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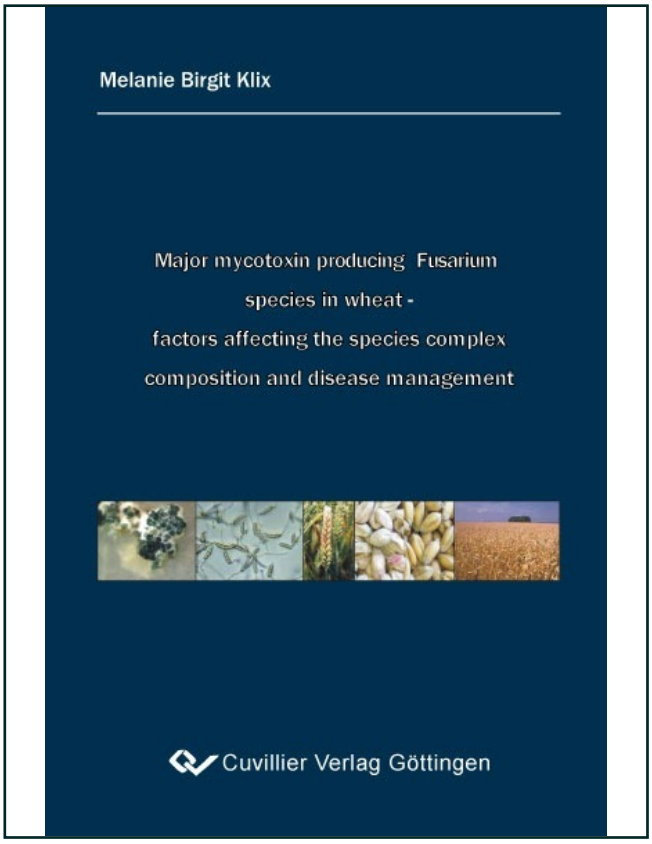
Major mycotoxin producing Fusarium species in wheat - factors affecting the species complex composition and disease management



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1. Fusarium head blight in wheat

Fungal species of the genus *Fusarium* are of major importance worldwide. After first description of this genus by Link in 1809, an association of mycotoxicosis with the symptoms of Fusarium head blight (FHB) on wheat (*Triticum aestivum* L.) was reported from Russia in 1923 (Donunin, 1926). Later, it became clear that FHB is not caused by a single species but by a species complex. In Northwestern Europe the main causative agents of FHB described are *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schwein.) Petch], *Fusarium culmorum* (W.G. Smith) Sacc., *Fusarium avenaceum* (Fr.) Sacc. (teleomorph: *Gibberella avenacea* R. J. Cook), and *Fusarium poae* (Peck) Wollenw. (Parry et al., 1995; CABI, 2001; Bottalico and Perrone, 2002; Waalwijk et al., 2003; Büttner, 2006). In wheat, the most important grain as a food source for humans, yield losses caused by *Fusarium* infections are sporadic but can reach levels as high as 70% under favorable conditions (Bai and Shaner, 1994; Curtis, 2002). Furthermore, contamination of wheat kernels with mycotoxins produced by FHB species limits the marketability of wheat. The contribution of each species to FHB, subsequent yield losses, and mycotoxin contamination of the grain in a wheat crop depends on the epidemiological capacity of the pathogen and environmental conditions. These conditions can be divided in factors that can be influenced by the grower, such as type of previous crop, tillage system, fungicide treatment, nitrogen fertilization, growth regulators, and cultivar choice and factors that are given for a certain location but none the less affect the disease development, such as macro- and micro-climatic conditions.

2. Taxonomy

Species of the genus *Gibberella* are homothallic Ascomycota, class Ascomycetes, order Hypocreales, family Nectariaceae (CABI, 2001). For some *Fusarium* species, a teleomorphic stage was not identified. The anamorphic stage was therefore assigned to the subclade Deuteromycotina, class Hypomycetes, order Tuberculariales as an independent species. These circumstances resulted in the peculiar situation known for various fungi that two stages of the same fungus are classified dissimilarly. Recently, the taxonomy of fungi is challenged once more since molecular tools allow classification based on genomic rather than morphological similarity.

3. Epidemiology

Fusarium species have a broad host range covering many agriculturally important plant families, such as *Poaceae*, *Fabaceae*, *Solanaceae* and *Cucurbitaceae*. All developmental stages of *Fusarium* can further survive saprophytically on crop debris and other organic matter. Symptomless growth in leaves and stems of wheat has been described as well (Parry et al., 1995). For dispersal the anamorphic stages form macro- and/or microconidia. Many *Fusarium* species additionally produce clamydospores that serve as a resistant stage for survival. The shape, septation, and size of *Fusarium* spores are spore type and species dependent (Table 1). In the teleomorphic stage, fusion of hyphae induces the formation of the sexual organs ascogonium and antheridium that are required for recombination. Following a phase of nuclear division, meiosis, and postmeiotic separation, eight-spored asci are formed in brownish black, flask shaped perithecia (Fig. 1). The final ascospore release is a result of a buildup in turgor pressure inside the perithecium generated by ion fluxes and accumulation of mannitol (Trail et al., 2002). Perithecia can be formed throughout the year and can emerge from the stomatal cavities (Trail et al., 2005). Maturation of the perithecia and ascospore release are directly and indirectly influenced by a range of environmental factors including light intensity, temperature, humidity, wetness, and osmolarity of the ambient medium (Trail et al., 2002; Gilbert and Fernando, 2004; Del Ponte et al., 2005).

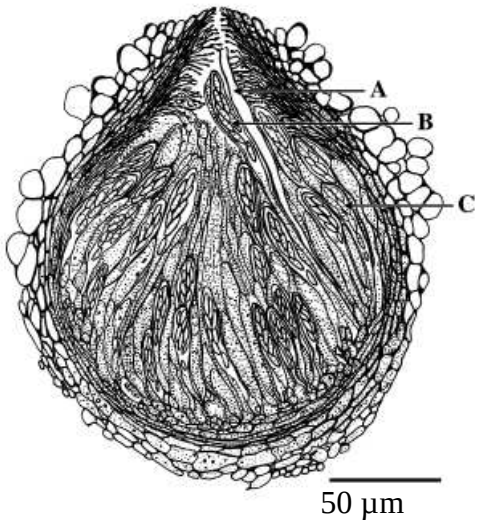

Figure 1: Cross section of a mature perithecium from *Gibberella zeae* (Trail and Common, 2000). **A)** Ascus after discharge of ascospores **B)** Mature ascus extending **C)** Ascus before extension to the ostiole.

After the forcible discharge, ascospores are transported to wheat heads by wind and air currents, and therefore are adequate for long-distance dispersal. In contrast to ascospores, the asexual macro- and microconidia are passively dispersed and depend on the kinetic energy of wind and rain-splash. In laboratory experiments, macroconidia of *F. poae* and *F. culmorum* were mainly observed at a vertical distance of 58 cm and a horizontal distance of 70-100 cm from the inoculum source, respectively (Hörberg, 2002). Since they are not initially accelerated, fewer conidia than ascospores were also sampled in the air layer above wheat fields (Fernando et al., 2000; Markell and Francel, 2003). Therefore, infections by conidiospores seem to rather depend on inoculum sources from neighboring locations. However, if a teleomorph exists, like for *F. graminearum*, then airborne ascopores are thought to be the primary source of inoculum (Shaner, 2003). Susceptibility of wheat heads to *Fusarium* species is concentrated to the period of anthesis (Strange et al., 1971; Sutton, 1982; Bushnell et al., 2003). After germination of the infecting spores, a dense mycelium develops on the adaxial surfaces of the lemma, palea, and glume. *Fusarium* species are able to penetrate the cuticle and later the plant tissue directly, followed by intra- and intercellular growth that is facilitated by cell wall degrading enzymes, such as cutinases, polygalacturonases, xylases and cellulases. Penetration by infecting hyphae is accompanied by the appearance of the first symptoms on wheat ears in the form of brown to purple lesions with a bleached centre (Kang et al., 2002; Bushnell et al., 2003; Kang et al., 2005). In the following stages of infection, the fungus spreads within the wheat ears through the vascular bundles, thus interrupting nutrient supply to the apical spiklets that result in shriveled kernels and typical bleached spikelets (Bushnell et al., 2003; Goswami and Kistler, 2004).

4. Effect of climatic conditions on Fusarium head blight species

Climatic conditions, such as temperature, relative humidity, leaf wetness and precipitation were reported to be the most important factors affecting FHB on wheat (Fernandez et al., 2005). In general, temperature requirements for FHB development are inversely correlated to the period of leaf wetness (Rossi et al., 2001). Highest disease severity and subsequent contamination of the grain by mycotoxins, such as deoxynivalenol (DON) were observed at temperatures between 20-32°C and prolonged wetness (Hooker et al., 2002; Bushnell et al., 2003). Nonetheless, *Fusarium* species differ in their requirements towards climatic conditions: Infections by *F. graminearum* were mainly described under warm and humid conditions (Fernandez et al., 2005; Shah et al., 2005; Büttner, 2006), whereas *F. culmorum*

and *F. poae* were assigned to cooler climates (Parry et al., 1995; Doohan et al., 1998; Bottalico and Perrone, 2002; Fernandez et al., 2005). On the other hand, *F. avenaceum* seemed relatively unaffected by the climatic conditions encountered in a region (Abramson, 1998). Some authors further suggested that the dominating head blight species at a given location is determined by temperature more than any other environmental factor (Cook, 1981; Miller and Greenhalgh, 1985).

Table 1: *Fusarium* species and spore types (Booth, 1971; Nirenberg, 1981; Joffe, 1986).

Anamorph	**Teleomorph**	**Macro-conidia**	**Micro-**	**Clamydo-**	**Asco-spores**
F. graminearum 	*G. zeae*	spindle shaped, curved 28-78 x 3.2-5.0 µm	absent	rare	1-4 septate 13- 28 µm
F. culmorum 	_	compact, dorsiventral mostly 5 septate 25-68 x 4.8-8.2 µm	absent	yes	absent
F. avenaceum 	*G. avenacea*	small, thin 3-7 septate 31-92 x 2.5-4.1 µm	rare 0-3 septate Spindle shape	no	elliptical 1-3 septate 12- 24 µm
F. poae 	_	sparse, small, curved 3 septate 17-36 x 4.0-6.6µm	globose to lemon shape	yes	absent

*Bars in spore figures equal 10 µm.

Temperatures below 16.5°C were limiting infections by *F. culmorum*, while infections and ascospore release by *F. graminearum* were solely restricted at temperatures below 10°C. The two species also differ in their optimal temperatures required for fungal growth and development, ranging from 18-26.5°C for *F. culmorum* and from 28-29°C for *F. graminearum* (Sutton, 1982; Rossi et al., 2001). Consequently, infections by *F. culmorum* are restricted to a more narrow range of temperatures as compared to *F. graminearum*. Further on, relative humidity correlated positively with infections caused by *F. avenaceum* and *F. graminearum*, but not with infections by *F. culmorum* (Rossi et al., 2001). Infection rates by this species were highest at a relative humidity of 65%, while for the other two species highest infection were observed at a relative humidity of 100%. Climatic conditions might also have varying effects on the different developmental stages of the fungus, like for example development of perithecia, ascospore discharge and germination as shown for *F. graminearum* (Trail et al., 2002; Beyer et al., 2005). Finally, the host plant wheat is also influenced by climatic factors, such as temperature, leaf wetness, and relative humidity, which determine both the duration and type of flowering (cleistogamous or chasmogamous) that can have an effect on FHB incidence (De Vries, 1971).

5. Effect of plant production measures used against Fusarium head blight

Plant production measures can affect FHB in different ways. They can influence both the overall infection incidence and infection rate by certain FHB species as well as subsequent progression of the disease. Consequently, they can also influence the mycotoxin content of wheat grain infected by *Fusarium*. Obviously, these parameters cannot be seen independently but they interact with one another. Methods of plant production measures to control FHB include crop rotation, tillage, choice of wheat cultivar, and application of fungicides. Studies focusing on the effect of plant production measures on individual *Fusarium* species are sparse, but can be quite important for understanding changes in the species complex composition at a given location. Most of the studies at hand focus either on overall infection rate and/or the mycotoxin content of wheat grain as an indicator for the success or failure of the plant production measures used (Dill-Macky and Jones, 2000). However, the mycotoxin content detected in the wheat grain obviously depends on the toxin producing ability of the infecting species. Mycotoxin contamination of the grain is the most important aspect with respect to food safety, and therefore has attracted most attention so far.

6. Mycotoxins produced by major *Fusarium* species

Mycotoxins are classified as secondary metabolites, since their biosynthesis is not required for fungal growth and reproduction. Trichothecenes and zearalenones are the major classes of mycotoxins produced by *Fusarium* species and are associated with mycotoxicosis in humans and animals (Desjardins, 2006). An additional metabolite not included in the two groups above but produced by various *Fusarium* species infecting wheat in Northern Europe is moniliformin (MON). The range of mycotoxins that are produced by fungi of the genus *Fusarium* is species dependent (Table 2). The mycotoxins most frequently detected in FHB damaged wheat grain is the trichothecene DON, followed by zearalenone (ZEA). Due to their adverse effect on eukaryotic cells, limits in the DON and ZEA contents tolerated in marketable cereals were laid down by national and European laws, in order to protect consumers from health risks associated with the intake of these mycotoxins (BDGB, 2004; EC, 2006). Overall, the most frequently detected *Fusarium* toxin in cereal-based products in Europe was DON (57%), followed by fumonisins (47%), ZEA (32%), and T2-toxin (20%) (SCOOP, 2003).

6.1. Trichothecenes

Trichothecenes are tricyclic sesquiterpenes with considerable differences in their oxygenation levels. In contrast to trichothecenes produced by other fungi, *Fusarium* trichothecenes contain a C-3 oxygenation. Trichothecenes are further characterized by a double-bond between C9 and C10 (Fig. 2), and a C12, C13-epoxid ring (Goswami and Kistler, 2004; Desjardins, 2006).

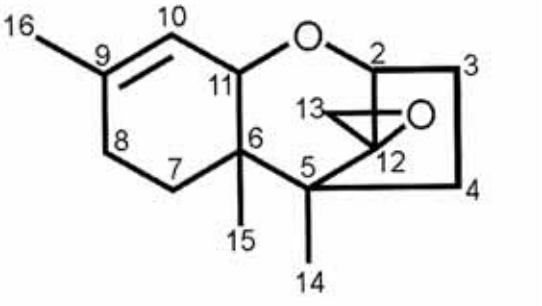

Figure 2: General structure of trichothecenes (Desjardins, 2006).

Two groups of trichothecenes can be distinguished depending on whether (Typ B trichothecenes) or not (Typ A trichothecenes) a keto group is present at the C8 position. The primary mode of action of trichothecenes is inhibition of peptidyl transferases and thereby

protein biosynthesis (Rotter et al., 1996; McCromick, 2003). The active moiety of trichothecenes is the C12, C13- epoxid ring.

Overall, DON was the most frequent trichothecene detected in wheat flour. Other frequently detected trichothecenes are Nivalenol (NIV) and the A-type trichothecenes diacetoxyscirpenol (DAS) and T2-toxin (Schollenberger et al., 2002; Desjardins, 2006). For trichothecenes and their main producers see Table 2. In animals their effect depends on both the type of toxin (e.g. oxygenation level at C3) and the animal system at hand (Desjardins, 2006). Typical symptoms of mycotoxicosis caused by trichothecenes especially in monogastric animals are nausea, vomiting, diarreha and immunosuppression. Next to animals and humans, trichothecenes are also toxic to a number of plant tissues. It was shown that trichothecenes are virulence factors in *F. graminearum*, especially during spread of the fungus within the wheat ear (Eudes et al., 2001; Lagevin et al., 2004; Desjardins, 2006). However, the impact of DON on the pathogenicity of *Fusarium* species led to ambiguous results (McCromick, 2003).

6.2. Zearalenones

Zearalenones (ZEAs) are estrogenic mycotoxins that were first isolated from *G. zeae*. The chemical structure of ZEAs is a resorcyclic acid lactone (Fig. 3), a nonaketid (Desjardins, 2006). In animal systems ZEAs have estrogenic effects since they can bind to estrogen receptors. The main source of ZEA intake by humans and animals is wheat, maize, and barley. ZEA did not show acute toxicity in animal systems but weak genotoxicity was reported. The adverse effect depends again on the animal system at hand, since ruminants are less sensitive than monogastric animals (Desjardins, 2006). With respect to the effect of ZEA on plants, no indications for phytotoxicity were found. Cereals contain detoxification genes that code for enzymes able to metabolize ZEA in the plant tissues. However, a direct correlation between activity of these genes and metabolism of ZEA has still to be established (Desjardins, 2006). Finally, since all ZEA producing *Fusarium* species also produce trichothecenes, the same management strategies can be applied to reduce contamination of wheat grain by these two metabolites.