Incidence of *Fusaria* and occurrence of selected Fusarium mycotoxins on *Lolium* spp. in Germany

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Abstract

Test plantings with varieties of *Lolium multiflorum* and *L. perenne* were harvested 4 to 7 times a year in 1991 and 1992. Samples were checked for the presence of *Fusaria*, the mycotoxins zearalenone, T-2 toxin, and diacetoxyarscinolenol (DAS). Spectrum of species and the incidence of *Fusaria* and fusariotoxins are discussed in relation to the influencing factors site, variety of *Lolium*, harvesting time and year. Depending on these factors, 41 % to 100 % of the samples were *Fusarium* positive. Differences in infestation with Fusarium among varieties of *Lolium perenne* were dependent on location and did not correlate with yield. The six species of *Fusarium* pathogenic to *Lolium* spp. (*F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. oxysporum*, *F. solani*, and *F. acuminatum*) totaled 35.7 % of all the isolated strains. 14 species could be isolated from *Lolium* samples (descending frequency): *F. culmorum*, *F. sambucinum*, *F. equiseti*, *F. acuminatum*, *F. semitectum*, *F. oxysporum*, *F. subglutinans*, *F. avenaceum*, *F. sporotrichioides*, *F. proliferatum*, *F. tricinctum*, *F. anthophilum*, *F. dimerum* and *F. graminearum*. For the detection of *Fusaria* a promising new immunological method is presented. It is based on the genus specific production of exopolysaccharides by *Fusarium* species.

Mycotoxin contents in grass ranged from 0.01 to 4.75 ppm for zearalenone with 67 % positive samples and 0.3 % samples above 1 ppm, 0.04 to 2.78 ppm for T-2 toxin with 25 % positive samples and 2.8 % samples above 1 ppm, and 0.003 to 0.06 for DAS with 21.6 % positive samples. In silages, no T-2 toxin was detectable. Isolated *Fusarium* strains were checked for the ability to produce the mycotoxins zearalenone, T-2 toxin and DAS in culture. Most of the strains were positive for at least one of the toxins.

Introduction

In recent years, the examination of mycotoxins has played an increasing role in food quality control. Up to date, more than 300 mycotoxins are described, produced in front of all by the genera *Aspergillus*, *Fusarium* and *Penicillium* (1).
Some Fusaria are phytopathogenic, and most *Fusaria* are able to produce mycotoxins especially in the field. Until harvest they have enough time to grow and produce secondary metabolites. Fusaria produce many mycotoxins.

Zearalenone is a frequent estrogenic substance that, depending on the animal species, provokes more or less strong disturbances in reproduction. The acute toxicity is low.

Trichothecenes are a chemical class with an epoxy group that is responsible for the high toxicity to cells. Frequent representatives of the trichothecenes are T-2 toxin (the most poisonous substance), diacetoxyscirpenol (DAS, less frequent) and deoxynivalenol (DON, most frequent, but less poisonous).

Mycotoxins may contaminate animal-derived food if they pass the border between intestine and blood after feeding considerable quantities. Fortunately, zearalenone and trichothecenes are not enriched in the body (2, 3).

The recording of the incidence of Fusaria and fusariotoxins in grass is important for assessing the risks to animal health and food contamination. *Lolium perenne* and *Lolium multiflorum* were chosen for examination because they are the economically most important species of grass for feeding purposes. A further aim of this examination was to seize the spectrum of species of Fusaria and to check the ability of the isolates to produce mycotoxins.

Furthermore a new immunological method for a quick detection of the genus *Fusarium* was tested for its applicability for grass samples.

**Materials and methods**

In 1991, 832 samples were taken from all plots of two trials with 17 varieties of *Lolium perenne* (Perennial ryegrass) and of one trial with 11 varieties of *Lolium multiflorum* (Italian ryegrass) at every sampling date. The former trials were located in Apfelbaum near Gummersbach (site A) and Dollendorf/Eifel (site B), the latter in Horbach near Aachen (site C, all sites in the Federal County of North Rhine Westfalia). By plating five necrotised and not surface sterilised leaves out of every sample on potato dextrose agar, 2486 strains of *Fusarium* were isolated. Plant pathogenic as well as saprophytic species of *Fusarium* were isolated with this method. Suspicious outgrowing fungi were isolated, single spore cultures were made, and pure cultures were identified to genus level. 250 randomly selected strains previously identified as *Fusarium* were identified to species level according to Nelson et al. (4), in combination with Nirenberg (5). The production of toxins by isolated strains was examined by plating them on potato dextrose agar and growing them at 22°C in black light/dark 12 h/12h for ten days. For mycotoxin analysis, the same samples were used as for mycological analysis, and additional samples from the same trials were taken in 1992. Hacked, not dried grass was used for analysis. Mycotoxins were extracted by methanol/water (70/30), partitioned against dichlormethane, the dichlormethane phase was evaporated. After resolving in methanol, samples were diluted with 0.0135 M phosphate buffer, defatted with heptane and detected by ELISA-procedure (1). Mycotoxin contents were calculated on the basis of dry matter. Antibodies kindly were made available by Prof. Terplan, university of Munich. Significant antibody cross reactions were for zearalenone: α-zearalenol 41.6 %, β-zearalenol 13.8 %, α-zearalanol 41.6 %, for T-2 toxin: acetyl T-2 toxin >100 %, HT-2 toxin 40 %, and for DAS: 3-acetyl DAS 597 % (1).

For the detection of genus-specific extracellular polysaccharides (EPS), hacked, not dried grass was extracted with 0.07 M phosphate buffer for ten minutes. The filtrate and dilutions of it were mixed with latex beads that were coated with antibodies. After ten