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THE INCIDENCE OF *FUSARIUM* FUNGI AND MYCOTOXINS IN WHEAT GRAIN AS AFFECTED BY GROWTH REGULATORS

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Abstract

Growth regulators have become an integral part of cereals cultivation technology. Their use is instrumental, since heavily fertilised crops are prone to lodging, especially in rainy years. When applied, active ingredients present in growth regulators can affect microbiological and biochemical processes occurring in plants and microclimate of crop stands. Growth regulators chlormequatchloride 750 g l^{-1} and ethephone 480 g l^{-1} were tested in the crops of winter wheat at the Lithuanian Institute of Agriculture during the period 2004–2007. Analyses on *Fusarium* fungi and mycotoxins produced by them – deoxynivalenol, zearalenone and T-2 toxin were performed. Grain samples for fungi and mycotoxin analyses were taken at harvesting. Internal grain infection with *Fusarium* fungi was determined by sowing disinfected grain on potato dextrose agar medium. The analyses of mycotoxins were performed by ELISA method, using *NEOGEN Europe Ltd* Veratox diagnostic test kits.

During the experimental period, the grain from the plots sprayed with chlormequat-chloride at BBCH 23–25 and ethephone at BBCH 39–45 and with chlormequatchloride at BBCH 23–25 and chlormequatchloride at BBCH 32–33 had the concentrations of the mycotoxin zearalenone by on average 32–33% higher compared with the grain from the unsprayed plots. Similar trends were revealed having done analyses of deoxynivalenol. The trends of deoxy-nivalenol and zearalenone concentrations increasing in grain as affected by the use of growth regulators have raised new tasks for further research.

Key words: *Fusarium*, deoxynivalenol, zearalenone, T-2 toxin, mycotoxins, winter wheat, growth regulators.

Introduction

Plant growth regulators (PGR) are exogenously applied chemical compounds that regulate stem elongation through inhibiting biosynthesis of gibberellins or releasing ethylene. PGRs are used in modern high input cereal management to shorten straw and increase lodging resistance /Rajala, 2003/. Lodging that occurs during early grain filling stage can cause considerable yield loss and can result in increased microbiological contamination and a risk of mycotoxin occurrence /Scudamore, 2000/.

The evidence on the effects of plant growth regulators (exogenic-origin hormones) on plant development is becoming increasingly numerous in the world literature, however, it is often very hard to reasonably interpret the obtained data, since the impact

on the biosynthesis of other biochemical processes is not clear /Kefeli, 1997/. Literature sources indicate that mycotoxins can exert a similar effect to that of PGR. In vitro tests with wheat showed that the mycotoxin zearalenone can be used as PGR, since it has a similar effect to that of auxins and even better than that of cytokinins /Szechynska-Hebda et al., 2007/. However, under natural plant growth conditions the temperature regime constantly varies – cloudiness, solar irradiation, wind velocity is constantly changing. Plants have also to adjust to the changes, and their hormonal activity has a great effect on this and causes unpredictable changes. It is likely that a shortage of nutrients can disturb the utilisation of PGR and their effect may dwindle. Moreover, some of the chain of biosynthesis can be inhibited as well as the activity of endogenic growth hormones. The evidence on the impact of hormones of exogenic origin on mycotoxin synthesis in grain is rather scarce in the world literature.

Numerous research has been done in Lithuania to study the efficacy of chlormequatchloride and ethephone agents in cereal crops, to identify their effects on plant stem length, lodging, yield and biometrical indicators /Auškalnienė, 2005a; Auškalnienė, 2005 b/. Experiments done during 2004–2005 in spring barley crops showed higher concentrations of mycotoxins deoxynivalenol, zearalenone and T-2 toxin to be present in the grain of PGR – applied barley /Supronienė et al., 2006/. Consequently, it was interesting to ascertain the variation of mycotoxin content in the grain of winter wheat whose crops had been applied with PGR.

Materials and Methods

Field experiments. The three varieties of winter wheat 'Širvinta', 'Ada', and 'Zentos' were investigated under high input cereal management at the Lithuanian Institute of Agriculture during the period 2004–2007. All crop stands were sprayed with PGR: for the first time chlormequatchloride 750 g l^{-1} (Cycocel, CCC) at BBCH 23–25, and for the second time with CCC at BBCH 32–33, or ethephone 480 g l^{-1} (Cerone, ETH) BBCH 37–39 or 39–45. Plant growth stages were recorded according to BBCH scale /Meier, 1997/.

Samples. Grain samples of winter wheat were collected at harvest during 2004–2007. The number of samples analysed is given in Table 1.

Table 1. The number of winter cereal grain samples tested for *Fusarium* fungi and mycotoxins contamination during 2004–2007

Indicator	Number of samples during 2004–2007
Fusarium fungi	216
Mycotoxins (total):	144
Deoxynivalenol (DON)	54
Zearalenone (ZEN)	54
T-2 toxin	36

Fusarium spp. *analysis.* To determine the grain internal contamination with mould fungi the agar plate method /Mathur, Kongsdal, 2003/ was applied. The *Fusarium*

species were identified on the basis of their morphological and cultural characteristics according to Bilai (1977), Gerlach, Nirenberg (1982), Nelson et al. (1983), Leslie, Summerell (2006).

Analysis of mycotoxins. Contamination with deoxynivalenol (DON), zearalenone (ZEN), and T-2 toxin was tested. Part of each sample was subjected to toxicological contamination, and the other part (about 50 g) was air-dried, milled in a mill IKA A11 Basic and kept at –20^{o} C until analysis. The wheat samples were analysed by the ELISA (enzyme-linked immunosorbent assay) method /Wilkinson et al., 1992/. The method is based on the antibody antigen interaction. The Veratox test kits (Neogen Corporation, Scotland), approved by the AOAC Research Institute (Certificate N 950702) were used for the analysis. Mycotoxin extraction and tests were performed according to manufacturer's instructions. The optical densities of samples and controls from standard curve were estimated by a photometer Neogen Stat Fax®303 Plus, using filter of 650 nm. The measured absorbance was automatically converted to the mycotoxin concentration units – μg kg^{-1}.

The results were estimated taking into account the lowest calibration curve's mycotoxin concentration value (LOD-limit of detection), which is for:

DON – 100.0 μg kg^{-1} (ppb); ZEN – 10.0μg kg^{-1} (ppb); T-2 toxin – 7.5 μg kg^{-1} (ppb)

While assessing our data with regard to food and forage safety we referred to the EU document No.856/2005 for DON and ZEN, and global research recommendations for T-2 toxin /Eriksen, Alexander, 1998/.

ANOVA was applied for the statistical processing of data. For data significance the Fisher test was used. Averages for the other data were calculated /Tarakanovas, Raudonius, 2003/.

Meteorological conditions recorded at the Dotnuva Weather Station during intensive growth and flowering period of plants were similar in the 2004–2005 experimental years (Table 2). The year 2006 was noted for extremely dry weather. The drought that started in July severely affected cereals at milk maturity stage. The rainy end of July and August interfered with harvesting.

In the spring of 2007 warm and dry weather prevailed, however, excess of moisture was felt from May throughout the entire plant growing season.

Results and Discussion

The tests were done in different years, however, no obvious symptoms of *Fusarium* head blight were observed in the winter wheat crops tested. In 2004–2005 grain contamination with *Fusarium* spp. fungi was similar. The year 2006 was distinguished by the lowest grain contamination, while the year 2007 was noted for the highest contamination since the conditions were conducive to the spread of *Fusarium* fungi (Table 2). However, regardless of the differences in the weather conditions between the experimental years, the trends of the spread of *Fusarium* fungi and mycotoxins produced by them – DON, ZEN, and T-2 toxin were similar. As a result, we presented the data averaged over the period 2004–2007, which reflect the obtained results.

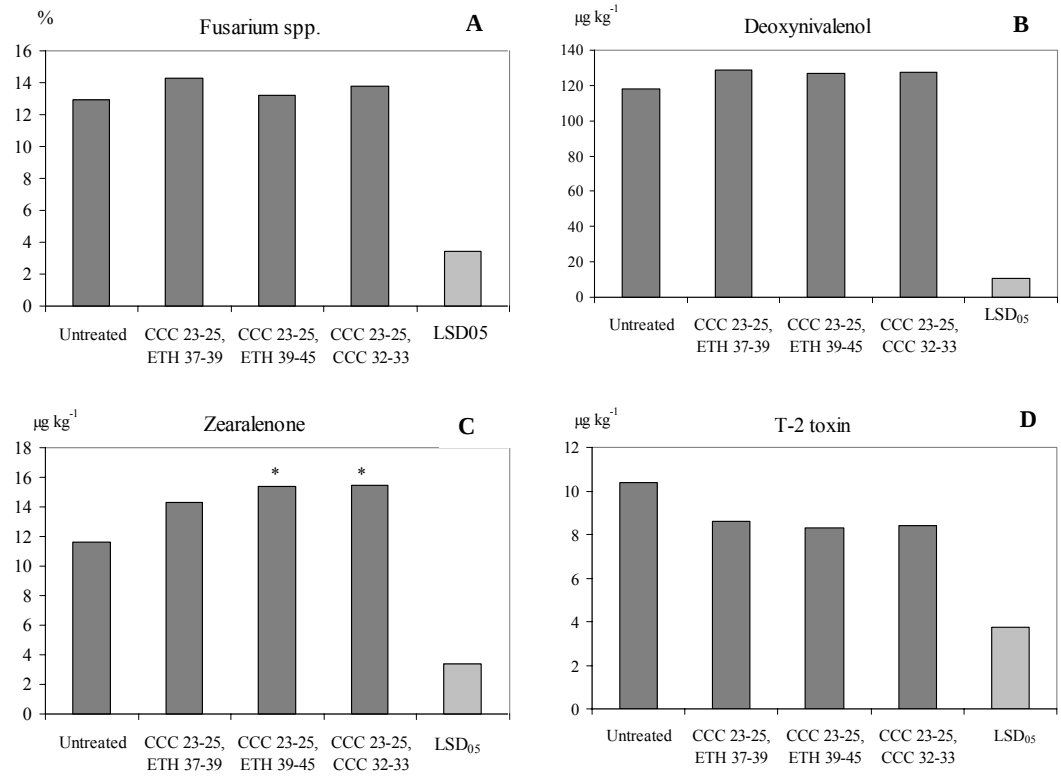
Table 2. The weather conditions during the 2004–2007 period

Year / Parameter	April	May	June	July	August
2004					
Average temperature ºC	7.6	11.2	14.2	16.8	18.1
Average temp. 1924–2004º C	5.7	12.2	15.6	17.6	16.6
± deviation over previous period	+1.9	−1.0	−1.4	−0.8	+1.5
Average rainfall in mm	11.1	27.8	44.2	81.6	94.5
Average rainfall 1924–2004 mm	37.8	52.0	62.1	73.8	73.4
% from average previous period	29.4	53.5	71.2	110.6	128.7
Days with rainfall ≥ 1mm	2	6	13	9	13
2005					
Average temperature ºC	7.6	12.4	15.3	19.3	16.8
Average temp. 1924–2005º C	5.7	12.2	15.4	17.6	16.6
± deviation over previous period	+1.9	+0.2	−0.1	+1.7	+0.2
Average rainfall in mm	23.9	46.1	50.3	46.3	75.5
Average rainfall 1924–2005, mm	37.7	52.1	61.9	73.8	73.4
% from average previous period	63.4	88.5	81.3	62.7	102.9
Days with rainfall ≥ 1mm	4	8	12	8	12
2006					
Average temperature ºC	6.7	12.6	16.8	21.3	18.1
Average temp. 1924–2006º C	5.7	12.2	15.6	17.6	16.6
± deviation over previous period	+1.0	+0.4	+1.2	+3.7	+1.5
Average rainfall in mm	19.2	45.0	6.8	40.4	105.0
Average rainfall 1924–2006, mm	37.4	52.0	61.2	73.0	73.8
% from average previous period	51.34	86.54	11.11	55.34	142.28
Days with rainfall ≥ 1mm	7	8	4	7	16
2007					
Average temperature ºC	6.9	13.5	17.6	17.2	18.7
Average temp. 1924–2007º C	5.7	12.2	15.6	17.6	16.6
± deviation over previous period	+0.4	+1.3	+2.0	−0.4	+2.1
Average rainfall in mm	15.8	98.2	61.5	118.1	50.8
Average rainfall 1924–2007, mm	37.2	52.5	61.3	73.6	73.6
% from average previous period	42.5	187.1	100.3	160.5	69.0
Days with rainfall ≥ 1mm	4	12	9	16	11

The incidence of *Fusarium* fungi during the 2004–2007 experimental period varied within 12.9–14.3% range (Figure 1. A), however, a trend was revealed suggesting that the content of these fungi was higher in the treatments applied with PGR. Under the action of PGR, the plants become shorter, the content of chlorophyll increases, the plant growing period becomes longer and this is a perfect medium for the spread of *Fusarium* and other fungi /Broschewitz et al., 2000; Bartel, 2001/. The highest *Fusarium* contamination level 14.3% was identified for the grain from the plots sprayed with CCC

BBCH 23–25 and ETH at the BBCH 37–39, whereas the contamination level for untreated plots was 12.9%. Similar trends were revealed having done analyses of DON – mycotoxin of trichothecene group. In the grain of the same treatment higher concentrations of DON amounting to on average 128.5µg kg^{-1}, were identified (Figure 1. B). In the grain from untreated plots the content of DON was on average 118.3 µg kg^{-1}. Higher concentrations of this mycotoxin compared with the grain from untreated plots were identified also in the grain of other treatments sprayed with CCC BBCH 23-25 and ETH BBCH 39–45 (126.6µg kg^{-1}) and CCC BBCH 23–25, and CCC BBCH 32–33 (127.6 µg kg^{-1}). Lower ZEN concentrations were identified also in the grain from the plots not sprayed with PGR (on average 11.6 µg kg^{-1}), however they were significantly higher (15.4 and 15.5 µg kg^{-1}) in the grain from the plots treated with CCC BBCH 23–25 and ETH BBCH 39–45 and CCC BBCH 23–25, and CCC BBCH 32–33 (Figure 1. C).

Contrary to DON and ZEN, higher T-2 toxin contents (10.4 µg kg^{-1}) were found in the grain from the plots not sprayed with PGR (Figure 1. D). The content of T-2 toxin in the grain from sprayed plots was very similar (8.3–8.6 µg kg^{-1}).



*Significant difference at 95% probability level from untreated

Figure 1. The variation of the content of *Fusarium* spp. fungi (A), deoxynivalenol (B), zearalenone (C) and T-2 toxin (D) in freshly harvested winter wheat grain as affected by PGR applied at different growth stages over the period of 2004–2007

Literature sources indicate that the content of mycotoxins produced by *Fusarium* fungi in most cases directly depends on the content of *Fusarium* fungi in grain /Schachermayr, Fried, 2000; Schollenberger et al., 2002; Xu, 2003; Mankevičienė et al., 2007/ but the contamination level is most often determined by *Fusarium* species diversity /Schachermayr, Fried, 2000; Schollenberger et al., 2002; Desjardins, 2006/. Species analysis of *Fusarium* spp. fungi revealed the trends that the highest species diversity was in the grain from the plots not sprayed with PGR (Figure 2. A). PGR might have had some effect on the development of *Fusarium* fungi and on their morphological changes /Bilai, 1977/. The content of *Fusarium* fungi in the grain from the treated plots increased and they were difficult to identify since they did not form micro and macro conidia, chlamydospores etc. (Figure 2. B, C, D) As a result, there might have been DON and ZEN producers (*F. graminearum, F. equiseti* or others) between the unidentified *Fusarium* species, since the concentrations of these toxins in the grain from the PGR – sprayed plots were higher compared with the grain from unsprayed plots. *F. culmorum* has been found to actively assimilate pesticides and sensitively respond to the changing substrate composition /Lugauskas et al., 2002/. A greater diversity of T-2 toxin producers (*F. poae, F. sporotrichioides, F. tricinctum, F. langsethiae*) was identified in the grain from unsprayed plots (Figure 2. A). This might have determined higher concentrations of this toxin.

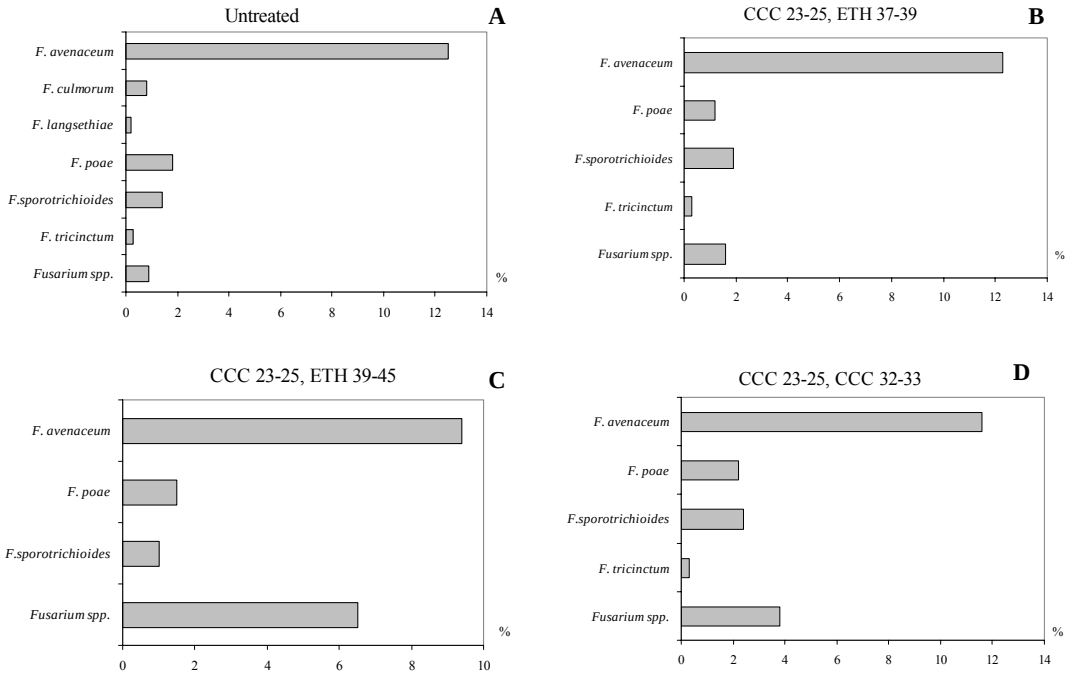


Figure 2. The variation of *Fusarium* species composition in winter wheat grain as influenced by PGR application at different growth stages during the 2004–2007 period

Conclusions

1. The application of plant growth regulators chlormequatchloride and ethephone for winter wheat protection against lodging during the period 2004–2007 revealed the trend of *Fusarium* spp. fungi increasing.

A greater *Fusarium* species diversity was identified in the grain from unsprayed plots where *F. poae*, *F. sporotrichioides*, *F. tricinctum*, *F. langsethiae* – potential T-2 toxin producers prevailed. This might have determined higher concentrations of this toxin in the grain from unsprayed plots.

2. During the 2004–2007 experimental period, the grain from the plots sprayed with chlormequatchloride at BBCH 23–25 and ethephone at BBCH 39–45 and with chlormequatchloride at BBCH 23–25 and chlormequatchloride at BBCH 32–33 had the concentrations of the mycotoxin zearalenone by on average 32–33% higher compared with the grain from the unsprayed plots. Similar trends were revealed having done analyses of deoxynivalenol. The trends of deoxynivalenol and zearalenone concentrations increasing in grain as affected by the use of PGR have raised new tasks for further research.

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