Fusarium spp. on maize in the Region wallonne: biodiversity and mycotoxin production

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Introduction

In Belgium, 8.3 million tons of maize plants are harvested annually from 222.000 hectares of farm land and mainly used as livestock feed (www.statbel.fgov.be, 2007). However, crop quality is often reduced by ear and stalk rot due to *Fusarium* spp., and their associated mycotoxins are a serious problem for human and animal health (Wilson *et al.*, 1990; Rheeder *et al.*, 1992; Chu & Li, 1994).

A large number of *Fusarium* species has been associated with ear and stalks rots (Bottalico, 1998; Logrieco *et al.*, 2002). Gibberella red ear rot and Fusarium stalk rot are caused by *F. graminearum* and *F. culmorum* respectively, often associated with many additional *Fusarium* species. Maize pink ear rot is caused by *F. verticillioides* and *F. proliferatum*, sometimes associated with *F. subglutinans* and *F. sporotrichioides*.

Some of those *Fusarium* species are able to produce mycotoxins of which important amounts are often found in maize silages. Nevertheless, it is established that the physico-chemical properties existing in silages (pH, aerobiose, t °) prevent the growth of *Fusarium* spp. (Scudamore and Livesey, 1998). Mycotoxins are thus produced in the field.

The present project supported by the Région Wallonne** aims to understand the dynamics of the various *Fusarium* species development during the cultural season, their biodiversity as well as their mycotoxin production. Two partners are associated with BCCM™/MUCL: the CARAH (Centre Agronomique de Recherches Appliquées du Hainaut) and the CIPF (Centre Indépendant de Promotion Fourragère, UCL) who are in charge of field assays. The CARAH team also performed the mycotoxin detection and quantification.

Materials and methods

Three repeated assays have been performed in five geographic situations under different tillage and crop rotations, in 2005, 2006 and 2007. A total of 225 plants from three maize varieties presenting different ranges of susceptibility to fusariosis have been collected at four physiological stages (R1-flowering stage, R4-dough stage, R5-dent stage, R6-maturity stage).

Small fragments of the stalk, ear and silks (up to R4 stage) from each maize plant were surface sterilized and put on Petri dishes for isolation of the fungal flora. Pure *Fusarium* strains were preserved on PDA and SNA in glass tubes, under sterile mineral oil.

Cultural characteristics of *Fusarium* species were assessed macroscopically and identification of isolates at species level was achieved by sequencing of several genes.

For mycotoxin detection, entire plants were mixed and homogenized at R6 stage. Deoxynivalenol (DON), zearalenone (ZEA), fumonisins and T-2 toxin were quantified by HPLC.

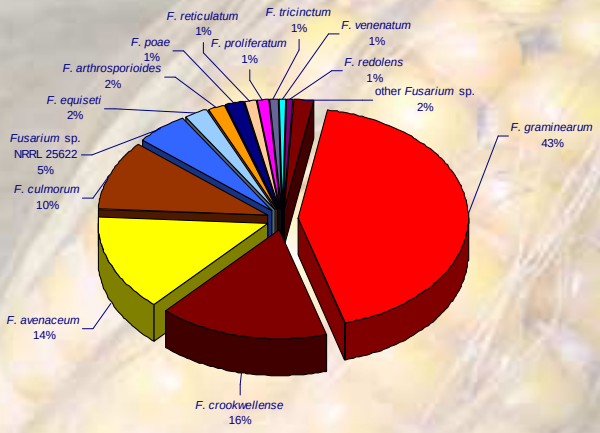

Figure 1. *Fusarium* species distribution in MUCL collection

Results and discussion

Approximately 5700 *Fusarium* spp. strains were isolated and are preserved at BCCM™/MUCL. A total of 23 *Fusarium* species were identified during this 3-year survey of which *F. graminearum*, *F. crookwellense*, *F. avenaceum*, *F. culmorum* and *Fusarium* sp. sim. NRRL 25622 were the most abundant (**Fig. 1**). Less than 2% of strains are represented by *F. oxysporum*, *F. verticillioides*, *F. sporotrichioides*, *F. torulosum*, *F. subglutinans*, *F. lateritium*, *F. sambucinum*, *F. ramigenum*, *F. flocciferum* and *F. solani* (section "other *Fusarium* sp." in figure 1).

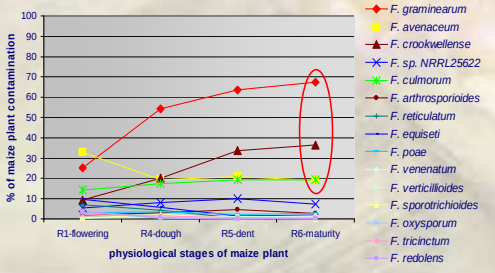

Figure 2. Maize plant contamination with *Fusarium* sp. during the three growing seasons

An important number of maize plants were already contaminated at the R1 stage (88% of plants in 2005, 79% in 2006 and 72% in 2007), while no symptoms were visible. For the three years, the mean detection frequency of *F. graminearum*, *F. crookwellense* and *F. culmorum* increased all along the growing season (**Fig. 2**). Less than 33% of maize plants were contaminated with *F. avenaceum* at R1 stage but the frequency decreased to 19% at R6 stage. The percentages of occurrence of all other *Fusarium* spp. isolated in this 3-year survey stayed or decreased along the cultural season.

The different species of *Fusarium* tend to develop on different parts of the plant (**Fig. 3**). Indeed, *F. crookwellense*, *Fusarium* sp. sim. NRRL 25622, *F. culmorum* and *F. equiseti* develop mainly on maize stalks while *F. reticulatum*, *F. poae* and *F. avenaceum* are almost exclusively observed on ear and silks. Nevertheless, the predominant species *F. graminearum* is detected with similar percentages on both stalks and ears.

Mycotoxin analyses for the years 2005 and 2006 reveals deoxynivalenol and zearalenone contamination, but under the recommended levels (**Fig. 4**). No fumonisin and no T-2 toxin were found in the homogenized samples, although various producing species were present. Deoxynivalenol and zearalenone levels were partially correlated with the detection of *F. graminearum* (DON and ZEA producer) and *F. crookwellense* (ZEA producer).

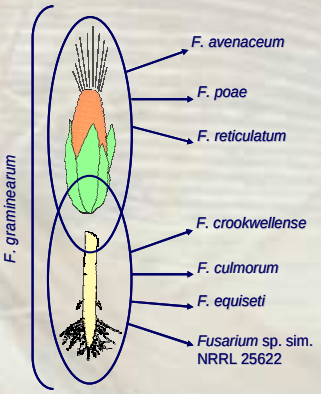

Figure 3. *Fusarium* species distribution on plant

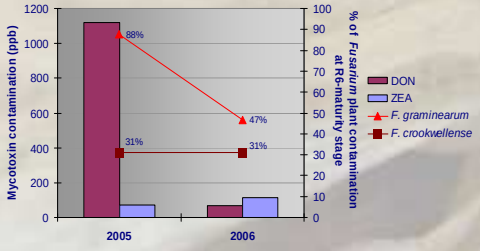

Figure 4. Mean DON-ZEA contamination and *F. graminearum*-*F. crookwellense* detection on maize plant at R6 stage in 2005 and 2006

Conclusion

Results of this 3-year investigation revealed a wide range of *Fusarium* spp. present on maize crop in Belgium. Furthermore, most of them have already been detected on plants from the flowering stage. This raises the question of a possible underestimated contribution of several species to the final fusariosis symptoms observed on plant at harvest.

F. graminearum and *F. crookwellense* appeared to be more and more present along the growing season and represent the two major *Fusarium* species recovered from maize plants at harvest.

Results of an extensive 15-yield assay in 2008 will allow to analyse and to integrate the effect of varietal, cultural and climatic factors that concurs to the final expression of fusariosis. Greenhouse experiments and mycotoxinogenic profiles description in progress at MUCL will allow a better understanding of the relationships between the *Fusarium* spp. involved in the pathogenic complex as well as of their mycotoxin production.

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