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INFECTION PROCESS AND MYCOTOXIN PRODUCTION IN *FUSARIUM CULMORUM*-INFECTED MAIZE EARS

ABSTRACT

Red ear rot of maize is an important disease in Europe caused by toxigenic *Fusarium* species like *F. graminearum* and *F. culmorum*. To get detailed information about the pathogenesis of the disease and the *Fusarium* toxin production in infected ears a field study was conducted with maize which was artificially inoculated with *F. culmorum* at the stage of female flowering. Every fortnight after inoculation, maize ears of two varieties were harvested and analysed for the progress of visual signs of the disease and related *Fusarium* toxin contamination. During the last harvest in mid October, external infection symptoms showing some small pale or brown-marbled kernels with dark brown rachillae were only observed at the ear tip, whereas internal symptoms visible within the rachis were much more pronounced and showed greyish –brownish or pink discolouration of the pith. The symptoms observed in rachis and kernels corresponded with the toxin contamination showing considerably higher concentrations in the rachis compared to the kernels and a top-down gradient from high to low toxin levels within the ear. This suggests that *F. culmorum* first infects the rachis from the tip towards the bottom, as it subsequently does the kernels via the rachillae connected to the rachis. As infection symptoms and mycotoxin production were much more pronounced in the rachis than in the kernels, red ear rot evaluation should be improved by observing signs of the disease in both kernels and the rachis.

Key words: Acetyl-deoxynivalenol, deoxynivalenol, kernels, nivalenol, pathogenesis, rachis, red ear rot, zearalenone

INTRODUCTION

Predominant *Fusarium* species causing an important disease in European maize called red ear rot are *F. graminearum* and *F. culmorum*, which are capa-

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ble to produce mycotoxins like trichothecenes and zearalenone in pre-harvest infected plants (Logrieco *et al.*, 2002). The infection of maize ears may either result from fungal entry via the silks or via wounds from birds, insects or damage by hail (Reid *et al.*, 1996). In the absence of physical injury, red ear rot of maize usually starts at the ear tip after silk emergence and moves towards the base (Reid *et al.*, 1999). Characteristic symptoms are pink to reddish coloured mould layers spreading downwards on the kernels underneath the husks (Reid *et al.*, 2002). When maize is cultivated under practical conditions of Central Europe, typical and heavy signs of the disease develop seldom and in addition late in the vegetation period. Moreover, other *Fusarium* species or black fungi co-infecting the ears interfere with the symptoms of red ear rot. The weakness and complexity of external symptoms observed under natural infection often lead to difficulties in disease evaluation. Furthermore, high ear rot ratings are usually necessary to obtain satisfying correlations between ear rot severity and the corresponding mycotoxin contamination of the kernels (Löffler *et al.*, 2010). To get more detailed and fundamental information on the time course of the infection process and the spectrum of *Fusarium* mycotoxins produced in the ears, a field study was conducted with maize artificially inoculated by *F. culmorum* to induce red ear rot.

MATERIAL AND METHODS

Two maize varieties were cultivated at Sickte near Braunschweig (Northern Germany) in experimental field plots of 100 m^2 each during the season of 2009. Sowing was carried out in late April with Mesurol-treated seed establishing 12 rows per variety with a 0.75 m row and 13 cm seed spacing. At the stage of female flowering (BBCH 65, Meier 2001) in late July, approximately 240 plants per variety were artificially inoculated with 1 million *F. culmorum* conidia suspended in 0.5 ml water, which was placed into the tip of the silk channel of each ear with a pipette. Every fortnight after inoculation, ten infected ears per variety were harvested by hand in two replications until the end of cultivation in mid-October.

Immediately after harvest, the husks were removed manually from the ears and visually examined for external disease symptoms. To follow the infection path of the fungus, each of the ten ears representing one replicate were chopped crosswise to obtain four slices of approximately equal size. Subsequently, the corresponding quarters of each ten ears were pooled and freeze-dried for at least 3 days. After the kernels were manually separated from the rachis sections, each fraction was ground with a chopper disc mill.

HPLC analysis

Subsamples of 25 g were taken with a sample divider PT 100 and extracted with

100 ml of a mixture of methanol/acetonitrile/water, (75/5/7, v/v/v) by turbulent shaking for 30 min. The extraction solvent was shaken thoroughly before each removal of an aliquot, which was transferred on a column containing celite, alumina, activated carbon (Darco G-60), and DOWEX 50 W-X8 resin. An aliquot of the eluate (5.0 ml) was evaporated to dryness and redissolved in 1.0 ml of the HPLC eluent in an ultrasonic bath. 40 µl of the purified filtrate were analysed by HPLC. An Agilent 1100 Series system (Agilent Technologies, Waldbronn, Germany) consisting of a membrane degasser, a binary pump, a thermostated autosampler with a 100 µl loop, a thermostated column compartment, a diode array detector set at 230 nm was used for analyses. The column was a Lichrosphere RP-18, 25 mm × 4.6 mm, particle size 5 µm, purchased from Merck (Darmstadt, Germany). The mobile phase consisted of solvent A (methanol/water, 5:95, v/v) and solvent B (methanol). A gradient procedure was used as followed: starting with 10% of B: up to 100%. The column temperature was set at 50°C.

RESULTS

Evaluation of disease symptoms

The first infection symptoms were visible after 4 weeks of inoculation, showing desiccated and lightly brown-coloured florets at the ear tips. Within the following 2 weeks, the brownish colouration became more intensive and co-infection with black fungi was observed. During the successive 4 weeks, small kernels situated at the tip area of the rachis began to pale or showed beige brown-marbled spots or white smears. Simultaneously, the piths of the rachis tip turned from whitish-yellowish to greyish-brownish colouration. At the last harvest (81-83 days from inoculation) the rachillae of infected kernels situated at the ear tip coloured dark brown and the subjacent part of the rachis showed intensively pink or greyish-brownish colouration. A few cases saw white coloured mycelium on the outside of an ear tip. The black spore layers on the ear tips deriving from black fungi infection were most intense at the latest harvest. In principle, these infection symptoms developed similarly in both maize varieties, but the discolouration through the rachis was more accelerated in variety B compared with variety A, indicating a faster spread of the infection.

Fusarium toxin production correlated with infection progress

Mycotoxin production started 4 weeks after inoculation, beginning at the rachis tip and showing traces of acetyl-DON and DON. Successively, contamination of the rachis increased but was mainly restricted to the tip in variety A, reaching a very high level of acetyl-DON, DON and zearalenone (mean 75, 67 and 3.4 mg × kg^{-1}, respectively) at the last harvest (Fig. 1).

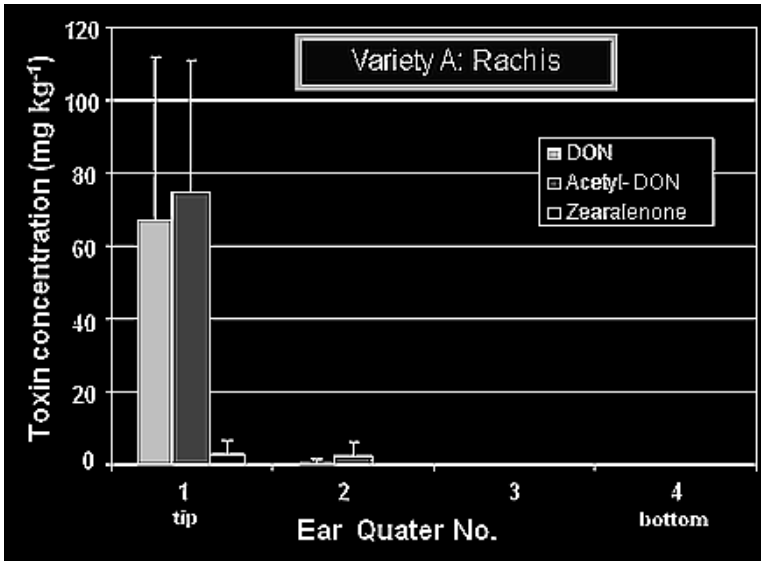

Fig. 1: *Fusarium* toxin concentrations in the rachis of variety A.
The error bars represent the standard deviation of the mean of two replicates.

In contrast, the kernels connected with these contaminated parts of the rachis showed considerably lower amounts of DON, acetyl-DON and zearalenone (mean 3.1, 1.4 and 0.03 mg kg^{-1}, respectively) (Fig.2).

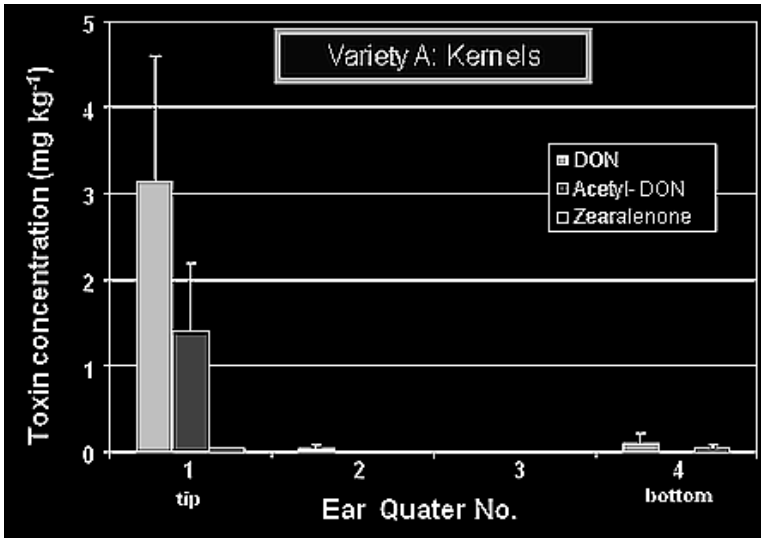

Fig. 2: *Fusarium* toxin concentrations in the kernels of variety A.
The error bars represent the standard deviation of the mean of two replicates.

In variety B, toxin production was even more severe with contamination of DON, acetyl-DON and zearalenone within the whole rachis, being highest at the tip (109, 39 and 24 mg kg^{-1}, respectively) and lowest at the bottom (Fig. 3).

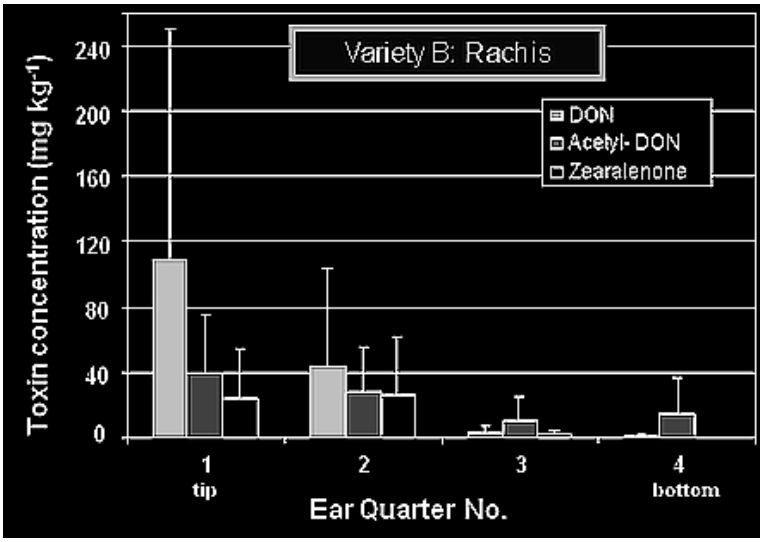

Fig. 3: *Fusarium* toxin concentrations in the rachis of variety B.
The error bars represent the standard deviation of the mean of two replicates.

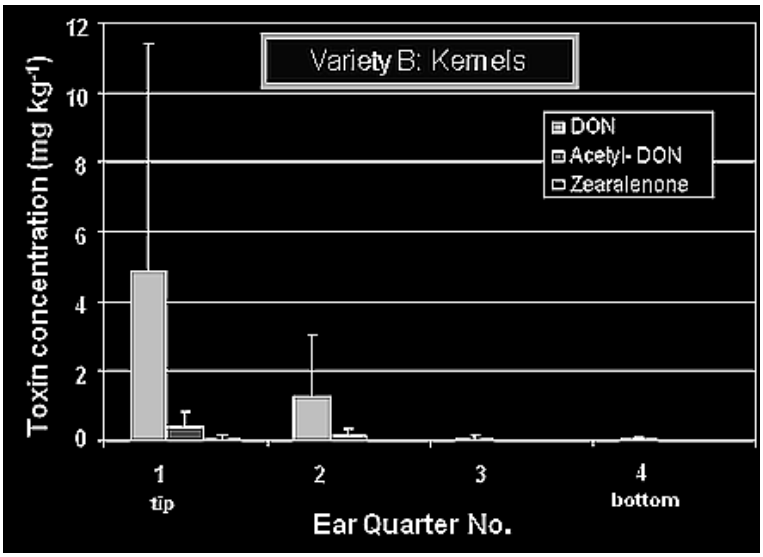

Fig. 4: *Fusarium* toxin concentrations in the kernels of variety B.
The error bars represent the standard deviation of the mean of two replicates.

Toxin production in the corresponding kernels was mainly detected in the upper half of the ear, showing highest amounts of DON, acetyl-DON and zearalenone (mean 4.9, 0.4 and 0.08 mgkg^{-1}, respectively) at the tip (Fig. 4).

It was only occasional that nivalenol was observed either in the rachis (max. 3.1 mg × kg^{-1}) or the kernels (max 0.08 mg × kg^{-1}) of both varieties.

The higher *Fusarium* toxin contamination especially in the rachis of variety B were in accordance with the more severe disease signs observed in the rachis of variety B in comparison with variety A.

DISCUSSION

The results of this study suggest that *F. culmorum*, after entering the ear through the silks, first infects the rachis tip and initially moves down towards the rachis bottom. The infection progress can be visually followed by observing the occurrence of a pink or greyish-brownish discolouration of the infected parts of the rachis. Subsequently the kernels are infected top-down the ear via the rachillae connected to the rachis but only some heavily infected kernels situated on the ear tip show visible signs of the disease.

The comprehension of the infection symptoms much more pronounced in the rachis may improve disease diagnosis, e.g. for the purpose of resistance characterization of maize hybrids. To evaluate *Fusarium* ear rot resistance of maize, at least two components should be analysed: the resistance to entrance of the fungus via the silks and the resistance to the spread of symptoms (Lemmens, 2010). The former is usually assessed after spray inoculation of the silks with *Fusarium* spores without wounding, the latter after injection (wounding) of *Fusarium* spores into the ear tip or kernels (Lemmens, 2010; Toldi and Mesterhazy, 2010).

It is suggested that a combined evaluation of disease signs outside the ear (ear tip and kernels) and inside the ear (rachis) after inoculation of the silks without wounding may facilitate the method to prove the susceptibility of maize hybrids against red ear rot. A successful primary infection via the silks might be deduced from visible disease signs at the ear tip (resistance to entrance of the fungus) and the progress of infection (resistance to the spread of symptoms) might be detected from the area of discoloured parts of the rachis.

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