From plants to silage: the mycotoxin problematic

« Previous article Newsletter contents Next article »

In Belgium, silage constitutes the basic cattle forage during winter and, in part, also during summer. There are different crops used for ensilage such as whole corn plant, CCM (corn cob mix), grass, and sugar beet pulp.
Among the various clients of BCCM/MUCL, farmers or industries often ask for help with health problems encountered in livestock fed with fungal and presumably mycotoxin contaminated silage. However, little information is are available that could be used to assess the risks of such contaminations by fungal toxic secondary metabolites, largely because most of the suspected effects are non-specific (reduction of fertility, reduction of productivity, immunosuppressive effects, etc.) and subject to influences other than simply feed quality. However, the presence of molds is a good indication of the likelihood of mycotoxin problems.
Several mycotoxin-producing genera such as Penicillium, Aspergillus, and Byssochlamys have often been isolated from silage samples. Those genera are perfectly adapted to silage conditions and can be correlated with the presence of mycotoxins. In addition, fusarial toxins (toxins produced by various species of Fusarium) have been detected in significant quantities in silage samples, although the living conditions for Fusaria are not optimum in such matrices. Therefore, the presence of the mycotoxins could be accounted for by fungal production while the plants were growing.
In that context, BCCM/MUCL introduced two research projects in order to analyze both silage and plants at the fungal and the mycotoxigenic levels.

Characterization of fungal species and mycotoxins contaminating silage in Belgium[1]

In the first part of the project, farms of various geographic regions and using various cropping systems were selected in order to establish the state of ensilage in Belgium. Information was compiled on the raw material of silage (plant species, variety, phenologic state of the plants at harvest), the ensilage system (silo type, silo design, etc.), and on husbandry (diseases, fertility problems, allergic reactions, etc.) in order to obtain background information related to the potential effects of mycotoxins.
The core research of this project relates to the biological and metabolic diversity of silage-contaminating fungi. Morphological characterization of the isolated strains will be included in later molecular taxonomic and phylogenetic studies. Data for the diversity within the different species isolated from the different types of silage will be analyzed using RAPD, AFLP and sequence analysis. Morphological, taxonomic and diversity studies will enrich the database of a BCCM/MUCL subcollection.
New methods are being developed for the extraction and HPLC analysis of selected mycotoxins from different silages. Analyses of mycotoxins will also be performed on samples from in vitro cultures of the fungi, and special attention will be given to lesser known mycotoxins or even hitherto unknown fungal metabolites. The information related to mycotoxin and metabolite production of the isolated strains will be useful for refining the characterization of the fungal strains.
Toxicological analyses will be performed on the silage extracts and on the pure fungal culture extracts in order to gauge the hazards related to some well-known mycotoxins and also less well-known and unknown compounds. Based on transgenic cell cultures pure mycotoxins and silage extracts will be evaluated for their potential to induce specific stress genes in both biosensor systems. This will enable mode-of-action analysis to be performed and consequently the grouping of contaminating mycotoxin families within the silage. As the technology uses living cells, mixture effects such as synergy, additivity, potentiation, and masking will be taken into account.
The private partners will obtain information about the overall problem of fungal contamination and mycotoxin production in silage. Several techniques will be optimized for routine application in silage monitoring, which will enable for preventive measures to be developed as opposed to the present common practice of post hoc analysis and evaluation. This will eventually improve the quality of silage feed by reducing contamination events and hence reduce costs.

Characterization and dynamics of fusariosis on maize in Belgium[2]

Fusariosis, which accounts for losses of up to 30% of the grain yield in Belgium, is the result of simultaneous contamination by several Fusarium species, mostly F. graminearum, F. verticillioides and F. proliferatum. Low frequencies have also been detected or other species such as F. avenaceum, F. subglutinans, F. sambucinum, F. sporotrichioides, F. crookwellense, F. culmorum, and F. poae. Important amounts of fusarial mycotoxins are often found in maize silage, although it has been established that the physical and chemical properties of silage prevent the growth of Fusarium spp. (Scudamore and Livesey, 1998). Hence, the mycotoxins are produced in the field.
The objectives of the present project are to chart the dynamics of the various Fusarium species during the cultural season in Belgium, the genetic variability within the individual species, and their mycotoxin production.

Field assays were set up by the CARAH3 and the CIPF4 in five different eco-climatic situations. Eight maize varieties presenting a range of susceptibility to fusariosis were analyzed at four different physiological stages (flowering, ~26% dry matter content, ~33% beginning harvest, ~40% end of harvest). The CARAH was also in charge of the detection and quantification by HPLC of four main Fusarium mycotoxins (zearalenone, deoxynivalenol, fumonisins, and T2 toxin).

Preliminary results for the flowering stage

In the first cultural season, a total of approximately 2200 Fusarium spp. strains were isolated, partially identified, and preserved at BCCM/MUCL under sterile mineral oil. Fusarium strains were obtained from the stalk, ear, and silks from each maize plant. At the flowering stage, 55% to 95% of the samples per field were contaminated by Fusarium spp., even though no symptoms were visible (data presented at the "Ninth European Fusarium Seminar", 19-22 September 2006, Wageningen, The Netherlands). This is positively correlated with the national fusariosis evaluation, which has shown that the year 2005 was particularly favorable for the development of Fusarium spp., Furthermore, a wide spectrum of Fusarium species has already been identified. F. graminearum, known to be the most important pathogen on maize, represented 35.5% of the identified strains. F. avenaceum, which is responsible of the "Fusarium head blight" on wheat, reached 23.1%. Important percentages of F. culmorum (13.9%), F. equiseti (8.6%), and F. poae (3.5%) were also detected. Other species were occasionally detected (<2%): F. redolens, F. arthrosporioides, F. venenatum, F. torulosum, F. crookwellense, and F. ramigenum. F. oxysporum, which is a pathogen of many plants and crops but which is not known to be an important threat to maize, reached 5% of the identified Fusarium strains.
The percentage of contaminated plant bases is higher than that of ear contamination, which suggests an extension of the Fusarium spp. from the base to the top of the plant. F. graminearum, F. equiseti, and F. culmorum develop mainly on maize stalks (Fig. 1a, 1b), whilst F. poae and F. avenaceum were observed almost exclusively on ear and silks (Fig. 1c, 1d).

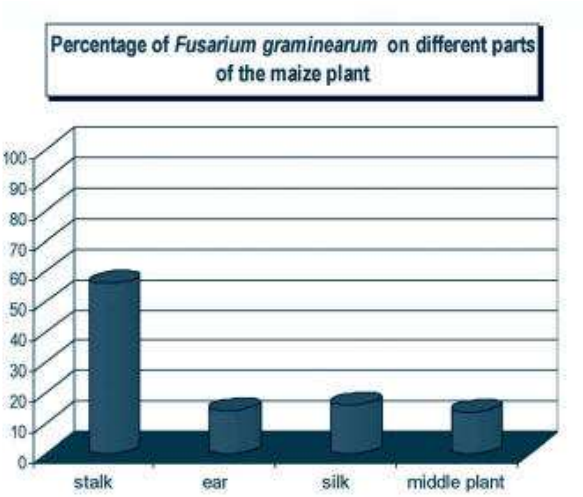

Fig. 1a.

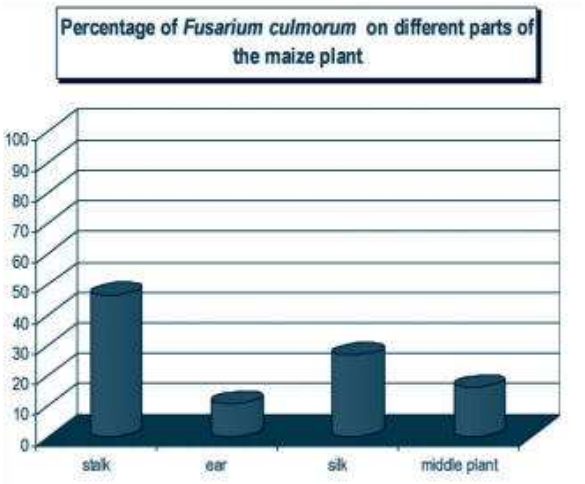

Fig. 1b.

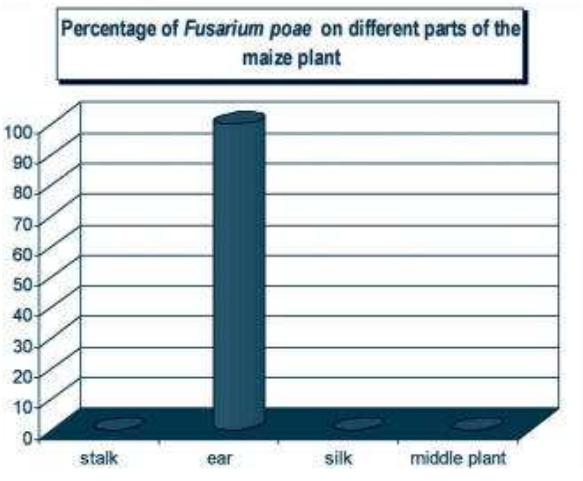

Fig. 1c.

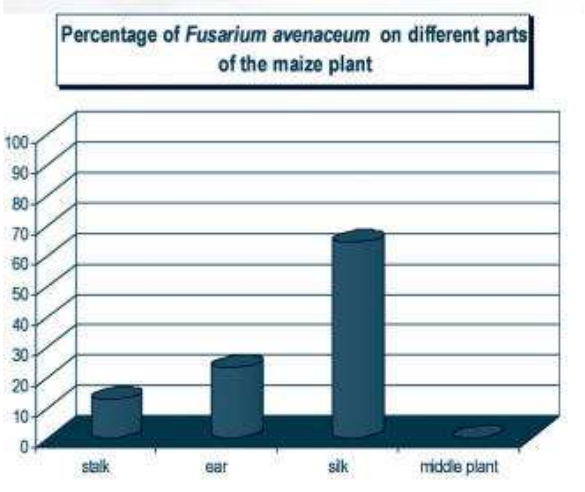

Fig. 1d.

The preliminary mycotoxin analyses in plant organs from which the Fusarium spp. strains have been isolated do not enable zearalenone to be detected although various producing species were present. In conclusion, an unsuspected level of Fusarium contamination was observed on maize plants from the early stage of the crops. Together with the observation that a very large number of species were detected, it raises the question of a possible under estimation of the pathogenicity and mycotoxin threat caused by Fusarium in fields.

Notes

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3. Centre Agronomique de Recherches Appliquées du Hainaut, Rue Paul Pastur 11, B-7800 Ath, Belgium.
4. Centre Indépendant de Promotion Fourragère, Faculté d'Ingénierie biologique, agronomique et environnementale, Université catholique de Louvain, Croix du Sud 2 boîte 3, B-1348 Louvain-la-Neuve, Belgium.

Literature

1. Scudamore KA and Livesey CT (1997). Occurrence and significance of mycotoxins in forage crops and silage: a review. J. of Sci. Food Agric, 77: 1-17.

Dr Françoise Munaut
Dr François Van Hove
Ir Jonathan Scauflaire