Agronomy Research **4**(Special issue), 397–401, 2006

The effects of growth regulators on spring barley (*Hordeum vulgare L.*) morphological indicators and grain contamination with fungi and mycotoxins

S. Supronienė, O. Auškalnienė, Z. Dabkevičius and A. Mankevičienė

Lithuanian Institute of Agriculture, Instituto str. 1, LT–58344 Akademija, Kedainiu distr., Lithuania; e-mail: skaidre@lzi.lt, ona@lzi.lt,

Abstract. The effects of growth regulators Modus 250 EC (trinexapac–ethyl 250 g l^{-1}) and Cerone 480 (etefon 480 g l^{-1}) on the morphological characteristics of the spring barley varieties *Henni* and *Luokė* and on grain contamination with fungi and mycotoxins were studied at the Lithuanian Institute of Agriculture in 2004 and 2005.

Spring barley was sprayed with the retardant Modus at growth stages 32–33 BBCH and with the retardant Cerone at - 39–43 BBCH. Plant morphological measurements were made before harvesting. Percent of internal grain contamination with fungi was determined. The concentrations of the mycotoxins deoxynivalenol (DON), T-2 toxin and zearalenone (ZEN) in grain were measured by ELISA method, using Neogen diagnostic mycotoxin determination tests.

The retardants reduced plant height from 66.0 to 58.0 cm, length of the last node from 19.4 to 17.9 cm for the variety *Henni*, and from 74.1 to 63.8 cm, and from 22.4 to 16.9 cm, respectively, for the variety *Luokė* compared with the unsprayed plants.

The content of *Fusarium*-affected grain in the retardant-sprayed and not applied treatments was similar, however, *Fusarium avenaceum* (Fr.) Sacc was more prevalent in the barley sprayed with retardants. *F. avenaceum, F. sporotrichioides* Sherb*., F. poae* (Peck) Wollenw*, F. tricinctum* (Corda) Sacc*., F. culmorum* (W. G. Smith) Sacc. dominated in the grain samples in 2005.

The contents of DON, ZEN and T-2 toxin determined were low, however, higher contents were identified for barley sprayed with the retardants.

Key words: barley, plant growth regulators, fungi, mycotoxins

INTRODUCTION

Plant growth regulators (PGRs) are commonly used in high input cereal management to shorten stems and thereby to increase lodging resistance. Reduction in plant height as a consequence of PGR treatment is associated with the reduced elongation of internodes (Rajala & Peltonen – Sainio, 2000).

However, it is likely that shortened stems and heads of cereals are more readily contaminated with fungal diseases (Bartel, 2001).

Research done in Lithuania showed that freshly thrashed cereal grain was contaminated with mould fungi of *Alternaria, Fusarium*, *Cladosporium, Bipolaris, Aspergillus, Penicillium* species (Lugauskas et al., 2004; Dabkevičius et al., 2005).

Most of them produce mycotoxins such as trichotecenes, zearalenone, ochratoxins, aflatoxins and others (Škrinjar & Kocic–Tanackov, 2004; Schollenberger et al., 2006).

Fusarium species are the most important group of mycotoxigenic moulds. They are generally encountered as contaminants of cereal grains and produce trichotecenes: deoxynivalenol (DON), nivalenol (NIV), T-2 and others. The main *Fusarium* genera that produce mycotoxins are *F. graminearum, F. pseudograminearum, F. culmorum, F. poae, F. equiseti, F. acuminatum, F. sporotrichioides*, (Surai & Mezes, 2005).

The production of mycotoxins in grain is often influenced by moisture, temperature and other factors and mycotoxin levels can vary significantly from field to field (Rankin & Grau, 2001).

The aim of our study was to evaluate the impact of stem shortening by PGR on the spring barley grain contamination with mould fungi and mycotoxins.

MATERIAL AND METHODS

Field experiments. The two varieties of spring barley *Henni* and *Luokė* were investigated in high input cereal management at the Lithuanian Institute of Agriculture during the years 2004 and 2005. The crop stands were sprayed with trinexapac – ethyl (Modus) and ethephon (Cerone) at the recommended times. Plant growth stages were recorded according to BBCH scale (Meier, 1997). Modus was applied at BBCH 32–33 DK, and Cerone at 39–43 DK.

Height and length of the last node of 80 stems per treatment were measured before harvesting. Spring barley grain samples were taken at harvesting.

Laboratory experiments. Internal grain contamination with fungi was estimated as follows: spring barley grain was surface sterilised for 5 minutes in 1% NaOCl solution, rinsed three times in sterile distilled water and dried before plating. The sterilized material was plated on Petri dishes with potato dextrose agar (PDA), incubated for 7–8 days at 26±2°C (ISTA, 2003) and identified according to the manual of Mathur & Kongsdal, (2003). The infection level of barley grain was evaluated in percent (0 – all grain healthy, 100% - all grain infected). The overgrown *Fusarium* colonies were isolated, purified and identified according to the manual of Nelson et al., (1983) by visual and microscopic observation of single spore cultures.

The level of mycotoxins DON, T-2 toxin and ZEN was determined by the ELISA method. Neogen diagnostic tests were used for the estimation of mycotoxins. Multiskan MS was used for reading of immunoenzymic microstrips.

ANOVA was applied for the statistical processing of data. For data significance the Fisher test was used. Averages for the other data were calculated.

RESULTS AND DISCUSSION

The height of the spraed spring barley plants decreased from 66.0 to 58.0 cm for the variety *Henni*, and from 74.1 to 63.8 cm for the variety *Luokė*, and the length of the last node from 19.4 to 17.9 cm, and from 22.4 to 16.9 cm, compared with the unsprayed plants (Table 1).

Table 1. The influence of PGR on the biometrical indicators of spring barley. Averaged data from 2004, 2005.

Treatment dose, l ha^{-1}	Growth stage BBCH at treatment	Henni		Luokė	
		Plant height, cm	Length of the last node, cm	Plant height, cm	Length of the last node, cm
Untreated		66.0	19.4	74.1	22.4
Modus 0.4	32-33	64.1	17.9	69.4	19.8
Modus 0.2 & Cerone 0.5	32-33 39-43	58.0	16.4	63.8	16.9
LSD_{05}		**1.64**	**0.96**	**3.80**	**2.17**

Table 2. The internal contamination (%) of spring barley grain with prevalent fungus.

Fungi	*Henni*				*Luokė*			
	U	M	M C	LSD_{05}	U	M	M C	LSD_{05}
				2004				
Fusarium spp.	39.8	43.0	43.5	*14.48*	40.3	38.5	49.3	*11.17*
Alternaria spp.	71.8	86.5*	91.8*	*14.49*	88.3	91.5	94.5	*7.43*
				2005				
Fusarium spp.	32.5	38.3	31.8	*15.59*	25.0	22.5	22.5	*10.81*
Alternaria spp.	100.0	100.0	100.0	-	99.3	100.0	100.0	*1.50*

U – Untreated, M - Modus 0.4 (BBCH 32–33), M C - Modus 0.2 (BBCH 32–33) and Cerone 0.5 (BBCH 39–43), *- Difference significant at P = 0.05.

Table 3. *Fusarium* spp. (%) composition in the grain of spring barley in 2005.

Fusarium spp.	*Henni*			*Luokė*		
	U	M	M C	U	M	M C
F. avenaceum	25.6	32.6	34.2	36.7	44.4	70.4
F. sporotrichioides	25.6	8.7	34.2	36.7	33.3	14.8
F. tricinctum	7.7	8.7	2.6	10.0	14.8	0.0
F. poae	35.9	34.8	15.8	6.7	7.4	0.0
F. culmorum	0.0	0.0	5.3	10.0	0.0	0.0
Other *Fusarium*	5.1	15.2	7.9	0.0	0.0	14.8

U – Untreated, M - Modus 0.4 (BBCH 32–33), M C - Modus 0.2 (BBCH 32–33) and Cerone 0.5 (BBCH 39–43)

Table 4. The amount in (μg kg^{-1}) of mycotoxins in the grain of spring barley.

Mycotoxins	*Henni*			*Luokė*		
	Not sprayed	Modus 0.4	Modus 0.2 Cerone 0.5	Not sprayed	Modus 0.4	Modus 0.2 Cerone 0.5
			2004			
DON	168.5	142.5	158.0	81.0	85.0	89.0
ZEN	22.2	7.15	19.8	14.0	54.4	72.7
T–2 toxin	14.8	14.0	22.3	0	1.9	1.7
			2005			
DON	69.0	73.5	97.5	79.5	95.5	101.0
ZEN	31.0	1.9	1.6	1.4	0	2.0
T–2 toxin	10.5	14.7	12.4	8.5	9.7	10.9

The internal grain contamination of spring barley with fungi differed between years. In 2004 the grain was more contaminated than in 2005 irrespective of the variety and the use of PGR (Table 2). The grain of the treated stands was more contaminated with *Fusarium spp*., but significant differences were not identified. In 2005 the differences between treatments were also not significant, but there were differences between *Fusarium* species composition.

Spring barley grain contaminated with *Alternaria* spp. fungus in both varieties of spring barley was recorded in both experimental years. Higher content of contaminated grain in 2004 was found in the treated plots (Table 2), especially in the plots treated with PGR twice, *Henni* 91.8 and *Luoke* 94.5%, respectively, while in 2005 all of the tested grains were 100% contaminated with *Alternaria* spp.

The content of *Fusarium*–contaminated grain in the retardant-applied and not applied treatments was similar, however, *Fusarium avenaceum* (Fr.) Sacc was more prevalent in the barley sprayed with retardants (Table 3).

The spring barley grain was contaminated with mycotoxins DON ZEN, T-2 in both experimental years. The amount of mycotoxins identified was not significant, but a higher concentration of DON (168.5 μg kg^{-1}) and ZEN (22.2 μg kg^{-1}) was found in the grain from the spring barley stands not applied with PGR. (Table 4).

In 2004 a higher content of T-2 toxins (22.3 μg kg^{-1}) was identified in the grain of the spring barley variety *Henni* in the plots treated with PGR twice. The grain of the spring barley variety *Luoke* was more contaminated with ZEN. A higher content of ZEN (72.7 μg kg^{-1}) was found in the plots treated twice with PGR. The grain samples of the variety *Luoke* from the treated plots contained traces of T-2, while in the grain from the untreated plots T-2 was not identified.

In 2005 the grain of the spring barley variety *Henni* was less contaminated with DON, however higher concentrations (97.5 μg kg^{-1}) were found in the grain from the treated spring barley stands. The same trend was observed in the grain from the variety *Luoke*. The grain of the spring barley variety *Henni* from the plots untreated with PGR was more contaminated with ZEN (31.0 μg kg^{-1}). The content of T-2 in both varieties of spring barley in 2005 was similar, however a higher content was observed in the grain from the PGR- treated plots.

CONCLUSIONS

The length of spring barley stems and last node was shortened by 3–8 cm in the plots sprayed with plant growth regulators.

Spring barley grain from the treated plots was more contaminated with the fungi of *Alternaria* species. The PGR used did not affect the occurrence of *Fusarium* spp. fungi, however, species composition of *Fusarium* changed: *Fusarium avenaceum* (Fr.) Sacc was more prevalent in the barley sprayed with retardants. The amount of mycotoxins deoxynivalenol, T-2 toxin and zearalenone was not significant and PRG had an inappreciable influence on the occurrence of mycotoxins.

REFERENCES

Bartel, M. 2001. Mischungen verbessern die Wirkung. *Top Agrar* **1,** 86–91.

Dabkevičius, Z., Sinkevičienė, J., Semaškienė, R. and Šaluchaitė, A. 2005. Fungal infection of the Lithuania–grown spring barley seed. Z*emdirbyste* **92** (4), 106–19.

International Rules for Seed Testing (ISTA). Annexe to Chapter 7. Seed Health Testing Methods. – Bassersdorf, Switzerland, Bassersdorf, 2003.

Lugauskas, A., Krasauskas, A. & Repeckiene, J. 2004. Ekologiniai veiksniai, lemiantys mikromicetu paplitima ant javu grudu ir soju seklu. *Ekologija* **2,** 21–32.

Mathur, S.B. & Kongsdal, O. 2003 *Common laboratory seed health testing methods for detecting fungi*. Denmark, Copenhagen, pp. 102–137, 176–177, 234–255.

Meier, U. 1997. Growth Stages of Mono- and Dicotyledonous Plans. BBCH – Monograph. Berlin – Wien.

Nelson, P.E., Tousson, T.A. & Marasas, W.F.O. 1983. *Fusarium Species: an Illustrated Manual for Identification.* The Pennsylvania State University Press, University Park and London, 193 pp.

Rajala, A. & Peltonen – Sainio, P. 2000. Manipulating yield potential in cereals by plant growth regulators. In Binghamton, A.S. (ed.): *Growth Regulators in Crop Production*. Basra, New York, pp.27–70

Rankin, M. & Grau C. 2001. Agronomic Considerations for Moulds and Mycotoxins in Corn Silage and High Moisture Corn. http://www.uwex.edu/ces/crops/MouldsMycotoxins.htm.

Schollenberger, M., Muller H. M., Rufle, M., Suchy, S., Plank, S. & Drochner, W. 2006. Natural occurrence of 16 *Fusarium* toxins in grains and feedstuffs of plant origin from Germany. *Mycopathologia* **161**, 43–52.

Škrinjar, M. & Kocic-Tanackov, S. 2004. Fungal infection and occurrence of zearalenone in barley harvested in 2003 in Serbia. *Acta agriculturae slovenica* **1,** 233–238.

Surai, P.F. & Mezes, M. 2005. Mycotoxins and immunity: theoretical consideration and practical applications. *Praxis veterinaria* **53**(1–2), 71–88.