-TGT TCCACCTCAGTGACAGGTT-3') (Parry and Nicholson, 1996); and Y13NF/R (5'-ACCAGCCGATTTGTGGTTATG

- 3'5'GGTCACGAGGCAGAGTTCG-3') (Nicholson *et al.*, 1996). Amplification was done in a T3 thermocycler (Biometra, Göttingen, Germany) under the conditions described in the protocols of the literature cited above.

## Results

### Mycological analysis of the wheat heads

Morphological characterization is very time-consuming. Since 2001 we have therefore used molecular diagnostics, which allows a much faster and more precise identification of fungal species. The primers used in this work proved their effectiveness and confirmed the morphological identification.

The *Fusarium* species found that were most frequently associated with FHB symptoms were: *F. graminearum* (Figure 3), *F. culmorum* (Figure 4) and *F. poae* (Figure 5).

The frequency of each species is shown in Figure 6. Each graph shows, for the main fungal species isolated, the year in the abscissa, and the frequency (%) in the ordinate. The straight line expresses the mean frequency and the dotted line the tendency.

*Fusarium graminearum* was consistently isolated, with a frequency varying from 15.7% (2007) to 67.5% (1996) (Figure 6a) except for 1997, when the isolation percentage was 6.2%. The mean frequency was 32.1% (straight line).

*Fusarium culmorum* had a mean frequency

of 25.2% (straight line). Its frequency declined in 1995, 1996 and 1998 (4.0, 6.2, 1.3% respectively) and peaked in 1997 at 59.8% (Figure 6b).

*Fusarium poae* was isolated since 1996, with frequencies of 5% in 1999 and 23.2% in 2007, and peaking in 2005 at 55.4% (Figure 6c). Its mean frequency was 17.8% (straight line).

*Fusarium avenaceum*, when it occurred, had a frequency of less than 15%, except in 1998, when it shot up to 39.7% (Figure 6d); its mean frequency was 6.4% (straight line).

*Microdochium nivale* was highly frequent, at 59.2%, in 1995. It was not found at all in 1997, 1998, 2001, 2002, 2005, 2006 or 2007 (Figure 6e), and its mean frequency was 5.7% (straight line).

Other *Fusarium* species such as *F. tricinctum* (Corda) Sacc., *F. equiseti* (Corda) Sacc., *F. semitectum* Berk. & Ravenel, *F. crookwellense* L.W. Burgess, P.E. Nelson & Toussoun, *F. proliferatum* (Matsush) Nirenberg ex Gerlach & Nirenberg, *F. sambucinum* Fückel, *F. compactum* (Wollenw.) W. L. Gordon were also detected (mean frequency 12.7%) (data not shown).

## Discussion

The survey on the causal agents of FHB in durum wheat grown in the Bologna countryside reflects the situation for durum and bread wheat in Italy and northern Europe as reported by other authors (Pancaldi and Torricelli, 1999; Pancaldi

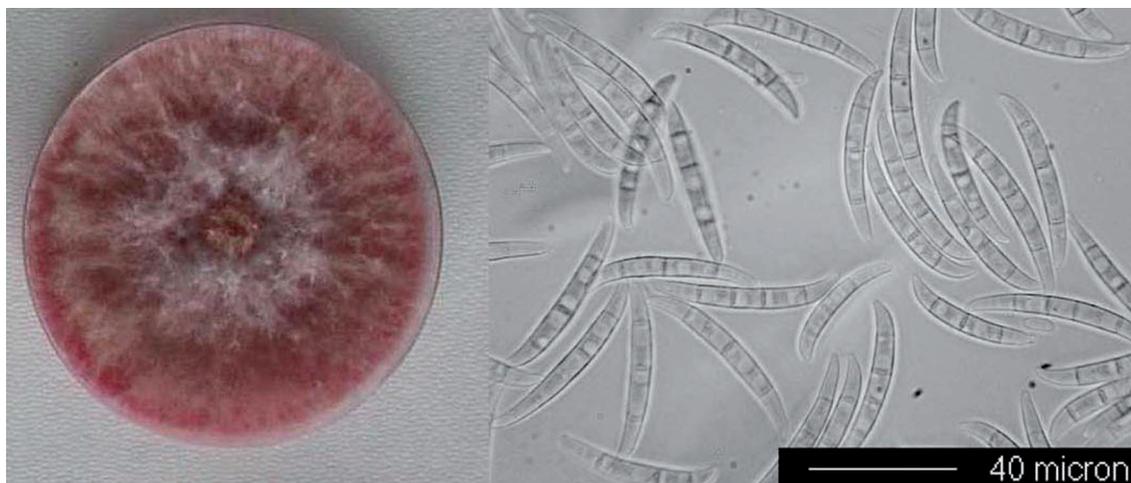


Figure 3. *Fusarium graminearum* on potato dextrose agar (PDA) and macroconidia.

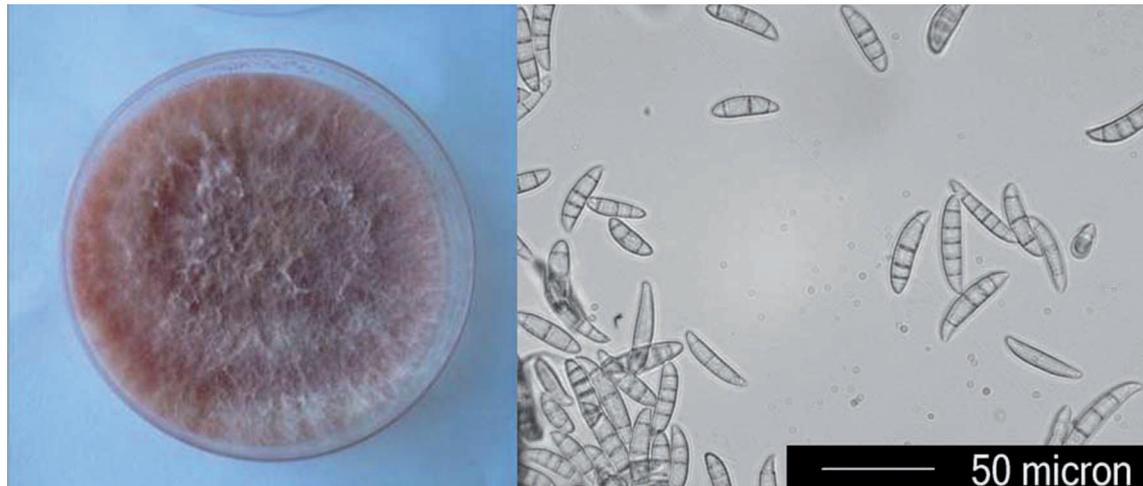


Figure 4. *Fusarium culmorum* on potato dextrose agar (PDA) and macroconidia.

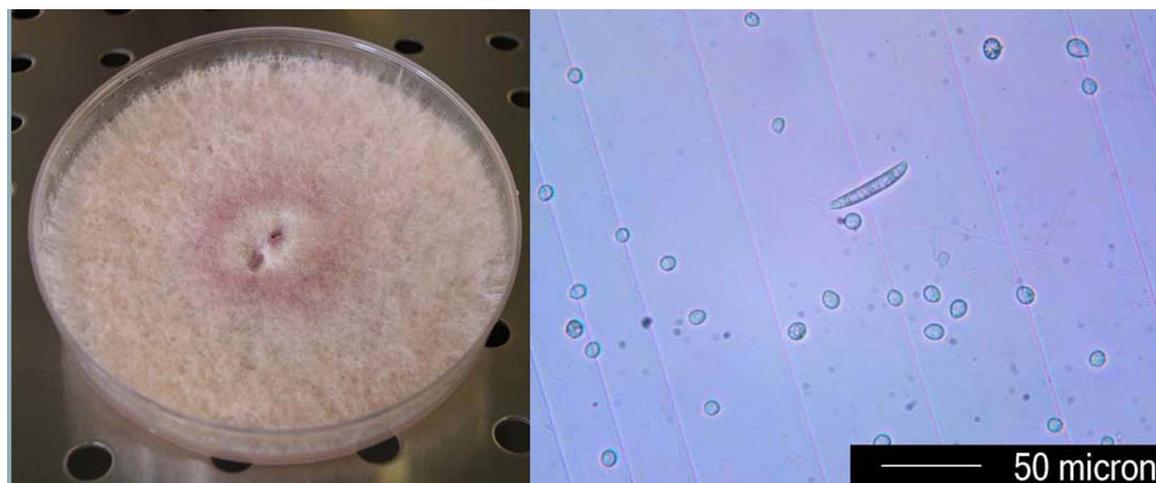


Figure 5. *Fusarium poae* on potato dextrose agar (PDA) and micro/macroconidia.

and Alberti, 2001; Pasquini *et al.*, 2001; Bottalico and Perrone, 2002; Waalwijk *et al.*, 2003; Xu *et al.*, 2005; Osborne and Stein, 2007).

The frequency data from 1995 to 2007 confirmed that *F. graminearum* and *F. culmorum* are still the most common species causing FHB, with *F. graminearum* becoming less frequent and *F. culmorum* more frequent (Figure 6a and b, dotted lines).

*Fusarium graminearum* is the most common FHB agent in northern and central Italy according

to Balmas *et al.* (1998), Balmas *et al.* (2000) and Infantino *et al.* (2005). Prodi *et al.* (2009), in a field study carried out between 2006 and 2008 in the same area as the present work, also found that *F. graminearum* was the most frequent, but these researchers mainly studied *F. graminearum* from a toxicological point of view. They found that the most frequent chemotype was 15-acetyl deoxynivalenol – 15ADON (87.2%), followed by 3-acetyl deoxynivalenol – 3ADON (8.1%) and nivalenol – NIV (2.7%).

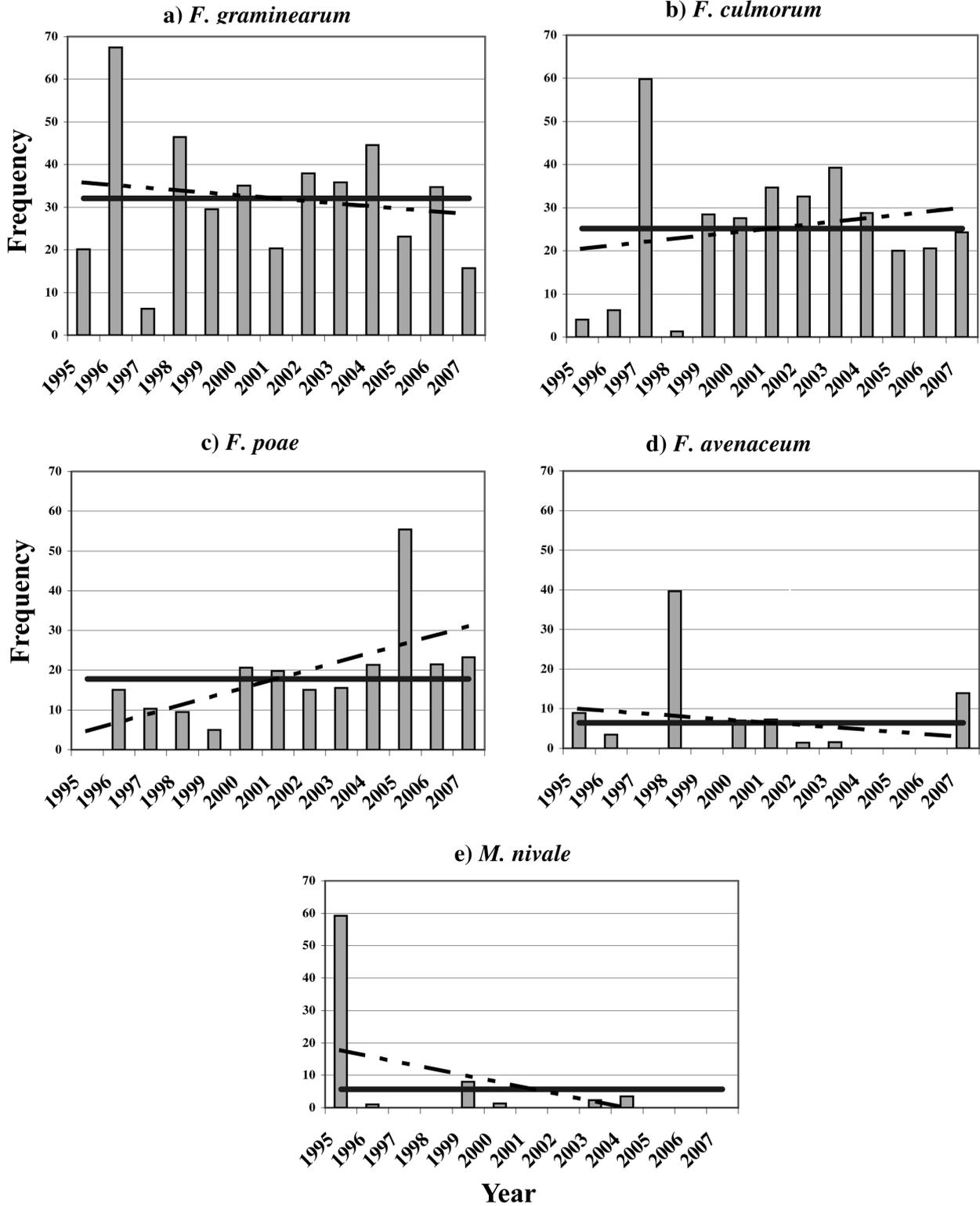


Figure 6. For each graph the straight line shows the mean value of frequency; the dotted line shows the linear tendency.

Pasquini *et al.* (2006) reported that *F. culmorum* prevailed in southern Italy, but according to our findings this fungus is also frequently associated with FHB in northern Italy. The prevalence of *F. graminearum* or *F. culmorum* in an area is usually related to the weather conditions of that area, and to the time of flowering (Bottalico and Perrone, 2002). The influence of climatic factors on *Fusarium* infection is complicated by the fact that a *Fusarium* species sometimes causes the disease by itself and sometimes while being associated with other *Fusarium* species. There are numerous reports on how individual *Fusarium* species vary in response to environmental variations, particularly temperature and humidity (Köhl *et al.*, 2007).

This work also found that *F. poae* has also become more frequently associated with FHB infected heads since 2000, and this finding is supported by Pasquini *et al.* (2006) and Infantino *et al.* (2005). Xu *et al.* (2005) stated that *F. poae* is becoming more frequent in the FHB complex in

various European countries. In Belgium its predominance has been reported by Audenaert *et al.* (2009) and our data also indicate that it is becoming more frequent (Figure 3c). Our findings were also consistent with Parry *et al.* (1995), who reported that *F. poae* was more frequent in years when *F. graminearum* was less frequent, and *vice versa* (Figure 7).

In our study, *F. avenaceum* and *M. nivale* were found discontinuously with *M. nivale* having decreased more than *F. avenaceum* (Figure 3d and e), confirming the findings of Pancaldi and Torricelli (1999) on durum wheat in several parts of Italy over a number of years.

*Fusarium semitectum*, *F. equiseti* and *F. tricinctum* were sporadically found. This was consistent with Bottalico and Perrone (2002).

In the present work we examined those *Fusarium* species that are most representative of the species associated with FHB symptoms. Accurate morphological characterization requires time, often as much as several weeks, while a molecular

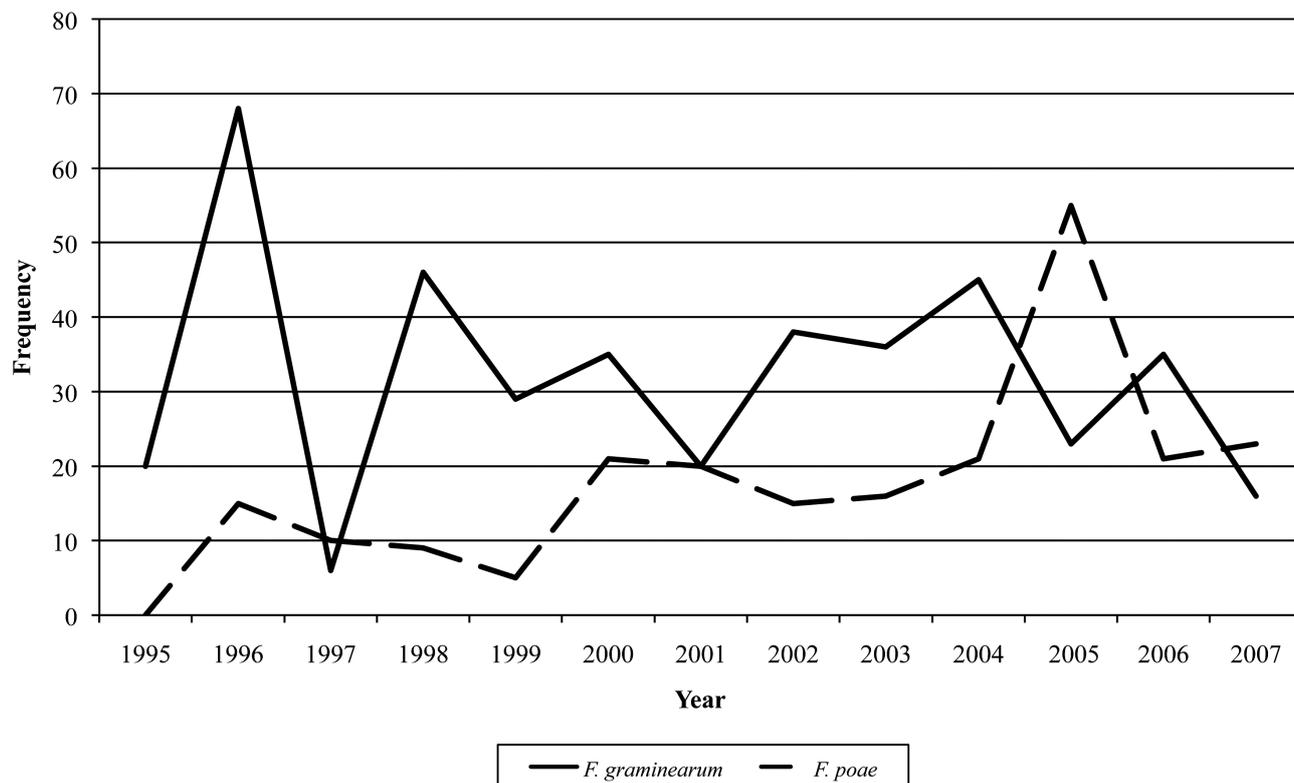


Figure 7. Frequency (%) of *Fusarium graminearum* and *F. poae* from 1995 to 2007.

diagnostics is much faster but needs to be carefully evaluated. It is important to identify and characterize the main species involved in FHB, in order to set up control strategies that will contain the disease and the levels of mycotoxins in food and animal feed.

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