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DTU Systems Biology
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# Fungi and their mycotoxins in maize and maize silage

**Storm IMLD, Sørensen JL, Rasmussen RR, and Thrane U**

**Introduction** Maize silage is a major feed product for dairy cattle. A dairy cow can eat up to 40 kg a day. Unfortunately maize silage can be contaminated with mycotoxins both pre-harvest and post-harvest (Storm *et al.* 2008). This is potentially harmful to cattle, farmers and consumers of dairy and meat products. In cooperation between DTU Systems Biology, DTU Food, Aarhus University-Faculty of Agricultural Science, Danish Plant Directorate and Danish Cattle Association the extent of this problem has been examined and possible consequences evaluated.

**Pre-harvest** A screening of the presence of *Fusarium* in Danish maize revealed that the most common species was ***F. avenaceum*** (Fig. 1). This species produces moniliformin , enniatins and beauvericin, but none of the commonly surveyed *Fusarium* mycotoxins. A semi-selective medium was developed for isolation of *Alternaria* and *Phoma* (Sørensen *et al.* 2009) and used to survey maize samples collected at harvest. ***Alternaria infectoria*** was the predominant *Alternaria* species-group followed by *A. tenuissima* and *A. arborescens*, whereas ***Phoma pomorum*** was the only *Phoma* species isolated from maize.

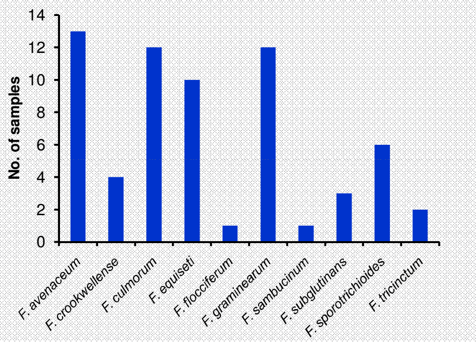



**Fig. 1** Presence of Fusarium species in 28 maize samples collected at harvest 2005 and plated on the Fusarium selective medium Czapek-Dox iprodion dichloran agar . The isolated Fusarium strains were grown on Yeast Extract Sucrose agar , potato dextrose agar and Spezieller Nährstoffarmer Agar and identified to species level based on morphology.

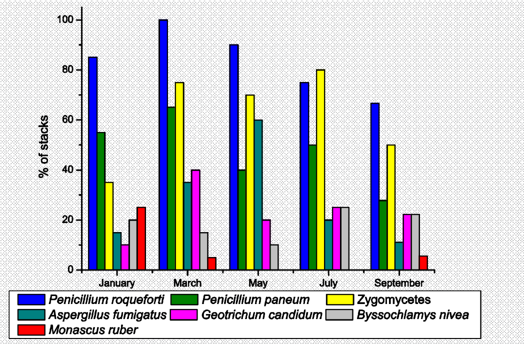



**Fig. 2** The percentage of maize silage stacks from which specific filamentous fungi have been isolated in a whole-season study of silage mycobiota . 20 silage stacks were examined by repeated sampling over a 9 month period. Both core samples and hot-spots with visible fungal growth were collected and plated on V8-juice agar and dichloran glycerol 18% agar . All apparently different filamentous fungi were isolated on suitable media and identified according to Samson *et al.* (2002).

**Post-harvest** The species of filamentous fungi occurring in Danish maize silages and their frequency *in situ* was examined by **monitoring 20 maize silage stacks over a whole season** (Storm *et al.* in press). Viable propagules of filamentous fungi were present in all silage stacks at all times during the study. The most frequent species where ***Penicillium roqueforti* and *Penicillium paneum*, Zygomycetes**, and ***Aspergillus fumigatus***. *Byssochlamys nivea/Paecilomyces niveus*, *Monascus ruber* and *Geotrichum candidum* occurred less frequently. Yeasts and lactic acid bacteria were also enumerated but not identified. The average counts of all microbial parameters were shown to vary significantly over a storage season. **The amounts of colony forming units of fungi were highest in 5-7 month old silage and significantly lower in 11 month old silage**. The occurrence of hot-spots with visible fungal growth showed the same tendency.

**Mycotoxins** Methods for mycotoxin detection in maize and maize silage have been developed and validated for:

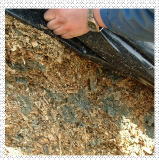
- Simultaneous extraction and analysis of **27 mycotoxins and other secondary metabolites** from the most important toxigenic fungal species pre- and post-harvest. A simple pH buffered sample extraction was developed on the basis of a very fast and simple method for analysis of multiple pesticide residues known as QuEChERS and raw extracts analysed by LC-MS/MS (Rasmussen *et al.* 2010). The method was applied to 99 Danish fresh and ensiled maize silages (Table 1) and hot-spots of fungal growth.
- **Moniliformin** using hydrophilic interaction chromatography with UV and MS detection (Sørensen *et al.* 2007). Fifteen out of 28 analysed samples contained moniliformin at trace levels (<12 ppb)
- **Enniatins A, $A_1$, B and $B_1$** and the related compound **beauvericin** by LC-MS/MS. The method was applied to 76 maize samples(Sørensen *et al.* 2008).

**Table 1** Mycotoxins and other secondary fungal metabolites detected in a survey of 99 samples of maize silage and fresh whole-crop maize. The samples were examined for 27 analytes by a rapid buffered extraction method followed by LC-MS/MS (Storm *et al.* submitted).

| | Compound | $n_{pos}$ | $avg_{pos}$ ($\mu g \cdot kg^{-1}$) | max |
|---|---|---|---|---|
| Quantitative | Alternariol monomethyl ether | 3 | 8.8 | 11 |
| | Andrastin A | 15 | 169 | 691 |
| | Alternariol | 2 | 18 | 24 |
| | Deoxynivalenol | 7 | 1841 | 2974 |
| | Enniatin B | 28 | 75 | 365 |
| | Mycophenolic acid | 2 | 43 | 52 |
| | Nivalenol | 16 | 263 | 758 |
| | Roquefortine C | 2 | 173 | 189 |
| | Zearalenone | 34 | 71 | 666 |
| Qualitative | Citreoisocoumarin | 8 | | |
| | Marcfortine A | 6 | | |
| | Marcfortine B | 1 | | |
| | Roquefortine A | 9 | | |

**Conclusion**

- Mycotoxin producing fungi are ubiquitously present in maize and maize silage .
- In Denmark maize samples rarely contain any of the regulated *Fusarium* toxins in concentrations above the EU recommended levels for cattle feed.
- The observed occurrences of mycotoxins in 99 samples of maize and maize silage do not suggest that they are the cause of general problems observed at dairy cattle farms
- Fungal hot-spots in silage can contain substantial levels of e.g. PR-toxins, mycophenolic acid, citrinin and gliotoxin.
- More knowledge on long-term exposure and possible synergistic effects is needed.



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