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ARE RUMINANTS REALLY LESS SENSITIVE TO THE MYCOTOXIN DEOXYNIVALENOL (DON)? WHAT THRESHOLDS ARE REASONABLE?

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Abstract: Mycotoxins (secondary metabolites of microscopic fungi) can contaminate animal feeds, potentially resulting in decreased animal production as well as veterinary and human health problems.
This article gives information about the toxicological aspects of deoxynivalenol (DON) in farm animals, specifically ruminants. A summary of current laws and regulations regarding tolerable DON content in animal feed in the EU and Hungary is given. The latest research with ruminants suggests a need for stricter thresholds for DON content.

Keywords: mycotoxin, deoxynivalenol, ruminant

Introduction

Approximately 25% of the world's crops are affected by mycotoxins annually (Council for Agriculture Science and Technology, 1989). Deoxynivalenol (DON) is produced by the species of *Fusarium* (Hudec and Rohačik, 2009). It has been long known to cause severe toxicosis in humans and farm animals following ingestion of mould-contaminated cereal grains. DON occurs predominantly in grains such as wheat, barley, and maize and sometimes in oats, rice, rye, sorghum and triticale (Creppy, 2002). DON is a very stable compound, during both storage and the processing/cooking of food, and does not degrade at high temperatures. Frequent contamination of grain crops, combined with resistance to processing, enables DON to persist in the human and animal food sources (Rotter et al, 1996). The purpose of this review is to summarize information about the toxicological aspects of DON in farm animals, especially in ruminants and to give a summary of current laws and regulations regarding tolerable DON content in animal feed in the EU and Hungary.

Toxic effects

DON, a type B trichothecene has various toxic effects. Cytotoxicity of trichothecenes has been attributed to their potent inhibition of protein, RNA- and DNA-synthesis. Other toxic effects of trichothecenes include disruption of membrane transport and function, suppression of the immune response, and abnormal blood function. Proliferation of human lymphocytes in cultures was shown inhibited by DON. In an in vitro study DON was shown to inhibit the phagocytic activity, microbicidal activity, and superoxide anion production. The actions of trichothecenes at the hematological level have been illustrated in several studies (Hussein and Brasel, 2001).
Deoxynivalenol is considered the most common trichothecene but also one of the least toxic. (Newmann and Raymond, 2005). Despite this, sensitivity to DON can vary

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greatly between different species of animals. Poultry were published the least sensitive in general, while swine were found the most sensitive among mammals. In comparison to monogastric species, ruminants are generally considered less susceptible to the adverse effects caused by contamination of feeds with mycotoxins. This is based on the assumption that the rumen microflora degrades and deactivates mycotoxins. A number of mycotoxins, however, resist rumen degradation, causing distinct clinical signs of intoxication (Fink-Gremmels, 2008).

In swine within the trichothecene group, deoxynivalenol is associated with emesis, feed refusal and depressed feed intake in pigs reduced total protein, albumin, Ca and P levels in blood (D'Mello et al, 1999). This effect of DON in swine has led to DON commonly being called vomitoxin when used in the context of swine.

Broiler chicken is less sensitive to DON. In chickens, DON can cause immunosuppression, increase in relative weights of gizzard, bursa of Fabricius and the heart. In laying hens the transmission to eggs following oral administration was shown (D'Mello et al., 1999).

Although horses are less sensitive to DON contaminated feed, the long term exposure can cause weight loss (Newman and Raymond, 2005).

In humans, symptoms include nausea, vomiting, gastrointestinal upset, dizziness, diarrhoea and headache (Hussein and Brasel, 2001).

In trials with dairy cattle no negative effects on animal health were found with the following doses: 66 mg DON kg^{-1} dry matter (DM) feed over 5 days (Cote et al., 1986), 6,4 mg kg^{-1} DON kg /dietary DM (Tenholm et al., 1985), 5,2 and 8,1 mg DON 100 kg^{-1} bodyweight (Dänicke et al., 2005) or 21 mg DON kg^{-1} DM in the feed (Dicostanzo et al., 1995). Contrary to this, Schuh found that doses of 5 mg DON kg^{-1} DM in the diet caused reduced feed intake, uneven hair coat and lower meat quality in the cattle (Schuh, 1996). Keese et al. (2008) showed that milk production was greater in cases of higher DON contamination in feed than if lower DON contamination was applied in the diet. According to Dänicke et al. (2005) in concentrations of 3,1 and 3,5 mg DON kg^{-1} daily ration (88% DM) did not cause lower feed intake or milk production (Alkaasem, 2009).

In the study of Korosteleva et al. (2007) a total mix ration containing a blend of feedstuffs naturally contaminated with Fusarium mycotoxins (with DON as major contaminant up to 3,6 μg g^{-1} dry matter) was fed for 56 days to 18 mid-lactation Holstein cows. Total serum protein and globulin levels increased significantly on day 42 of the experiment. Serum urea concentrations were significantly elevated throughout the experiment in cows fed the contaminated diet.

Seeling et al. (2006) examined the effect of DON and possible interactions between DM intake and feeding Fusarium toxin-contaminated wheat and the effect of the toxin on ruminal fermentation (8,21 DON mg kg^{-1} DM). An increased amount of crude protein degradation and a lower molar percentage of propionate in the rumen fluid were observed when feeding the Fusarium toxin-contaminated wheat at an increased OM intake in comparison with the control diet containing no DON contaminated wheat.

Exposing sheep to DON (15.6 mg kg^{-1} of feed) for 28 days had no effects on average daily gain, hemacytology parameters, or liver function (Harvey et al., 1986).

Defence against mycotoxins

Since mycotoxins have a wide range of toxic effects on both animal and human consumers, prevention of the contamination is the most efficient defence against them. Important components of prevention include good agrotechnical methods, breeding of resistant crops, and reasonable storage of crops after harvest.
A further component of defence against mycotoxins is low regulation. Despite the severe toxic effects of Fusarium mycotoxins, statutory regulations do not exist for these or any of the other Fusarium mycotoxins. In contrast, stringent directives are in place for the Aspergillus-derived aflatoxins (1999/29/EC, 2002/32/EC). However, a selection of advisory and tolerance limits for the Fusarium mycotoxins are available (D'Mello, 1999; Weber, 2008).
The European Union recently issued recommended maximum levels for the presence of DON in animal feeds (Table 1: Part of Commission Recommendation 576/2006/EC). The Committee on Veterinary Science of the Hungarian Academy of Sciences issued recommendations regarding depressive and toxic levels in animal feed for certain prominent mycotoxins (2003). Table 2 shows the values pertaining to DON for ruminants.

Table 1. Limits of DON present in ruminant feed in 576/2006/EC Commission Recommendation

Items	Guidance value in mg kg^{-1}
Cereals and cereal products	8
Maize by products	12
Complementary and complete feedstuffs with the exception of	5
- complementary and complete feedstuffs for pigs	0,9
- complementary and complete feedstuffs for calves, lambs and kids	2

The indicated values are provided as guidance only, since in the absence of sufficient data the potential interaction between different toxins and toxicological synergy of different mycotoxins is not well understood (Weber , 2008).

Table 2. Part of the Recommendation of the HAS's* Committee on Veterinary Science (2003)

DON in feed (mg kg^{-1})	Depressive	Toxic
Cattle	5,0	-
Calf	0,2	-
Swine	0,4	1
Chicken	2	-
Goose, Duck, Turkey	0,5	-

*Hungarian Academy of Sciences

The utilization of feeds already contaminated with mycotoxins is often a topic of debate. The use of various absorbent additives makes the use of contaminated feeds possible, although this process is not effective for all mycotoxins. In the past, it was common practice to use the contaminated feeds with less sensitive animals (e.g. ruminates). Recent examinations and our own results suggest that despite ability of these animals to degrade mycotoxins in the rumen, these toxins can be detected in the

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animal products.Numerous calculations exist to determine safe thresholds for mycotoxins. According to D'Mello (1999), estimates of advisory or tolerance limits for DON in cattle are 10mg/kg grain, in dairy cow 15mg/kg barley or 31mg kg^{-1} body weight. On the other hand, numerous research results show that DON is largely metabolised (Seeling, 2005). Our own, current study with ewes shows that DON can be detected even in the milk of dairy sheep fed a diet containing as low as 2-4 mg kg^{-1} DON. The research of Seeling (2006) with cattle showed that feeding a dietary level of 8.21mg DM kg^{-1} DON, and de-expoxy DON resulted detectable concentrations of this toxin in the milk. These results published recently seem to suggest the need for the introduction of a stricter regulation of mycotoxin content in animal feeds.

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COMBINING ABILITY OF IN VITRO DOUBLED HAPLOID MAIZE PARENTAL LINES

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Abstract: From the breeding point of view, the development of genotypes with better adaptability to environmental conditions and soil properties, what makes the resilience of the agro-ecosystem more effective, is the main task. Irrespective of whether the plants are developed using conventional or biotechnological methods, it is these traits that determine whether the new genotype will prove satisfactory in general cultivation. This is also true of maize hybrids involving doubled haploid (DH) parental components. In recent years an efficient tissue culture system has been developed for maize in our department, allowing a large number of DH lines to be developed. The aim of the present work was to analyze the combining ability of the DH maize lines developed using this system in order to clarify the role these lines could play in field maize production. Field performances of maize hybrids with DH background were examined in Martonvásár, over three years. The performance of fifty-two hybrid combinations was compared with that of two standard maize hybrids with commercial value. Compared with the grand mean of the experiment, the majority of hybrids performed well below the level of the standard mean, though it was possible to find a DH line that resulted in hybrids capable of producing yields equivalent to that of the standards and a combination whose agronomic value and yield potential were as good as the standard mean. The importance of these results is that the DH lines were developed within an *in vitro* plant regeneration system and were tested in performance trials after long years of selection. Up till now only DH lines developed *in vivo* have been introduced into cultivation.

Keywords: adaptability, doubled haploid, maize, field performance

Introduction

The efficiency of anther and tissue cultures in most cereals such as maize has reached the stage where it can be used in breeding programmes to some extent and many new cultivars produced using this system have now reached the market (Pepo, 2008). The use of DH lines improves the efficiency of line development, while reducing the labour requirements involved in line maintenance (Röber et al., 2005). The *in vitro* androgenic response could be successfully transferred into hybrids from a single cross between doubled haploid lines and genotypes with high commercial value (Barnabás, 2002)

The efficient use of doubled haploids requires the optimization of the entire breeding strategy in order to maximize progress from selection (Longin, 2006). As there is no need to make a separate evaluation of sublines, considerable savings can be achieved by utilising DH line development. Not only can the size of the nursery be reduced, but smaller seed lots have to be exchanged between the main programme and the secondary winter generation, and the time required to develop commercial hybrids can be reduced by several years.

DH lines can be successfully incorporated in the breeding of commercial hybrids. Breeding based on *in vivo* or *in vitro* DH line development consists of the following basic steps: (i) crossing selected lines to produce new combinations, (ii) haploid induction from the F_1 generation, (iii) chromosome doubling, (iv) planting out and self-pollination of DH plants, (v) evaluation of progeny lines in single-row observation plots, and simultaneously (vi) initiation of a seed multiplication programme, the evaluation of test crosses in multi-location performance trials and the development of experimental hybrids. This breeding cycle takes approximately eight years, but can be reduced to half with the help of a winter nursery (Röber et al., 2005).

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In addition, the best DH lines can be used in further combinations, forming the basis for new cycles of recurrent selection. This requires a thorough knowledge of the combining ability of the DH lines, which can be estimated by joint selection and combining ability analysis.

In recent years an efficient *in vitro* tissue (anther) culture system has been developed for maize in our institute's Cell Biology Department, allowing a large number of DH lines to be developed each year. The aim of the present work was to analyse the combining ability of the DH maize lines developed using this system.

Materials and methods

DH maize lines were used as parental components in crossing experiments carried out in three consecutive years (2005-2007). The DH lines were developed from combinations of exotic Chinese lines with good haploid induction ability and Martonvásár inbred lines, including MvLine, used to produce commercial hybrids. The latter were chiefly of Iodent and Mindszentpusztai Yellow Dent origin. The *in vitro* DH maize lines were developed in such a way that they contained varying proportions of the commercial lines (in F_1 and BC_1 combinations).

The testers were 'sister line crosses' (SLC) of Iodent (IodSLC), Lancaster (LancSLC) and Iowa Stiff Stalk Synthetic (ISSS SLC) origin, together with a fourth SLC tester unrelated to any of the above (NRSLC). FAO 390 and FAO 450 standards were used in the experiments, which were laid out in a random block design with three replications in the nursery of the Agricultural Research Institute of the Hungarian Academy of Sciences. Yields ($t \cdot ha^{-1}$) were converted to 15% grain moisture content. Statistical evaluation of data was performed according to Sváb (1981).

Results and discussion

Statistical analysis of the three-year data series with three replications at a single location, including the effect of genotype and year (data not shown), is summarized in Table 1. The grain yields, converted to 15% grain moisture content, are given in Table 1/A and the actual grain moisture content of each combination at harvest is presented in Table 1/B. The four genotypes at the heads of the columns are the Martonvásár testers used as male components in the crosses, while the rows are the 12 DH lines used as female parent, together with the commercial Martonvásár line used as the control. The last column contains the general combining ability (GCA) of the lines, while the last row gives GCA values for each tester. The intersections of the rows and columns represent the yield or moisture content obtained for each hybrid combination. As the Martonvásár testers and the original 'non responsive' Martonvásár line have real commercial value, the various combinations can also be considered as top-crosses. Four out of the 12 DH lines exhibited improved general combining ability for yield (as compared to the grand mean) but none of them was able to compete with the control Martonvásár line (*Table 1/A*). Combinations developed using the non-related tester (NRSLC) exhibited the greatest heterosis, which was significantly greater than the means of the other male testers. Half the combinations (i.e. 23 hybrids) had yields above the experimental mean, but with two exceptions they did not approach the yield of combinations developed using the control Martonvásár line. The grain moisture at harvest averaged around 20% (Table 1B).

Table 1 General combining ability of twelve DH and one commercial Martonvásár (Mv) line in maize hybrids for yield and moisture content

Table 1/A. Yield (t ha^{-1})

Genotypes	Iod SLC	Lanc SLC	ISSS SLC	NR SLC	GCA
DH 109	9.32	7.94	7.57	8.83	-0.53
DH 384	8.79	7.97	7.80	9.21	-0.51
DH 136	9.16	9.16	9.69	9.30	**0.38**
DH 143	8.59	8.96	8.82	9.82	**0.10**
DH 31	9.38	8.80	8.82	7.97	-0.21
DH 141	9.03	9.69	7.31	9.47	-0.07
DH 105	8.69	8.25	8.34	9.68	-0.21
DH 57	8.62	9.84	9.27	9.26	**0.30**
DH 64	7.57	8.05	9.81	9.61	-0.19
DH 56	8.50	8.10	*10.21*	*10.62*	**0.41**
DH 53	9.11	7.83	9.16	9.58	-0.03
DH 63	7.18	7.40	6.91	8.01	-1.58
MvLine	9.99	*10.71*	9.48	*11.42*	**1.45**
GCA	-0.19	-0.28	-0.24	**0.49**	

Table 1/B. Moisture content at harvest (%)

Genotypes	Iod SLC	Lanc SLC	ISSS SLC	NR SLC	GCA
DH 109	19.21	22.94	22.75	23.86	1.83
DH 384	*18.56*	22.77	*19.78*	20.11	**-0.05**
DH 136	18.85	21.02	22.14	22.79	0.84
DH 143	19.57	20.32	21.03	21.92	0.35
DH 31	20.61	24.23	21.51	23.83	2.19
DH 141	18.45	19.67	21.61	20.91	**-0.20**
DH 105	18.54	22.47	19.59	20.94	0.02
DH 57	19.20	22.88	20.01	20.90	0.39
DH 64	18.28	20.05	20.24	20.67	**-0.55**
DH 56	18.06	19.89	19.39	20.23	**-0.97**
DH 53	18.75	21.76	20.20	20.41	**-0.08**
DH 63	*16.95*	*17.88*	*17.43*	*17.94*	**-2.81**
MvLine	22.14	19.60	18.56	20.14	**-0.25**
GCA	**-1.35**	0.83	**-0.03**	0.77	

Grand mean for yield: 8.95 t·ha^{-1}
$LSD_{5\%}$ = 0.56 (yield)
Standard mean: 10.66 t·ha^{-1}

Grand mean for moisture: 20.36 %
$LSD_{5\%}$ = 0.71 (moisture)
Standard mean: 19.74%

DH or tester lines with favourable general combining ability (as compared to the grand mean of both traits) are indicated in bold. Genotypes with significantly better yield or moisture content indicating superior specific combining ability are set in italic.

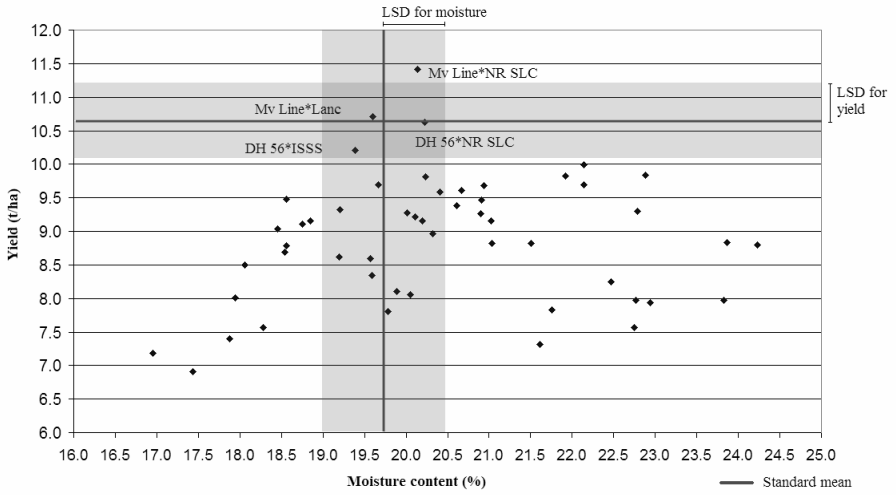

Figure 1. Combined yield and moisture content of hybrid combinations containing DH lines or the control MvLine, and of FAO 390 and FAO 450 standards (2005-2007). Horizontal and vertical lines to indicate standard means for yield and moisture, respectively.

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When the data are plotted on a yield-grain moisture diagram (Fig. 1), including those of the two standards, it can be evaluated how the hybrids of DH origin perform compared with the commercial hybrids currently cultivated. Hybrids developed using the control line had yields close to the average for the two standards (MvLine*IodSLC and MvLine*LancSLC) or better (Mv Line*NRSLC), again depending on the vegetation period. The yields of DH-derived hybrids were below the standard mean (with two exceptions), but the difference was not always significant.

Conclusions

The yields obtained in hybrids using DH lines were significantly lower than those recorded for hybrids involving the control Martonvásár line. Also, the majority of the combinations had substantially lower yields than the standard mean, though certain genotypes approached or even surpassed this level. The grain moisture contents corresponded to the maturity groups. However, although values considerably higher than that of the FAO 390 standard were found, very few hybrids also had substantially higher grain yields. Compared with the FAO 450 standard, where the grain moisture content was unfavourable due to the longer vegetation period, the experimental hybrids had lower grain yields. The combining ability analysis indicated that hybrids with DH parental components responded very diversely in test crosses, but in all the analyses genotypes with better general and specific combining ability could be clearly distinguished, despite the small sample number. In summary, in favourable cases the DH-derived hybrid combinations tested may equal, but not exceed the mean of standards. Though they require further improvement with respect to vegetation period, the expansion of the experiments to include new genetic sources could lead to the *in vitro* DH technique being suitable for use in the development of commercial hybrids.

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CORRELATION BETWEEN GLUCOSE AND FRUCTOSE FOR CHARACTERIZATION OF RELATIONSHIP BETWEEN PLANTS AND ENVIRONMENTAL CONDITIONS

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Abstract: The plant metabolism as a controlled system continuously interacts with the environment. The alterations occurring in environmental conditions can bear on the metabolic pathways and, as a consequence, on the intensity of the metabolism. By measuring the values of biochemical variables, to detect this relation between the plant and its environment is not easy in many cases. An alternative detection of the physiological state alteration can be performed on the base of linear correlation of some biochemical variables. The glucose and fructose levels in the plant leaves are regulated in a synchronized way. Their values are in linear correlation to each other. Regression straight lines of glucose-fructose correlation are sensitive to both abiotic and biotic environmental effects. By tracking the parameter alteration of the state-dependent regression, the effects of temperature on maize (*Zea mays* L.) and *Cercospora beticola* infection on sugar beet (*Beta vulgaris* L.) could be detected. Before the manifestation of morphological change, the early phase of this infection could be indicated by the parameter alteration in the state-dependent regression.

Keywords: state-dependent correlation, environmental effect, sugar beet, maize

Introduction

Plant carbohydrate metabolism is very sensitive to the changes of environmental conditions. Drought, irrigation, chilling temperature, heat shock, radiation in UV and VIS ranges, NaCl stress, air pollutants, nutrients and nitrogen supports as abiotic effects is able to induce specific alterations in the amounts of various carbohydrates. It is a well known that water deficit (Erdei et al., 2009; Németh et al., 2009a) and enhanced temperature (Sato et al., 2006) decrease the soluble carbohydrate levels, while cold effect (Plazek et al., 2003), sulphur-dioxide (Kainulainen et al., 1993), salt stress (De Costa et al., 2007) and sunlight intensity (Streb et al., 2003; Bunce and Sicher, 2003) increase them. The effects of biotic stresses are diverse. The direction and extent of their alterations are peculiar to specific relationships between pathogens and hosts. The contents of glucose and fructose are regarded as stress indicators. Similarly to the other biochemical variables (e.g. peroxidase, polyphenol oxidase) synchronic controlled in the metabolism, their values are in linear correlations with high regression coefficients (Németh et al., 2009a; Németh et al., 2009b). The concentrations of glucose and fructose in the foliage can reflect the physiological states of the plants. The variable values belonging to the same sampling time determine a state-dependent regression straight line. The alterations occurring in the physiological state can be tracked with the serial of state-dependent regressions of biochemical variables (Németh et al., 2009c). Sometimes, there can also be detected a linear relationship among the centre points of state-dependent correlations (Németh et al., 2009a; Németh et al., 2009d), which is called centre points correlation. Applied the conception of state dependent and centre points correlations to the contents of glucose and fructose measured from plant leaves, significant differences of the resistance against *Botis vinifera* infection (Németh, 2009b)

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have been revealed. Moreover, the manifestation of drought (Németh, 2009a) could also be followed with this novel idea. In the summer of the year 2009, the vegetation period of two plant species, that of the sugar beet (*Beta vulgaris* L.) and the maize (*Zea mays* L.) were characterized by state-dependent correlations of glucose and fructose. Relations were established between the state-dependent as well as the centre point regressions and the environmental condition.

Materials and methods

Extraction: the mixture of 2g of powdered plant leaf, 2g of quartz sand and 0.5 g of the pulp is extracted with 0.75 ml H_2O-MeOH solution (1:4). After 1 hour of the extraction the suspension is centrifuged (6000 rpm; 10 min). The carbohydrates of the supernatant are to be separated by thin-layer chromatography. *Stationary phase*: MERCK OPLC Silica gel F254. *Eluent*: 85 % ACN-15 % H_2O. *Equipments*: CAMAG, Linomat 5; BIONISIS OPLC 50; CAMAG TLC Scanner 3 (540 nm); MERCK TLC Sprayer; DESAGA Thermoplate S (118 ^oC, 5 min). *Separation method*: After finishing the separation the thin layer is dried (40 ^oC, 5 min) and the separation is repeated; rapid phase: 250 µl; flow rate: 350 $\mu l.min^{-1}$; eluent volume: 2 x 4500 µl. *Derivatization*: 1 g diphenyl amine, 1 ml aniline and 5 ml cc H_3PO_4 in 50ml acetone.

Results and discussion

In our correlation monitoring investigation, sugar beet and maize plantations of BETA Research Institute Ltd. (Sopronhorpács, Hungary) were sampled and glucose and fructose contents of plant leaves were determined by thin layer chromatography. During this investigation the field of the maize was only exposed to abiotic effect. From beginning of August, the sugar beet plants had got an infection of *Cercospora beticola*. Thus, their results were grouped and evaluated according to the states before and after infection, too. Belonging to the same sampling, state-dependent regression straight lines (*Figures 1a* and *2a*) were determined from the data set. The regressions obtained were compared to each other by covariance analysis (ANCOVA; StatsDirect v. 2.6.5). The slopes of these regressions can be considered as unchanged under abiotic environmental condition. The significant differences among the fitted straight lines originate from the deviation of the intercepts. Based on the novel conception of state-dependent correlation, physical meanings can be related to the parameters of regression straight lines. The slope of relationship of theoretical state-dependent correlation has been defined by the ratio of theoretical standard deviations of the variables. Its intercept depends from the standard deviations and expected values (Németh et al., 2009b).

$$(1)\quad y_2 = \frac{\sigma_2}{\sigma_1} y_1 + \frac{\sigma_1\mu_2 - \sigma_2\mu_1}{\sigma_1}$$

where: y_i – i^{th} biochemical variables, μ_i – i^{th} expected value, σ_i – i^{th} theoretical standard deviation. To predict the values of theoretical slope and intercept is possible by determining the empirical standard deviations and the means. State-dependent correlation of biochemical variables is sensitive to the environmental condition.

Table 1. Relation between carbohydrate centre points and cloudiness

Sampling	T (°C)	Cloud octa at 10 am	Centre points (*Zea may*)		Centre points (*Beta vulgaris*)		
			Glucose (μg/g)	Fructose (μg/g)	Glucose (μg/g)	Fructose (μg/g)	
14.07.2009	25.8	0.0	1051.00	1533.57	1783.20	1608.93	A
23.07.2009	26.0	0.0	1243.70	1705.95	-	-	B
27.07.2009	21.3	2.0	1411.43	1469.14	799.26	1143.54	C
30.07.2009	26.7	1.0	1648.14	1894.90	1174.98	1216.35	D
05.08.2009	20.8	5.5	893.20	1254.10	1624.74	1114.25	E
10.08.2009	22.8	4.5	616.72	824.62	616.72	824.62	F
13.08.2009	21.6	7.0	624.83	580.38	624.83	580.38	G
24.08.2009	19.1	3.0	778.33	564.49	778.33	564.49	H
27.08.2009	23.7	4.5	929.36	593.21	929.36	593.21	I

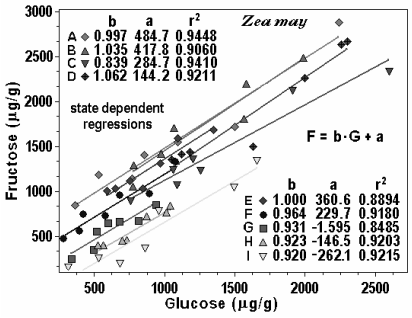

Figure 1a. State-dependent regressions of glucose (G) and fructose (F) in maize.

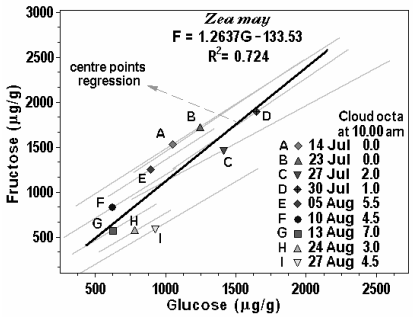

Figure 1b. Centre points correlation of glucose (G) and fructose (F) in maize.

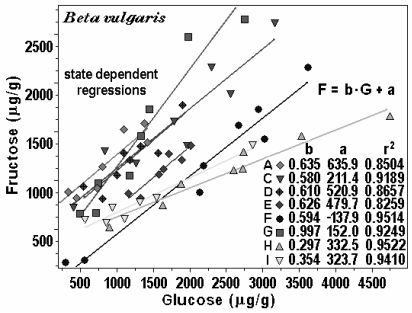

Figure 2a. State-dependent regressions of glucose (G) and fructose (F) in sugar beet.

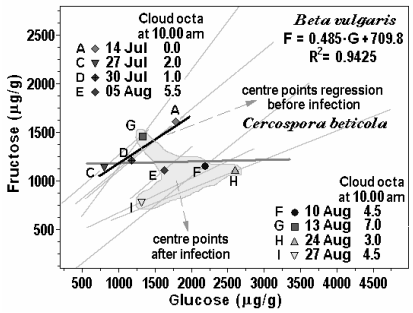

Figure 2b. Positions of centre points of glucose (G) and fructose (F) before and after infection.

Environmental factors can influence the slope and intercept of state-dependent regressions. An example of interaction between plant and its environment is present in the data shown in Figures 1b and 2b. With augment of the sunlight intensity, centre points of state-dependent regressions are shifted to higher contents of glucose and fructose. Moving of the centre point within coordinate system correlates to cloudiness of overall sky dome (cloud octa). Notice in these examples that without using the correlation approach, separately evaluations of glucose and fructose contents are not

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able to reveal the significant changes supported by correlation analysis. Applying the state-dependent correlation conception extra information can be obtained out of the data. In the case of infection by *Cercospora beticola*, both the intercept and the slope are modified. First, the slope had been increased at the beginning of biotic stress and afterwards, when the infection was spreading over the whole leaf surface, it was gradually decreased. This biotic effect annulled the centre-points correlation being typical of abiotic condition. Due to significant alteration in the slope of state-dependent regression, the presence of this biotic stress could be detected before development of the metamorphosis.

Conclusions

State-dependent correlation of glucose and fructose is resulted from their synchronized metabolic regulation. The values of its parameters are sensitive to the intensity of solar radiation and *Cercospora* infection. Application of state-dependent and centre points correlation conception can reveal extra information about the relation between plant and its environment.

Acknowledgements

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CORRELATION BETWEEN STALK ROT CAUSED BY MAIZE FUSARIUM AND THE LEVEL OF CELLULASE ACTIVITY

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Abstract: Fusarial stalk rot is expected to represent severe problem in the future as a result of the increased occurrence of extreme weather conditions. The development of stalk rot is more likely under drought stress due to the propagation of saprophytic fungi. According to our results, the tolerance of genotypes to stalk rot is in close correlation with the cellulase enzyme activity measured in infected stalk tissue extracts. Stalk rot is more easily develops when the stalk is damaged by for example pests, cultivation devices, hail, therefore, the mechanical parameters (thickness, strong epiphloem) have also to be taken into consideration during selection. The determination of stalk tissue cellulase enzyme activity of maize genotypes can be a suitable method to obtain information on the susceptibility-tolerance of different genotypes. Droughty years are proved to significantly raise the risk of stalk rot. In case drought is accompanied by increased incidence of hot days, stalk rot epidemic has to be reckoned with as well.

Keywords: *Fusarium spp*., maize, corn stalk rot

Introduction

Maize is one of the most important crops in Hungary and in the world, it is annually grown on an area of 1.0–1.2 million hectares in our country. Today, the yield stability of maize is endangered most by the increase in the occurrence of droughty years. The risk of stalk rot is significantly raised by dry conditions (Schneider and Pendery, 1983), therefore, the improvement of stalk rot resistance becomes more important than before. Drought stress generates stalk tissue decay before its time, which significantly increases the chance of stalk rot development. Stalk rot in maize is caused by a variety of microorganisms (bacteria and fungi), and in Hungary *Fusarium* species are responsible for most of the cases (Mesterházy and Vojtovics, 1977; Fischl and Halász, 1990). As a consequence of the process, stalk tissues decay also due to the cell wall degrading enzymes produced by *Fusarium* species (Szécsi, 1973; Riou et al., 1991; Lorenzo et al., 1997, Yamamura and Shim, 2008; Szőke et al., 2009). In the first stage of pathogenesis, these enzymes assist the fungal organisms in introducing and colonizing in the host plant, and later they are involved in degrading the tissues, which prevents the flow of nutritives from the root to the cobs. A correlation was found between the infecting ability and the enzyme activity of pathogens causing fusarial stalk rot (Chambers, 1987). This disease may result in a yield loss of 13-20%. The level of fusarial stalk rot infection is determined mainly by environmental factors, the relation between genotype and environment, as well as the tolerance of the given maize genotype to pathogens (Sutton, 1982; Mesterházy, 1983; Kovács et al., 1988; Todd and Kommedahl, 1994; Mesterházy et al., 2000; Keszthelyi et al., 2009).
This paper deals with the correlation between fusarial stalk rot greatly affecting stalk strength and cellulase enzyme activity.

Materials and methods

Three single-cross hybrids (sensitive, average, tolerant) were inoculated with two *Fusarium graminearum* isolates (FG36, FGH4) in 2006–2008. The two isolates were

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selected on the basis of previous pathogenicity tests in the phytotron, where they proved to be the most aggressive. The genotypes were sown in a split-plot design in four replications, with the maize genotypes in the main plots and the treatments (FG36, FGH4, sterile, natural infection) in the subplots. Inoculation was carried out on the 12th day after flowering by placing infected wheat grains in the second internode from the roots on six plants per plot. The grains were sterilised in a 60°C water bath for 2×5 min, after which they were placed in test tubes with 2 ml of a 10^6 conidia/ml suspension of the above isolates at 27°C for 14 days. Sterile wheat grains were placed in the maize stalks as a control, and natural infection was scored on the fourth plot. The collection and processing of samples was begun on October 10th. The stalk samples were cut in half lengthwise and all the samples were photographed with a digital camera to determine the area of the lesions on the pith using the Colim 4.0 image analysing program. Percentage values were calculated from the complete area of the internode and the infected area. The enzyme activity in the stalk tissue extracts was determined using the modified cup-plate method in 2006-2008. The assay medium contained 1740 mg dipotassium phosphate, 840 mg citric acid, 1500 mg agar (Sigma), 100 mg AZCL-HE-Cellulose (Megazyme), 50 mg Na azide (Sigma) in 100 ml water, pH 5.0. Wells (5mm diameter) were made in the assay medium (5mm thick), and filled with 0.2 ml of enzyme preparation. Plates were incubated at 37 ^{o}C for 24 hrs. The cellulase activity was determined from the diameter of the activity rings using the Colim 4.0 image analysing program followed by the calculation of the ring area in mm². The data were evaluated using analysis of variance (Sváb 1981).

Results and discussion

As a result of artificial inoculation, the infection caused by the two *Fusarium graminearum* isolates in the genotypes examined was significantly greater than in the control and the sterile grain treatments (Table 1). From the two isolates, FGH4 proved to be more pathogen, however, the difference was not significant. In the field, artificial inoculation generated less severe infection in the tolerant hybrid (MV2) than in the susceptible hybrid (MV3) and the hybrid with average tolerance (MV1). The cellulase activity was also measured in the three genotypes. The enzyme activity in the tolerant MV2 hybrid with the least severe infection was found to be low, while it was high for the susceptible MV3 hybrid (Table 1). The regression between the infections developed as a result of field treatments and cellulase enzyme activity was also determined. On the basis of the three-year data, it can be stated that there is close positive correlation between the two factors (R=0.81). The isolate FGH4 was found to be more pathogen on the basis of both field inspection and enzyme activity data, followed by the enzyme activity of the sterile grain treatment and that of the control. The relatively high infection level and tissue enzyme activity of the sterile grain treatment used as a control are noteworthy (Table 1). The would we inflicted on the stem was likely to be enough for the *F. verticillioides* often present in the stem without causing symptoms (Bacon and Hintón 1996), which is less pathogen than the *F. graminearum* used in our experiment, to induce visible symptoms. In 2007, cellulase activity was detected in the control treatment as well. This was caused on the one hand by the wounds inflicted by pests, and also because this year was ideal for the development of fusarial stalk rot, making the less pathogen *F. verticillioides* capable of causing infections. Enzyme activity was

not observed in healthy stalk tissues. Table 2 summarizes the main weather characteristics during the maize growing season of the three examined years.

Table 1. Table of variance data relating to the field treatments and enzyme activity data (2006-2008)

		Infection in the field	Activity of the cellulase enzyme	*Genotype (A)*	Level of infection (%)	Enzyme activity (mm^2)
Factor	df	MS	MS	MV1	40.85*	135.83*
Genotype (A)	2	2117.99***	20291.27**	MV2	32.87*	111.06*
Treatment (B)	3	15039.68***	127815.38***	MV3	51.59*	169.01*
Year (C)	2	2122.27***	22166.70***	$LSD_{5\%}$	6.68	24.579
A*B	6	301.68ns	3316.36ns	*Treatment*		
A*C	4	280.35ns	2386.78ns	FG36	59.22	203.43
B*C	6	448.94ns	11296.22**	FGH4	65.49	208.45
Residual	47	190.34	2574.92	KONT	1.28*	31.52*
				STERIL	41.08*	111.14*
*P=5%				$LSD_{5\%}$	7.71	28.3814
**P=1%				*Year (C)*		
***P=0,1%				2006	36.49	155.40
ns=not significant				2007	53.20*	156.95
				2008	35.60	103.55*
				$LSD_{5\%}$	6.68	24.579

Precipitation and temperature data were similar in 2006 and 2007, however, there was considerable difference in the number of hot days. The amount of precipitation in the year 2008 was much higher than in the previous two years. The number of hot days in 2008 was similar to that in 2006. The level of infection was the highest in 2007, followed by those in 2006 and 2008. Enzyme activity was also found to be the highest in 2007. As compared to the 30-year average, the years 2006 and 2007 are considered as dry, while 2008 was wet. In 2006 and 2008, average temperature corresponded to the 30-year average, while the year 2007 was somewhat warmer. The number of hot days was higher for all the three years of the experiment than the base period.

Table 2. The main weather characteristics during the maize growing season (2006-2008)

April-September	2006	2007	2008	30-year average
Precipitation (mm)	246.50	265.00	482.70	312.00
Average temperature (C°)	17.88	18.21	17.95	17.70
Number of hot days	40	58	42	37.25*
*1999-2007				

Conclusions

Due to the increased occurrence of extreme weather conditions today, special attention has to be paid to the development of maize genetic sources and hybrids tolerant to stalk rot. The risk of stalk rot development is raised by drought stress as it generates stalk tissue decay before its time, facilitating the propagation of saprophyte fungi. According to our results, there is close correlation between the susceptibility-tolerance of genotypes and the cellulase enzyme activity measured in infected stalk tissue extracts.

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The high level of infection observed with sterile grain treatment draws attention to the fact that the development of fusarial stalk rot is greatly assisted by stalk damage caused by for example pests, cultivation devices, hail, consequently, the mechanical parameters (thickness, strong epiphloem) have also to be taken into consideration during selection. As the infected tissue shows a certain level of enzyme activity in every case and as there is close correlation between the susceptibility-tolerance of genotypes and the cellulase enzyme activity measured in infected stalk tissue extracts, the determination of the level of enzyme activity can be used to obtain information on the susceptibility-tolerance of each genotype. It is proved that the risk of stalk rot is significantly increased by dry conditions. In case drought is accompanied by increased number of hot days, fusarial stalk rot epidemic has to be reckoned with as well.

Acknowledgements

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FIPRONIL INDUCED CHANGES IN ISOLATED ILEUM: HARMFUL EFFECS OF AGRI-ENVIRONMENT

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Abstract: Harmful effects caused by environmental factors may influence physiology and pathology of humans and animals as well. Anthropogenic pesticide use in agriculture has also some risks disturbing life processes. Pesticides occurring in the food chain can cause functional changes in parameters of the living organism. The proposed sensitive biotesting method evaluating quantified modifications of tone and contraction parameters of *in vitro* rat intestine (ileum) may characterize agrotoxic poisoning for a sound risk assessment.

Keywords: pesticides, fipronil, isolated intestine, biotesting methods *in vitro*

Introduction

Isolated *ex vivo* animal tissues can be used as model systems for monitoring potential harmful effects of agri-environment (Banczerowski-Pelyhe, 2002, 2006). The dangerous environmental effects can not be fully eliminated, but the right balance for the living organisms can be achieved. *In vitro* biotesting methods may be applied in detection of these effects and thus to enhance adaptive capacity of the living systems that are important components of the ecosystems. The main task is to find the right methods and tolerable toxic levels for the living systems: how to tolerate adverse environmental effects without collapsing into irreversible state. *In vitro* testing model systems are useful tools to determine quantitative characteristics of the threshold limits from what resilience can result in recovery of physiological processes.

There is a need for new approaches to determine the safety of pesticides and to introduce new risk assessment methods. Although it has been proved, that new generation of insecticides show higher affinity to invertebrate as compared to mammalian receptors, their toxic effect in vertebrates is not excluded. Fipronil is a widely used insecticide in the plant protection and veterinary medicine. The neurotoxic effects of fipronil was reported, that manifested in enhanced excitability and paralisis. Toxic effects were detected in insects, in birds, aqual invertebrates and fishes. Fipronil may considerably accumulate in fishes (Tingle el al., 2003). Fipronil may not appear in milk, but a metabolized compound of fipronil residues can be transferred to cow milk from feed containing fipronil. Food and feed may be contaminated by fipronil residues, and this pesticide can be detected in the gastrointestinal tract of humans and animals.

Acute gastrointestinal disorders caused by agri-environmental pollutants like insecticide residues can be modelled and extrapolated to human disorders making prognoses for prevention.

In these series of experiments the tone and contraction changes were studied and measured in *ex vivo* intestine isolated from adult rats as an effect of fipronil exposition.

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Acetylcholine (ACh) or histamin (HIS) were added to the bathing medium in order to enhance motility of isolated ileum segment *in vitro*.

Materials and methods

Adult male albino Wistar rats were sacrificed by decapitation and 1cm long segments of the distal ileum were removed. The samples were placed into an organ bath containing oxygenated Tyrode solution at 39 ^{o}C and fixed to a displacement sensor (Experimetria, Budapest). Measurements started after 30 minutes stabilization period. The ileum segments were perfused every fifteen minutes with fresh oxygenated Tyrode solution. Contractions were evoked by Ach and HIS in eighth concentrations each. Applied concentration for fipronil was 10 mg/l. At the end, 5 ml (1M) KCl solution was added to the organ bath to produce a maximal contraction. Amplitude data were calculated as a fraction of the maximal contraction. Contraction patterns were studied with the software 'Analyze'. Amplitude, as well as the tone of the contractions was measured.

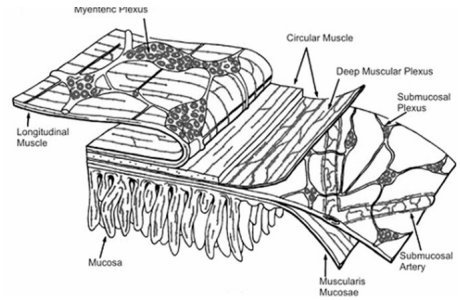

Figure 1. The anatomy of intestine

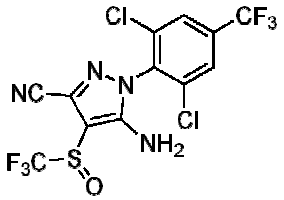

Figure 2. The structure of fipronil

Results and discussion

Amplitude and tone of contractions evoked by ACh was significantly decreased after application of fipronil to the bathing solution. Maximal contractions evoked by KCl were smaller as well.

An opposite effect was revealed in the case of contractions evoked by HIS: the tone was increased by fipronil except of maximal concentration.

Amplitude of contractions evoked by ACh or HIS was decreased by fipronil in both cases. The tone and amplitude changes were significant compared to controls.

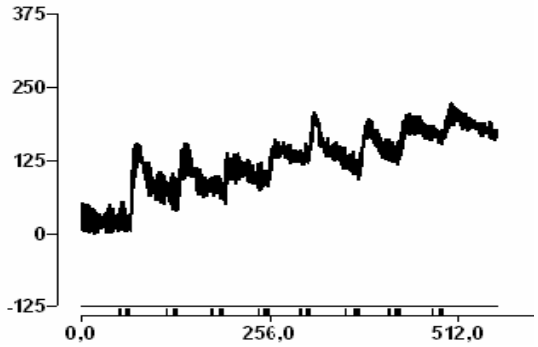

Figure 3. Contraction patterns evoked by acetylcholine (x: time in s, y: relative changes)

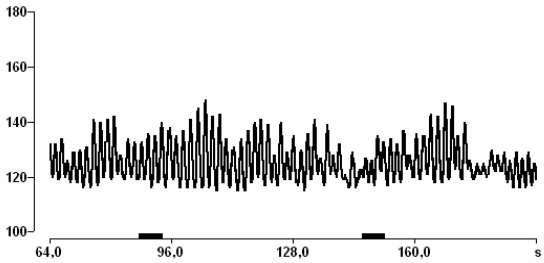

Figure 4. Contraction patterns evoked by histamin (x: time in s, y: relative changes)

Relative amplitudes were calculated as relation of amplitudes evoked by ACh or HIS to maximal amplitudes evoked by KCl. Relative contractions evoked by smaller concentrations of ACh were decreased after application of fipronil and increased at higher concentrations of ACh. In the case of HIS a decrease was revealed at all concentrations. Relative tone was calculated as relation of tone evoked by ACh or HIS to maximal tone evoked by KCl. After application of fipronil contractions evoked by ACh were increased in every case except of highest concentration. In the case of contractions evoked by HIS there were no significant changes in relative tone. To evaluate the risk of environmental agrotoxicity of synthetic agrochemicals, like pesticides in non-target mammalian organisms and to reveal the underlying processes is also necessary for an effective risk management. It is reasonable to investigate direct pesticide effects at moderate or high toxicity level to model low level exposition and an accidental exposure as well.

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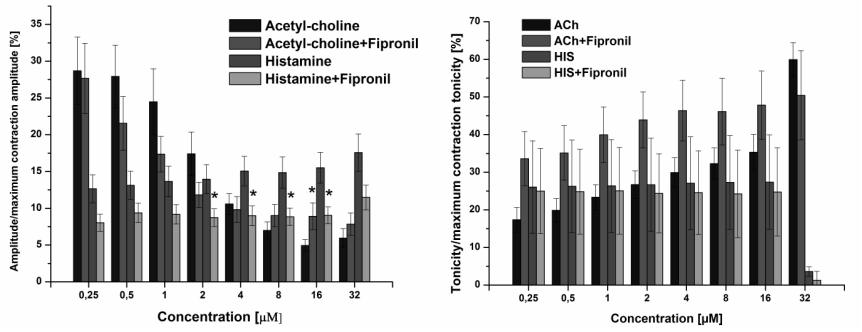

Figure 5. Relative amplitude and tone changes of contractions

Conclusions

Results enable to evaluate the effects of toxic substances introduced into the food chain on the exposed populations and identify danger points in terms of pesticide contamination. It will be possible to determine a physiologically based tolerable level of pesticides for an effective risk assessment and risk management. Main goals are to achieve environmentally sound agricultural production, to ensure a secure, healthy supply of food, to ensure monitoring of this. The proposed bio-sensing techniques, where living tissues are used as biosensors, can be applied in risk assessment of pesticides under analytical detection limit and to select them with less harmful effect (Szegedi et al., 2005). Our new quantitative *in vitro* bio-testing can reveal functional impairment of the ileum in an early, reversible stage of harmful agrotoxical agent exposure.

Acknowledgements

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GERMINATION AND SUGAR MOBILISATION OF MAIZE AS A FUNCTION OF *FUSARIUM* CONTAMINATION

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Abstract: Healthy and *Fusarium* affected ears were collected in Fészerlak, Somogy County at the end of vegetation cycle of maize (25-30 August). Each pattern contains 25-25 ears. We compared the samples on the basis of visual image of *Fusarium* affection. The ears were shelled and two 0,5 kg samples were formed: healthy and *Fusarium* contaminated. After surface sterilisation the uniform sized seeds were soaked in sterile distilled water for 24 hours and there were germinated for 7 days. The α-amylase activity was measured with Phadebas- α-amylase test. Seeds were extracted one by one three times under reflux using 10 cm^3 boiling water for 15 minutes. During our investigation germinating activity was detected to measure glucose, fructose, sucrose content and α-amylase activity. In the first seven days of germination the highest values were detected in control seeds followed by the affected seeds. Our results clearly show that stress conditions applied altered not only the saccharide content but decreased their germinating activity as well in the case of maize grain.

Keywords: *Fusarium* contamination, α-amylase activity, sucrose, glucose, fructose content

Introduction

Climatic conditions -rainfall and temperature- are the two essential factors determining near the maize growth the average yield mass development In Hungary (Izsáki, 2007; Surányi, 1957). In the recent years different biotic factors have contribute significantly to cultivation insecurity (Keszthelyi, 2007). One of these factors is the contamination with different *Fusarium* taxa. The infection of maize by *Fusarium* can result in highly variable disease symptoms ranging from asymptomatic plants to severe rotting and wilting (Oren et al., 2003). Several investigations were made on the mechanism of *Fusarium* contamination of maize (Mesterházy, 1974; Mesterházy and Vojtvics, 1977; Marín et al., 1996), as well as on the resistance of maize to *Fusarium* contamination (Mesterházy, 1982, 1983; Munkvold et al., 1997). A very interesting question is the eventual connection between Fusarium contamination, germination and sugar mobilisation in maize, important in beer-brewing, distilling industry, as well as in cosmetics industry (Tömöskei et al., 2000; Szél, 2007).

Materials and methods

Healthy and *Fusarium* affected ears were collected in Fészerlak, Somogy County at the end of vegetation cycle of maize (25-30 August). Each pattern contains 25-25 ears. We compared the samples on the basis of visual image of *Fusarium* affection. The ears were shelled and two 0,5 kg samples were formed: healthy and *Fusarium* contaminated.

After surface sterilization the seeds were soaked in sterile distilled water for 24 hours, and then were germinated for 6 day in the dark thermostat at 25 °C. Seedlings were

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removed at daily intervals for detection of α-amylase activity, glucose, fructose and sucrose content.

To determine the α-amylase activity, seedlings were homogenized in 0.01M phosphate buffer (pH 6.7) at 4 °C. The extracts were centrifuged at 3500g for 20 min. Phadebas-α-amylase test was used to measure the α-amylase activity.

In order to determine the sugar content, seeds were extracted in boiled water one by one three times under reflux. Fractions were collected and purified by filtering, dried under reduced pressured at 40 °C and dissolved in distilled water. Amount of glucose, fructose and sucrose were measured using Boehringer Mannheim GmbH Kits.

Results and discussion

As the figure 1. shows, *Fusarium* contamination caused well detectable changes in carbohydrate metabolism in germinated maize seeds.

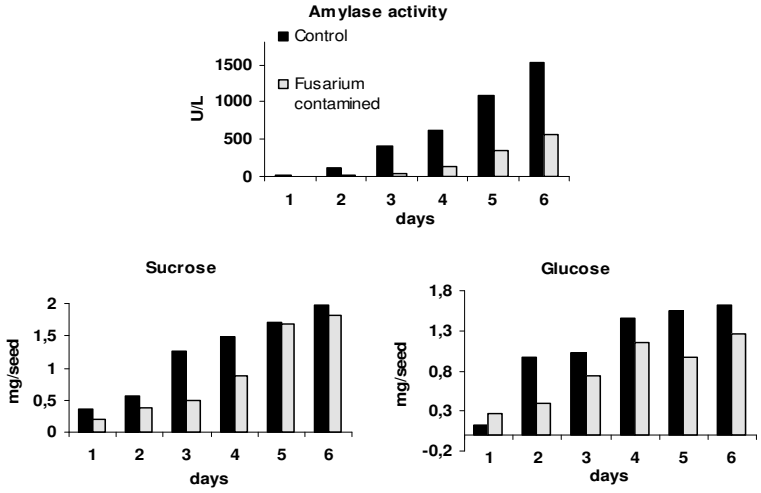

Figure 1. α-amylase activity, sucrose and glucose content in germinating maize seeds from plants exposed to *Fusarium* contamination.

The most characteristic alteration were measured in α-amylase activity and glucose content with significant higher values in control seeds to compare with *Fusarium* contaminated ones, while sucrose level was higher only in the first four days of germination.

Table 1. Fructose content in germinating seeds.

days	1	2	3	4	5	6
	mg/seed					
control	0,143	0,125	0,121	0,3	0	0
Fusarium contaminated	0,03	0,06	0,343	0,1	0,08	0,04

Changes in fructose content were not characteristic (Table1.)
These data were correlated our earlier results (Pál-Fám et al.2008), show a very sensitive interaction between the biotic and abiotic stress and carbohydrate metabolism.

Conclusions

The effect of *Fusarium* contamination as it was expected caused a sharp decrease in sugar content as well as α-amylase activity. The interpretation of these results needs further investigations.

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MYCOTOXIN CONTENT ASSESSMENT OF SOME FUSARIUM AFFECTED WINTER WHEAT (*Triticum aestivum* L.) SAMPLES BY ROSA METHOD

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Abstract: Fusarium infection on cereal grains causes an increasing problem through its probable mycotoxin production. These mycotoxins, like DON may endanger both human and domestic animals' health. For this reason we observed fusarium infection on winter wheat grain gained from a small plot experiment run at the Szent István University's Nagygombos experimental site. In this experiment we made some treatments with plant nutrients (nitrogen) and fungicides on five commercial wheat cultivars. In laboratory we examined which of the samples was infected by fusarium and also the DON content of them. For measuring DON concentration we used the ROSA method. *Fusarium spp.* produce mycotoxin in case of suboptimal environment. In samples affected by fusarium, but free of DON we tried to provoke fungi by inserting in a milieu suit for mycotoxin production for a period of various durations. After that treatment we measured DON concentration and the results were evaluated by the exposition time of stress. The concentration was studied also from a point of view of utilisation as grain with higher DON content still appropriate for several uses like bioalcohol production.

Keywords: Fusarium, mycotoxin production, winter wheat

Introduction

In the past years, a dangerous disease of cereals, the Fusarium head blight came into prominance. From amidst the species occuring in Hungary, the typical loss of quantity and quality is mostly caused by *Fusarium graminearum* and *Fusarium culmorum.*
While a decrasing thousand kernel weight presents the losses of quantity, the problems of quality are manifested by the occurrence of violently poisonous mycotoxins. The producer must reckon serious economic loss in both cases.
Fusarium species are poliphags, but the host-plants of these species are mostly cereals, the fungi live on their infectious residues.
The period of infection is chiefly the anthezis, but between favourable weather conditions (precipitation, high humidity, high temperature) the fungi may be contagious over 5 weeks, till the stage of wax-ripening (Békési 2010).
The greater danger is not the loss of quantity, but the qualitative damage, in other words the production of toxins (DON, trichotechenes, nivalelol (NIV), moniliformin, fumonisins) that are poisonous for warm-blooded organisms (Wagacha and Muthomi 2006).
These compounds generate damages in protein-synthesis and skin sensitivity or necrosis to the blue and green fluorescent colours produced extreme immunodeficiency, and may cause spontaneous abortion (Sweeney and Dobson 1998).
Both the genetic (plant breeding), agro-technical (crop rotation, nutrition supply) and also the chemical protective (using fungicides) methods must be used for the defence against pathogens. The significant problem of varieties currently known in purification and used as resistant is the poor baking quality. This is the reason why the usage of these varieties is limited (Laszlo et al. 2009). As a result of all the above, those factors

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must be emphasised that are impressible by the producer: the agro-technology and chemical protection (Klupács et al. 2007).
An experiment of winter-wheat was set up in the SZIE Research Station of Hatvan-Nagygombos in 2009 to investigate this combination of technologies. The goal of the research was to investigate the mycotoxin content of the harvest after applying different doses of N-fertilizers and various types of fungicides.

Materials and methods

The optimum temperature needed by *Fusarium* species has been discussed in several scientific works (Wagacha and Muthomi 2006). However, only some of them investigate those environmental factors that are favourable for the micotoxin production of these fungi. The samples harvested in the Experimental Area of Hatvan-Nagygombos were investigated for that purpose in the laboratories of SZIE Institute of Plant Protection and SZIE Institute of Crop Production.

The investigation had two parts. It has been carried out in parallel from the aspect of production technologies and storage after harvesting. During the time of storage mycotoxins may occur namely as a result of inappropriate technology even if they were eliminated during the technology of production.

1st phase (of production technology)
Varieties: (Alföld, Magdaléna, Suba, Csárdás, Toborzó)
From these varieties the most sensible for *Fusarium* spp was Mv Toborzó, Mv Csárdás was the less sensible.
Fertilization: Ø, 80, 120, 80+40, 80+40+30 kg N ha^{-1}
Fungicides used: Folicur (tebukonazol), Amistar (azoxistrobin)

We put down 75 grains of each sample in 3 repetitions, in 105 Petri dishes. Before putting them down, 25-25 grains were used in 2 % NaOCl solution for 10 minutes for the contamination (2 tablets for 2-3 dl water). Then the grains were cleaned in distilled water and were set on a filter-paper until they got dry. The crops were put on PCNB-agar breeding ground and were placed in a thermostat of the temperature range 18-20 degrees Celsius that is supposed to be the optimum, since below 17 degrees the growth slows down. The incubation period was 7 to 14 days.
Afterwards we counted the number of *Fusarium* beds emerged from the 25 grains by Petri dishes and now we also define the varieties with Árpád Szécsy by UV lights.
From the original items that were infected by *Fusarium* we chose such samples - applying the ROSE method - that were included in the phase II of the investigations and had not contained mycotoxins.

2nd (storage) phase:
For this investigation we chose the Csárdás and Toborzó varieties on the basis of their sensitivity for *Fusarium*. We settled the samples of all treatments on 4 temperature levels (15, 20, 25, 30 degrees Celsius). This means a total number of 2*7*4*4*3=672 grain samples of 20 g each (using a 2% of NaOCl solution for 10 minutes for the

contamination and after washing them twice with sterilized water). Finally, we put the samples in closed plastic bags with 30 ml water. We took out the samples four times, after one, six, eleven, sixteen days. We dried the content of the plastic bags, mixed them with which we got 224 items.
We measured the DON level of those items, that were infected by *Fusarium* in the first phase but had no DON applying ROSA M device.
The device demonstrates the toxin content of the sample of a suspension made out of wheat grist. The sample is heated in the incubator and the coloured medium is mobilized, which forms the colour of the test stripe according to the level of toxins, and provides a numerical result (Chrpova et al. 2008).

Results and discussion

Although all of the grain samples were infected by *Fusarium spp.* and most of them at a remarkable level, none of them contained DON in a significant level that occurred only after a longer period of treatment.
After the treatment that simulated a non adequate storage, the evaluated samples showed a considerable variation, but most of the samples had 0-100 ppb of DON content, except one of them, that had 6 ppm. That extremely high value was observed in a sample treated at 25 ^{o}C of incubation and that cause the high speak on curve that can be seen on figure 1. The second highest point of that curve belongs to the treatment with 20 ^{o}C of incubation.

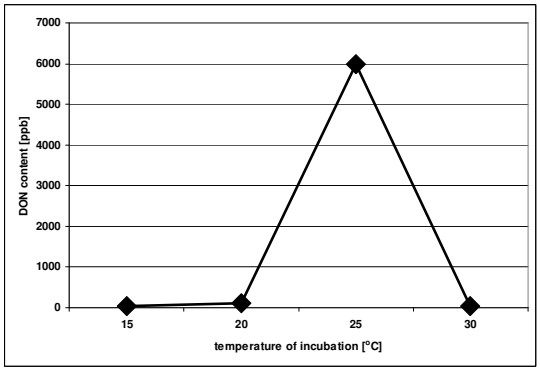

Figure 1. Total DON content of samples on each incubation temperature

Conclusions

There cannot be found direct connection between *Fusarium* infection and DON content in case of normal storage of wheat grain.
Connection can be found among the temperature of treatment and DON production of fungi but not on a reliable level. However 20 and 25 ^{o}C of incubation caused a notably

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higher DON production than 15 or 30 °C. And these issues meet the values that can be found in references.
The time dependence of DON production is unproven in this experiment. Except one very essential fact, that we have not found DON production before the treatment as well but not even after one day of incubation at any temperature. So that can be asserted that starting of DON production need more than one day of non adequate wheat grain storing.
After these series of incubation we can conclude that more samples should be treated for finding the starting point of DON production in time and also for making clear the temperature optimum of *Fusarium spp.* among extreme storing conditions.

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The authors would like to express their thanks for the scientific advices and the kind assistance of the SZIE Institute of Plant Protection.

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RESILIENCE OF WEED BEET (*COMPLEX BETA VULGARIS* L.) WITHIN AGRO-ECOSYSTEMS: THE IMPACT OF AGRICULTURAL PRACTISES ON GERMINATING CAPACITY

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Abstract: Thanks to its broad ecological valence, weed beet is capable of successfully surviving on various types of arable land both in Europe and Northern America. The effective suppression of its presence in the cultures of sugar beet consists in using a combination of preventive and direct protection methods. The effect of the selected direct methods on the germinating capacity of the weed beet glomeruli was tested. Among direct methods of protection, we include hoeing (this was not tested), manual selection (pulling out, snapping off, cutting off) and local application of non-selective herbicides. It has been established that snapping off, cutting off, mowing or causing any other damage to the plant has a partially significant effect on the germinating capacity of weed beet (only largest glomeruli). Significant differences in the germinating capacity were identified between glomeruli exposed by the non-selective herbicides and the glomeruli unexposed by these herbicides (correlation to the application of a herbicide/BBCH stage). On arable land with low presence of weed beet (up to 1.000 plants/ha), manual selection can be recommended. On arable land with stronger presence of weed beet (up to 10.000 plants/ha), a combination of measures carried out in the following order can be recommended: hoeing – chemical protection 2 times – manual selection with removal of the plants from the arable land. Combining the measures is important because if carried out individually, the measures may not guarantee the desired effect.

Keywords: beet, hoeing, BBCH, Roundup, glomerulus

Introduction

The first reports on the occurrence of weed beet in cultures came from Britain in 1970, where annual forms of beet with dominant seeds were found. Later the weed beet was also found in other European countries and the USA (Longden, 1989). Weed beet started to be present in the Czech Republic to a greater extent in late 1980s and it was included among quarantine weeds in 1992. The reason was not only the increase of the import of sugar beet seeds, but also the abandonment of mechanical inter-line cultivation (line weeding). At present, there is also a lack of workers for manual selection of bolters and weed beets. Successful suppression of the presence of weed beets on the plots of land consists in using a combination of preventive and direct methods of protection (Mücher et al., 2000; Sester et al., 2006). Due to frequent inclusion of sugar beet into sowing procedures, it is necessary to take the increased risk of the presence of weed beet into account and to intensify the methods of protection. The direct methods of protection are line weeding, manual selection and local application of non-selective herbicides to weed beets and bolters. Even if a chemical protection is used, some of the plants survive because the senescence tissues distribute the active substance rather poorly and the lower part of the plant can create germinant seeds; strong sclerenchymized pericarp of glomeruli also has its effect (Skalicky, 2009). The purpose of the experiment was to find out whether the direct methods of protection (breaking and cutting out the stem) and chemical protection methods based on the non-selective herbicides significantly reduce the germinating capacity of the weed beet glomeruli.

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Materials and methods

The defined issue was addressed experimentally. Weed beet (complex *Beta vulgaris* L.) was the experimental plant. Its glomeruli were collected from the beet growing areas in the Czech Republic in the years 2005 and 2006. A glomerulus (also called "seedball") is a cluster formed with two to six flowers aggregated during their maturation. Each flower forms a cavity called cell, which contains one seed if pollination is successful.

Various methods of direct protection – manual selection (pulling out, cutting out and breaking the stem) – and chemical protection methods were tested. The scheme of the experiment with the detailed descriptions of the individual alternatives is shown in Table 1. Variant B consisted of the glomeruli of weed beet in BBCH 65-69 (full flowering – end of flowering, fruit set visible; Meier, 1993), they were treated by a spray of a total herbicide (Roundup Klasik, 480 g l^{-1} Glyphosate-IPA) and they were collected at the stage of BBCH 89 (fully ripe, seed coat final colour, perisperm hard). For other variants, selected direct methods of protection were applied in the same BBCH phase and the glomeruli were also collected at the same point in time.

The germination capacity was being identified in the glomeruli of weed beet under the controlled conditions in climate boxes by means of the standard methods defined in the ISTA rules (Don, 2006). The laboratory-stored glomeruli were tested after dormancy ended (8 months – in April 2006 and 2007). For germination test in the dark, glomeruli were laid directly on pleated paper (pleated strips, 113 g m^{-2}, double folds, 2,000 mm × 110 mm, 50S) in a plastic box with 30 ml of water to ensure non-limiting water conditions (50 glomeruli in one box). The boxes were closed hermetically to avoid water loss and placed in an incubator at 20 °C. All the handling actions were carried out in a dark room illuminated by a green inactinic lamp without any stimulating effect on germination, as shown by Colbach et al. (2002). Every sample was prepared in 4 repetitions and within each taken sample (one plant), the glomeruli were divided into three size categories (see Table 1). Seed germination in the boxes in darkness was assessed every 7 days. The final count was carried out after 1 month. Germination assessment consisted in counting the newly protruding roots.

Table 1. Scheme of experiment.

Variants/size category	Selected methods of direct protection – characteristics
A	weed beet, manually pulled out, including the root; not exposed to non-selective herbicides
B	weed beet, manually pulled out, including the root; exposed to non-selective herbicides
C	weed beet with a cut-out stem without the root (hack, sickle); not exposed to non-selective herbicides
D	weed beet with a broken stem, including the root; not exposed to non-selective herbicides
glomeruli – size I.	larger than 4.5 mm
glomeruli – size II.	up to 4.5 mm
glomeruli – size III.	up to 3 mm

Exposure to Roundup in BBCH 65-69 (full flowering – end of flowering, fruit set visible; Meier, 1993), sampling in BBCH 89 (fully ripe, seed coat final colour, perisperm hard)

A mixed model procedure with a repeated statement for variants (STATISTICA 9.0, StatSoft Inc., Tulsa, OK) was used to analyze the germinating capacity of weed beet. Data from each size category of weed beet were analyzed separately. The analysis of variance (Wilks' lambda, P □ 0.05) was used to determine significant differences.

Results and discussion

The germinating capacity of the glomeruli of the weed beet, which was pulled out, including the root (variant A) was the highest one in all size categories (see Figure 1). In spite of the application of the Roundup herbicide, weed beet retained a high germinating capacity (this corresponds to the results of Sester et al., 2006), especially in the glomeruli larger than 3 mm, which is probably connected with the non-permeability of the sclerenchymized pericarp of the glomeruli (variant B). In the field conditions, this can be influenced by the technique of application of non-selective herbicides and other factors (climatic conditions, the height of the sugar beet crop, experience of the applicator operator, etc.). A significantly lower germinating capacity of variants after application of the manual direct methods of protection (variants C, D) can only be observed in the largest size group of glomeruli (size I); it is possible to presume that there is correlation to the lower content of stock substances.

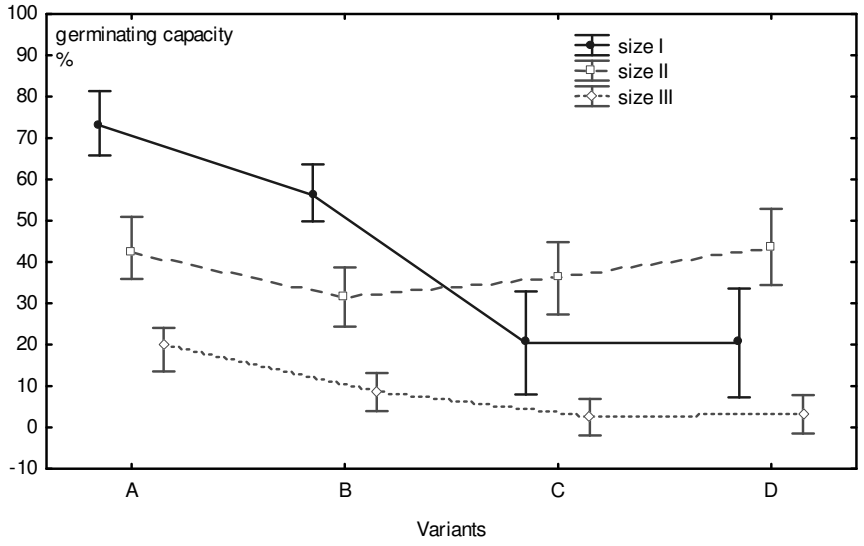

Figure 1. Comparison of the germinating capacities between the glomeruli exposed to direct and chemical methods of protection (variants: A – D; symbols: size I – III; see Table 1).

With other size categories of the glomeruli (size II, III) used in the variants (variants C, D), no reduction of germinating capacity was provable. The glomeruli (size II) exposed to Roundup (variant B) as well as the glomeruli from the weed beet exposed to breaking, cutting out or mowing (variants C, D) retained a relatively high germinating

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capacity (more than 31 %). The category III size of the glomeruli had the lowest germinating capacity in all variants (A: 19 %; B: 8 %; C: 2 %; D: 3 %). Undoubtedly, the lower content of stock substances in the glomeruli is an important factor. Based on these results, it is possible to recommend manual selection (pulling out, extracting) on the plots of lands with a lower density of the weeds. On the plots of land with a stronger presence of the weeds, the combination of all three measures is appropriate, used in the following sequence: line weeding, chemical protection applied twice, manual selection (pulling out the weed together with the root) and removal of the plants from the plot of land. With line-weeding, the surroundings of the line (up to 25 % of the area) remain unaffected.

Conclusions

Provable differences in the germinating capacity were identified in the glomeruli exposed to direct methods and chemical methods of protection. The differences in the germinating capacities in the same BBCH phase can be explained by the various sizes of the glomeruli. Large glomeruli (more than 4 mm) had a high germinating capacity; there is a direct connection with the higher content of stock substances in this respect. On arable land with a low presence of weed beet (up to 1.000 plants ha^{-1}), manual selection can be recommended. On arable land with a stronger presence of weed beet (up to 10.000 plants ha^{-1}), a combination of measures carried out in the following order can be recommended: hoeing - chemical protection 2 times – manual selection with removal of the plants from the arable land. Combining the measures is important because if carried out individually, the measures may not guarantee the desired effect. Manual selection cannot be used effectively on the plots of land with a strong presence of weeds. In addition to that, during the period of selection, the plants are already capable of maturing and creating seeds in emergency situations

Acknowledgements

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STRESS INFLUENCES ON THE PERFORMANCE OF EUROPEAN BROWN HARE (*LEPUS EUROPAEUS* PALLAS, 1778) POPULATIONS IN HUNGARY

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Abstract: European brown hare superpopulation all over Europe shows decreasing tendency, thus the Hungarian populations reduce as well. As hare is a typical member of agro-ecosystem, this chronic reduction can be observed from the mid-twentieth century, when agronomy and agricultural systems changed dramatically. In the same time changes in the climate manifested. All changes in population number should have a reason, in this case it can be biotic or abiotic stress factors. Agronomy and changing climate both have impact on hare populations. There could be three possible responses for it, namely resistance, resilience or total collapse of population. Analyzing population data in last century, we can state that hare populations have the ability of resilience as have not crashed irreversibly, they have very small uprising periods in some of the years. The reason of continuous decrease is the frequently repeating disturbations. Game management and environment conservation have the same objective to save and improve our hare populations. To reach that aim, correlations between each type of stress and the dimension and direction of change in the population level should be studied, just like the characteristic of the appearing resilience.
Results gained from this experiment may contribute to the development of agronomic practices and also to a better adaptation to climate change in favour of saving and improving hare populations.

Keywords: european brown hare. Lepus europaeus, yield, harvesting area, climatic conditions

Introduction

From the late twentieth century one of the most frequently mentioned idea in agriculture is the so complex term of sustainability. The concept of sustainable agriculture labels harmony with the regeneration of natural resources and the assimilation of environmental stress, while assisting the protection of human health and the improvement of life standards and the carrying capacity of rural areas (Győrffy, 1993; Várallyay et al., 1985). In agriculture and rural areas wildlife fauna performance is an indicator of sustainability of any system. All human activities may have an impact on agro-ecosystems. Especially agronomy is responsible for the outcome of that. Good agricultural practice is essential in maintaining sustainability (Jolánkai-Németh, 2002; Szöllősi et al., 2004). Game management should be considered as a part of agriculture; small game management is a special kind of land use (Faragó, 2002; Csányi, 2007). From this point of view we should evaluate game populations and their answer given on stress factors disturbing these populations.

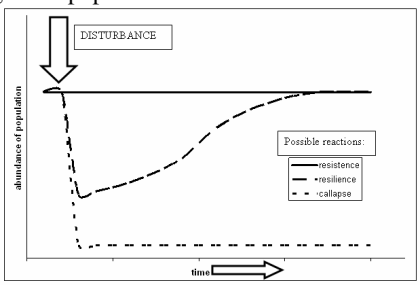

Figure 1. Possible reactions for disturbance in the abundance of a population

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One of the most important small games in our country is the European brown hare (*Lepus europaeus* PALLAS, 1778). Unfortunately, in the last decades the hare population of Hungary decreases similarly to other European populations.

Like all kind of populations hare population can give three sort of answers for a disturbance depending on its size, namely resistance, when the abundance of population does not change, resilience, when the abundance falls but returns to former level or collapse when population should stabilizing on a lower level (Figure 1.). Hare population seems to react with resilience but the frequency of disturbation does not allow returning to former level only to a lower level, when next disturbation comes.

This question should be studied in details as there exist a lot of false theories. We should study all components of agro-ecosystem just as the connections among them. If we would like to save our hare population and all the agro-ecosystem, we should learn more about them and we should analyze the data already collected.

Materials and methods

In an average population the dynamics are formed by the migration, the birth and the death (Csányi, 2007). In case of hare we found that migration is not typical (Angelici et al., 1999). So we can set an equation: $N_1 = N_0 + B - D$, where N_1 is the population level in given year, N_0 is the population level in last year, B is the number of individuals born and D is the number that died. According to long term studies number of births is invariable in a given period of the year, and geographical area (Blottner et al., 2001; Hacklander et al., 2001), so in Hungary we can calculate with 9 new born baby per year per mother. As it is well studied that the rate of sex is about 1:1 and there is no reproduction in first year (Kovács and Heltay, 1993; Faragó, 2002), so half of the N_0 is reproducing female. The equations can be changed for the following form:

$$N_1 = N_0 + ((N_0/2)*9) - D \text{ or } N_1 = 5.5*N_0 - D.$$

As we can count the number of hares (N_0 and N_1), we can calculate the number that died by transforming the previous equation: $D = 5.5*N_0-N_1$ for each year. The D covers several sources (also exploitation by hunting as well), and as we have data about hunting we can calculate with it: $D = H + M$, where H is the number exploited by hunting and M is the mortality. All these equations can be combined:

$$H + M = 5.5*N_0 - N_1 \text{ or } M = 5.5*N_0 - N_1 - H.$$

Data on hare population parameters are available in the National Game Management Databank (OVA) just as hunting statistics, so M can be calculated for each year. It surely has a considerable environmental influence, as some element of it could be a stress factor. To explore the strongness and appearance of this disturbation and the given answer we collected hare population data from OVA for the period 1960 to 1999, and calculated mortality for each year.

For the same period data for the agro-ecological and climatic environment were also collected (KSH and OMSZ), namely 13 series about area of harvestation, 17 yield data on representative crops, 14 kind of temperature and 14 on precipitation data and 4 series on radiation. All these factors can be found on the web site containing spacious tables and specifications of this paper (referred as website).

Statistical analysis was made by MS Excel programme packages. The mortality belonging to each year was assigned to the value of each parameter at the same year. For crop production data the deviation from the tendency were observed. For each

calculation one of the 62 environmental factor and mortality data gave a series of point on that a trend-line was fit and the square of the sample correlation coefficient (Pearson, r^2) was calculated. From the r^2 calculated we know how strong is the connection.

Results and discussion

In consideration of size limit in this paper only the summarized figures are shown, whole tables are on the website.

The sample correlation coefficients calculated were aligned by value and five groups were made. The position of sample correlation coefficients in ranking can be seen on website. In table it can be seen how much of environmental characteristics of each bigger categories (harvesting area, yield, temperature, precipitation, radiation) got into each fifth of the ranking. On Figure 2 the percentages are shown.

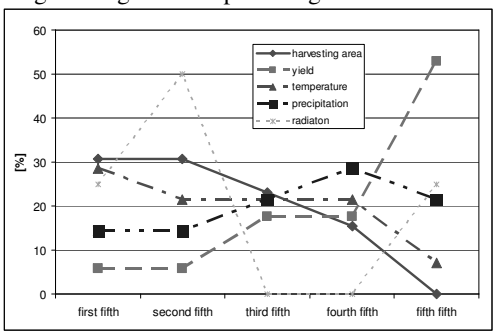

Figure 2. Distribution of sample correlation coefficients in ranking by %

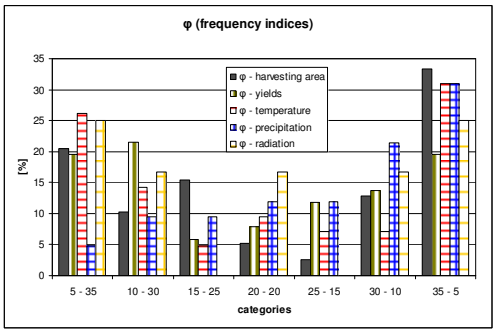

Figure 3. φ - frequency indices

After, for the better result, series of point were evaluated not only with one but two trendlines dividing each series in two groups by the independent variable. The first pair of groups was the lowest five with the highest 35 and other groupings were made by the step of five until the last pair of groups: the lowest 35 with the largest five. The distribution of points also gave the name of categories. In each of 62 cases in all seven categories the r^2 of trend-lines laid on groups of points were summed (Σr^2) and represented in tables on the website. In the tables the environmental characteristics are

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grouped by the bigger categories already introduced (harvesting area, yield, temperature, precipitation, radiation). In each big category the φ (frequency index) was calculated by the position of the biggest Σr^2 and second biggest Σr^2, namely counting the frequency of falling in each category (5-35, 10-30, etc.). The φ - frequency indices can be seen on Figure 3.

Conclusions

After analyzing the distribution of correlation coefficients in ranking (Figure 2) we can state that harvesting area has bigger effect than yields, as not the amount of food determinates the population dynamics but the quality and balanced distribution in time of it. Regarding climatic factors, temperature has more significant effect than precipitation and radiation shows almost no significance.

Based on the results received by calculating φ (Figure 3) it can be asserted that even the harvesting area has considerable effect only in case of remarquable deviation of the trend as only last column shows extreme height. Yields never give extreme results, not even with extreme yields we can make remarkable changes. Both very low and very high amount of temperature can make harm but between these cases hare populations has a remarkable force of resilience. Also shows a very good adaptation for the precipitation where only extremely high values have significant impact. As radiation has an indirect effect only the very extremities have visible influence.

After all we can conclude that European brown hare adapted well to our region's climate and it can be mark as an important stress factor only in case of extremities. Apropos of habitat we can state that qualitative parameters have more significant impact than quantitative ones.

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THE EFFECT OF RECOVERY HOP PLANTS FROM VIRUS DISEASES ON THE RESILIENCE OF THE AGRO-ECOSYSTEM OF A HOP-FIELD

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Abstract: The most widespread variety of hop in Czechia is Saaz semi-early red bine hop (Osvald clone No. 72). A big problem is the infestation of the vegetation consisting of this variety with viruses, particularly Hop Mosaic Virus and Apple Mosaic Virus. Their presence reduces the yield and quality of the hop. The technique involving meristem cultures makes it possible to eliminate viruses from plant tissues and to establish a virus-free (recovered) crop. In a three-year experiment (the years 2000–2002), the physiological characteristics of plants 5–7 years old, both recovered and non-recovered ones, were compared. Both types of plants were tested for the presence of viruses. The recovered plants gave negative results; in the non-recovered plants, the presence of Hop Mosaic Virus and Apple Mosaic Virus was identified. The recovered plants had a significantly larger area of the hop bine leaves and the leaves on the lateral branches of the bine. In the recovered plants, both types of leaves also manifested a significantly higher photosynthesis rate as compared with the non-recovered plants. The fact that the plants were recovered from the virus diseases also resulted in prolongation of the period of the photosynthetic activity of leaves. The result was an increased overall amount of dry matter of the plants and a higher yield of the dry matter of hop cones in the recovered plants (+ 34.3 % in comparison with the non-recovered plants). The agro-ecosystem of the hop-field was stabilised at a higher production level.

Keywords: *Humulus lupulus* L., hop plants, hop cones, virus diseases, recovery, leaf area, rate of photosynthesis, dry matter

Introduction

There is a hundred-year tradition of hop growing *(Humulus lupulus* L.) in Czechia. The variety most frequently grown in the Czech Republic is the Saaz semi-early red bine hop (Osvald clone No. 72). The variety was created through clone selection in the regional population of Saaz hop. It was permitted in 1952. As a result of many years of cultivation and the predominant use of traditional technologies to reproduce the hop seedlings, viruses, especially Hop mosaic virus and Apple mosaic virus, have spread significantly among the plants of this variety. Their colonisation is irreversible and there are no means of protection against them like against other diseases and pests. Their presence reduces the yield and quality of hop (Krofta and Kroupa, 1995). The resilience of the agro-ecosystem of a hop-field is manifested in that the plants attacked by the virus diseases gradually physiologically adapt. The change of the physiological characteristics results in a shift from initial stable domain into another one where a balance is reached at a lower level of production capacity (Hejnak et al., 2004).

Techniques involving meristem cultures, combined with thermal therapy, are used to eliminate the viruses from the plant tissues (that is, to recovery the plants) (Svoboda, 1992). The purpose of this paper was to examine the effect of recovery plants from virus diseases on the activity of the photosynthetic apparatus (on the size and duration of the existence of leaf area and on the leaf photosynthesis rate), production of dry matter and yield for the above-mentioned traditional Czech variety.

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Materials and methods

Monitored plants of the Saaz semi-early red bine hop (Osvald clone No. 72) were planted out and grown in the hop garden in the Czech hop growing region Žatec. The experiment was carried out using two variants of the hops. The first group was planted with the use of cutting transplants of the hop field clones of mother plants. The second group of individuals was obtained from recovered meristematic in vitro cultures. The plants were observed in the years 2000–2002. On the beginning of observing were plants 5 years old.

Health condition was done by evaluation of visual symptoms and of immunoenzymatic method DAS – ELISA. From fifty plants were taken leave samples on high 2 m. The plants were analyzed on the occurrence of viruses separately. Plants for following physiological parameters were selected from that collection.

The dates of plant sampling and measuring were chosen to represent the main growth periods of the hop plant. First sampling was in the period of establishment (30.5.), second sampling in the period of shoots and shooting (20.6), third sampling in the catkins stage (10.7.), fourth sampling in the flowering period (30.7.) and fifth sampling in the period of hop cones formation (20.8.).

One in 50 plants was chosen for sampling in each data collection time; every variant had five repetitions. The photosynthesis rate, the size of leaf area and the weight of the dry matter of the whole plant as well as the weight of the dry matter of the hop cones were identified. Thirds of the above-ground parts of the hop stems were separately evaluated due to a high heterogenity of the plant physiological processes. The photosynthesis rate was identified in the intact leaves by means of a commercial portable gasometric infrared analyser *LCA-4* (*ADC Bio Scientific Ltd.*, Hoddesdon, UK) with a leaf chamber *LC4/PLC4BT-1/E*.

The statistical assessment was carried out by means of a variance analysis with $P = 0.05$ in the computer software Statistica, version 6.1 CZ, module ANOVA. The tables show the mean levels obtained from measurements in three years of experiments, including the statement of standard deviations.

Results and discussion

The non-recovered hop plants were fully infected by Apple mosaic virus (ApMV) and Hop mosaic virus (HMV). These viruses were not identified on recovered plants. The others tested viruses were not detected on both variants.

The results stated in Table 1 show that the healthy, virus-free plants produced a significantly larger area of bine leaves and particularly a significantly larger area of the leaves on the lateral branches of the bine during the second half of the vegetation period as compared with the non-recovered plants. The leaves on the lateral branches of the bine are very important for production of assimilates, which are used to fill the maturing hop cones during the second half of the vegetation period (Hejnak et al., 2004). While in the non-recovered plants, the area of the leaves on the lateral branches of the bine stagnated between the 4^{th} and 5^{th} measurements, there was a significant growth in the recovered plants. It can be inferred from this that the act of curing the hop plants of virus diseases prolongs the photosynthetic activity of the leaf apparatus of plants. This

is also confirmed by the results obtained from measuring the photosynthesis rate (P_n) stated in Table 2.

Table 1. The leaf area of hop plants during growing season [m^2 $plant^{-1}$]

The types of leaves	The variants of trial	The dates of measuring and leaf area [m^2 $plant^{-1}$]									
		30.5.		20.6.		10.7.		30.7.		20.8.	
		Mean	SD.	Mean	SD.	Mean	SD.	Mean	SD.	Mean	SD.
Bine leaves	Recovered hop	0.73	0.05	2.01	0.08	3.00	0.13	2.89	0.11	2.74	0.16
	Non-recovered	0.70	0.04	1.81	0.04	2.35	0.11	2.51	0.20	2.19	0.11
Leaves on the lateral branche	Recovered hop	0.47	0.03	1.18	0.15	2.38	0.06	3.02	0.20	3.76	0.13
	Non-recovered	0.49	0.02	0.91	0.06	1.68	0.04	2.15	0.13	2.13	0.10

The differences during the last measurement were very significant. While the recovered plants still maintained high levels: P_n in the bine leaves was 8.12 µmol m^{-2} s^{-1} and P_n in the leaves on the lateral branches of the bine was 7.81 µmol m^{-2} s^{-1}, in the non-recovered plants, it was only 4.16 µmol m^{-2} s^{-1} in the bine leaves and 4.54 µmol m^{-2} s^{-1} in the leaves on the horizontal branches of the bine. This once again shows that the recovery prolonged the period of the photosynthetic activity of the leaves. This has a favourable effect especially on the production of the dry matter in the plants and the hop cones during the 2nd half of the vegetation period (Table 3).

Table 2. Net photosynthetic rate of hop leaves during growing season [µmol CO_2 m^{-2} s^{-1}]

The types of leaves	The variants of trial	The dates of measuring and net photosynthetic rate of hop leaves [µmol CO_2 m^{-2} s^{-1}]									
		30.5.		20.6.		10.7.		30.7.		20.8.	
		Mean	SD.	Mean	SD.	Mean	SD.	Mean	SD.	Mean	SD.
Bine leaves	Recovered hop	9.46	0.13	9.78	0.15	10.26	0.23	7.14	0.20	8.12	0.09
	Non-recovered	7.45	0.15	6.78	0.07	8.28	0.22	7.29	0.10	4.16	0.27
Leaves on the lateral branche	Recovered hop	-	-	-	-	10.07	0.26	6.87	0.25	7.81	0.29
	Non-recovered	-	-	-	-	4.79	0.16	6.05	0.17	4.54	0.15

Except for the first measurement, the weight of the dry matter in the recovered plants was higher in comparison with the non-recovered plants during all the other measurements. During the vegetation period, the recovered plants produced a higher amount of matter (dry matter) than the non-recovered plants. At the time of the last measurement, the difference was 31.4 %. Other results stated in Table 3 show that at the

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end of the vegetation period, assimilates are used primarily for production of the dry matter of hop cones, that is, for production of economic yield. The accumulation in the recovered plants was more intensive. At the 4th measurement, the difference was + 15.7 % and at the 5th measurement, the difference was + 34.3 % in favour of the weight of the dry matter of the hop cones on the recovered plants. This corresponds to the results obtained by Nesvadba et al. (1999).

Table 3. The dry matter formation of hop plants and hop cones during growing season [g plant^{-1}]

Dry matter	The variants of trial	The dates of measuring and dry matter of hop plants and hop cones [g plant^{-1}]									
		30.5.		20.6.		10.7.		30.7.		20.8.	
		Mean	SD.	Mean	SD.	Mean	SD.	Mean	SD.	Mean	SD.
Hop plants	Recovered hop	64.2	7.42	316.8	26.47	626.1	36.91	952.5	26.92	1,065.2	37.49
	Non-recovered	67.7	7.51	247.6	24.73	450.3	16.89	804.2	23.24	810.4	47.28
Hop cones	Recovered hop	-	-	4.5	1.29	21.6	3.73	184.7	12.25	290.3	11.17
	Non-recovered	-	-	1.7	0.48	26.8	4.03	159.7	16.67	216.1	16.48

Conclusions

The results proved that the act of recovery of the plants has a favourable effect on the size of the assimilation area, the photosynthesis rate, the prolongation of the period of the photosynthetic activity of leaves and apparently on the prolongation of the vegetation period. The result is a higher total amount of the produced dry matter of the plants and a higher amount of the dry matter of hop cones. Thus, the result is a higher biological and economic yield. The agro-ecosystem of the hop-field is stabilised at a higher production level.

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THE INFLUENCE OF GERMINATION TEMPERATURE ON THE EXPRESSION OF GERMINATION INHIBITION BY WATER SOLUBLE INHIBITORS FROM WHEAT BRACTS

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Abstract: Pre-harvest sprouting resistance in wheat depends on the level of grain dormancy at harvest time as well as on certain spike characteristics including the level of water soluble germination inhibitors found in wheat bracts. The objectives of the present study were: (i) to evaluate the influence of germination temperature on inhibitory effect of water bract extracts on wheat germination and (ii) to compare different germination criteria for measuring germination inhibition. Samples of bracts were taken at harvest ripeness from 30 wheat cultivars grown in the field. Germination tests were performed at 20°C or 30°C using nondormant seeds of the cultivar Banica and 5 ml of bract extracts of 30 genotypes or 5 ml distilled water as a control. Three criteria were used to characterise a seed as germinated: 1) rupture of the pericarp over the embryo (P), 2) at least one rootlet >3mm in length (1R) and 3) three rootlets 3mm in length (3R). Mean germination percentage (GP) and germination index (GI) in bract extracts were significantly lower compared to GP and GI in water for both germination temperatures but the inhibition was stronger at the higher germination temperature. Comparing the three germination criteria the highest inhibition of germination was observed using 3R and 1R for GP and GI, respectively. Significant differences among cultivars for germination inhibition were observed for both germination temperatures.

Keywords: wheat, germination inhibitors, germination temperature, germination criteria

Introduction

Pre-harvest sprouting resistance in wheat depends on the level of grain dormancy at harvest time as well as on certain spike characteristics including the level of water soluble germination inhibitors found in wheat bracts (Derera et al. 1977; Derera and Bhatt, 1980; Sarcevic et al. 1999, 2000; Grunberg et al. 2002;). Sarcevic et al. (1999) observed a stronger inhibitory effect of bract extracts on post-germination events (rootlet elongation) than on the appearance of the first visible sign of germination (rupture of the pericarp over the embryo). It has been shown that embryo response to ABA (Walker-Simmons, 1988) as well as the proportion of dormant grains at harvest time (Mares and Ellison 1990; Nyachiro et al. 2002) increases with increase of germination temperature. However, there are no reports published by now on the effect of germination temperatures on expression of germination inhibition caused by water soluble inhibitors from wheat bracts. The objectives of the present study were: (i) to evaluate the influence of germination temperature on inhibitory effect of water bract extracts on wheat germination and (ii) to compare germination and post-germination events as criteria for measuring germination inhibition.

Materials and methods

Thirty wheat cultivars from the collection of the faculty of Agriculture University of Zagreb (Table 2) were sown in 2001 at Zagreb, north-west Croatia, in a field trial set up

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as randomized complete block design with three replications. Each experimental plot consisted of three 120 cm long rows spaced 20 cm apart. All cultural practices were as usual for optimal wheat production. Bracts were taken from 50 random ears per genotype at harvest ripeness and stored for five months at room temperature. The role of germination inhibitors from milled bracts was assessed using common seed source (nondormant seeds of cultivar Banica, which had been after-ripened at room temperature for five months). To obtain water-soluble germination inhibitors from milled bracts, 6 g of bract material of each of 30 wheat cultivars were soaked in 72 ml of distilled water for 24 hours at 20°C in darkness. Bracts were than separated from the liquid extract using a glass funnel and a Whatman No.1 filter paper. The filtrate (bract extract) was used as medium for germination of nondormant seed of the cultivar Banica. Germination tests were performed in Petri dishes containing 30 seeds and 5 ml of bract extracts of 30 genotypes or 5 ml distilled water as a control. Petri dishes were incubated for 104 hours at 20°C or 30°C in darkness. The first count of the germinated seeds was after 24 hours and the subsequent counts were in the intervals of 12 hours giving eight counts in total. Three criteria were used to characterise a seed as germinated: 1) rupture of the pericarp over the embryo (P), 2) at least one rootlet >3mm in length (1R), and 3) three rootlets 3mm in length (3R). Germinated seed showing three rootlets >3mm long were removed after each count. Germination for all three criteria was expressed as final germination percentage (GP) as well as germination index (GI). Germination index was calculated as follows: $GI = (n_1 / 1 + n_2 / 2 + n_3 / 3 \ldots + n_8 / 8)$, where n_1, n_2 …, n_8 are germination percentages at the 1st, 2nd and subsequent counts until the 8th count and 1, 2, … 8 are weights added to the germination percentages of the 1st, 2nd and subsequent counts until the 8th count. Combinations of the three germination criteria with GP and GI gave in total six germination parameters. Germination inhibition by bract extracts for all germination parameters was expressed as the percentage of the control. ANOVA combined over germination temperatures and Duncan's Multiple Range Tests for means comparison were conducted using SAS statistical pakage (SAS Institute 2007).

Results and discussion

The combined analysis of variance revealed significant differences between the two germination temperatures as well as among cultivars (extract sources) for all germination parameters, whereas temperature x genotype interaction was not significant (data not shown). Mean germination percentage (GP) and germination index (GI) in bract extracts were significantly lower compared to GP and GI in water for both germination temperatures but the inhibition of germination was stronger at the higher germination temperature (Table 1). This is in agreement with results of Walker-Simmons (1988) who showed that embryo response to ABA increases with increase of germination temperature. Previous studies have shown an increase in the level of seed dormancy at higher germination temperatures (Mares and Ellison, 1990; Nyachiro et al., 2002). Results of the present study indicate that germination inhibitors from wheat bracts might improve pre-harvest sprouting resistance at higher germination temperatures at least in genotypes with low level of seed dormancy at harvest time.

Table 1. Germination (%) and germination index (GI) of nondormant seeds of cultivar Banica germinated in water and bract extracts (average of 30 genotypes) at 20°C i 30°C

Germination		20°C				30°C	
medium	P#	1R	3R		P	1R	3R
	Germination (%)				Germination (%)		
Water	95	92	90		90	87	85
Bract extract	87	83	81		76	69	65
Inhibition (%)	9	9	10		15	21	24
		GI				GI	
Water	67	34	27		74	32	24
Bract extract	51	23	19		40	16	13
Inhibition (%)	24	31	29		47	49	45

P-rupture of the pericarp; 1R and 3R - at least 1 and 3 rootlets (>3mm in length), respectively

Comparing the three germination criteria the strongest effect on germination inhibition was observed at 3R and 1R for GP and GI, respectively (Table 1). This confirmed previous results of Sarcevic et al. (1999), who observed a stronger inhibitory effect of bract extracts on rootlet elongation than on the appearance of the first visible sign of germination (rupture of the pericarp over the embryo).

Table 2. Inhibition (%) of the germination of nondormant seed of cultivar Banica by bract extracts of 30 wheat cultivars at germination temperatures of 20°C and 30°C

	Inhibition (%)#						Inhibition (%)			
Extract	Germination temperature					Extract	Germination temperature			
source	20°C		30°C			source	20°C		30°C	
Tina	29	CDEF*	47	CDEFGHI		Adrijana	37	EFG	57	FGHI
Kuna	29	CDEF	44	CDEF		Gabi	31	CDEFG	52	DEFGHI
Marija	33	CDEFG	47	CDEFGHI		Bosanka	31	CDEFG*	54	EFGHI
Mihelca	41	FG	50	DEFGHI		Skopjanka	37	EFG	60	I
Hana	31	CDEFG	51	DEFGHI		Obelisk	33	CDEFG	46	CDEFGH
Sana	29	CDEF	52	DEFGHI		Herzog	30	CDEFG	46	CDEFGH
Sžitarka	31	CDEFG	57	FGHI		Apollo	35	EFG	59	HI
Žitarka	32	CDEFG	49	CDEFGHI		Remus	32	CDEFG	56	EFGHI
Edita	37	EFG	60	I		Soisson	39	FG	58	GHI
Perla	26	BCDE	46	CDEFGH		Renan	42	G	60	I
Magdalen	34	DEFG	55	EFGHI		AUS1408	21	BC	37	BC
Patria	33	CDEFG	57	FGHI		Janz	36	EFG	40	BCD
Divana	34	DEFG	54	EFGHI		Spica	23	BCD	53	EFGHI
Barbara	34	DEFG	54	EFGHI		Water	0	A	0	A
Sivka	37	EFG	49	CDEFGHI		Min	21		37	
Lipa	34	DEFG	43	CDE		Max	42		60	
Rina	37	EFG	45	CDEFG		Average	33		51	

#Inhibition by the bract extract of a certain genotype was expressed as the percentage of the control; germination parameter was germination index; criterion for germination was 1R; *means followed by the same letter are not significantly different at P=0.05 according to Duncan's Multiple Range Test

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However, a high germinability of non-dormant seed used in both studies might have favoured rootlet elongation as more appropriate criterion. Inhibition (% of the control) of the germination of nondormant seed of cultivar Banica by bract extract varied in a broad range among 30 wheat cultivars at both germination temperatures (Table 2). The inhibition at 20°C varied from 21% to 42% with an average of 33%. The inhibition was enhanced at 30°C for all cultivars ranging from 37% to 60% with an average of 51%. For most cultivars the difference in germination inhibition between the two germination temperatures was about 20%. The highest and the lowest response to the higher germination temperature showed cultivar Spica and Janz with a relative increase in inhibition of 31% and 4%, respectively.

Conclusions

The present research showed that the effect of water soluble germination inhibitors from wheat bracts on germination inhibition is temperature dependent. Stronger inhibition of germination by bract extracts at the higher germination temperature was observed for all 30 cultivars included in this study. The use of after-ripened seeds of the cultivar Banica as a common seed source for testing the inhibitory effect of bracts on germination suggests the possibility of prolonged testing period (after dormancy release), especially if higher germination temperatures are applied.

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