EFFECTS OF SPIKE INFECTION DEGREE ON THE CONTENT OF MYCOTOXINS, GERMINATION AND 1000 KERNELS WEIGHT IN WINTER WHEAT

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ABSTRACT

Three winter wheat varieties, PKB-Lepoklasa, Jugoslavija and Francuska, differently susceptible to *Fusarium* spp. and widely used on farms and in bread-making were selected for the experiment. Three treatments were applied: the artificial inoculation with the isolates of *Fusarium* spp., wheat protection with a fungicide on the basis of active ingredients of flutriafol and carbendasim (85+135 g l^{-1}) and monitoring of spontaneous infection over different wheat phenophases. The same variety not treated in the same location was the control. Trials were performed during 2000-2003. The phytopathological estimation was done twice during the growth period. The varieties Jugoslavija, Francuska and PKB-Lepoklasa had, on average, 85%, 65% and 100% plants infected with the fungi of *Fusarium* spp., respectively, during the three years. The presence of fungi reduced germination, as mycotoxins inhibited the protein synthesis. The development of fungi in wheat plants resulted in grain shrivelling due to damages of the conducting system and therefore to the statistically significant germination and 1000 kernels weight decrease. The fungi of the genus *Fusarium* produce many mycotoxins, mainly of trichothecene type B-deoxynivalenol. Additionally, zearalenone and fumonisin were detected. These mycotoxins were detected in kernels of each variety under artificial inoculation conditions. The content of each mentioned toxin was higher in kernels completely (100%) infected with fungi.

Key words: *Fusarioses*, germination, 1000 kernels weight, mycotoxins, wheat.

INTRODUCTION

The increase of the population density resulted in a need to increase the food production and to store it under as good as possible storage conditions in order to provide hygienically safe food. According to FAO data, one fifth of losses of the produced food originates from insects, microorganisms and other pests. The lower these losses are the smaller problems related to famine in the world.

Mycotoxins are secondary metabolites of fungi with adverse effects on human and animal health. Wheat can be infected by fungi of *Fusarium* spp. not only in the field, but also in the storage. Fumonisins, zearalenone and trichothecene (deoxynivalenol, nivalenol and T_2) are the most important *Fusarium* toxins, very often occurring in significant concentrations in cereals. Recently, the Working Group on the Evaluation of the Carcinogenic Risk has considered toxins of *Fusarium moniliforme* (together with fumonisins) as a possible carcinogen factor for humans, the group 2B.

Considering Serbia and Montenegro in relation to its geographical location and climatic characteristics, mycotoxins originating from the fungi of *Fusarium* spp., zearalenone and deoxynivalenol, are the most important, while there are no data on fumonisins present in wheat. At the same time, these mycotoxins are globally mainly related to maize.

MATERIAL AND METHODS

Three varieties of winter wheat (Jugoslavija, Francuska and PKB-Lepoklasa) were used for studying mycotoxins in wheat kernels and products. Three treatments were applied:

1) the wheat crop protection with a fungicide on the basis of the active ingredients of flutriafol and carbendasim (85+135 g l^{-1});

2) artificial inoculation with isolates of fungi of *Fusarium* spp. during the wheat flowering stage;

3) monitoring of spontaneous infection. The same varieties from the same location but not treated were the control.

The trials, carried out under field conditions, were set up according to the four-replicate block design. The plot size was of 5 m^2 (1 x 5) and the inter-row distance was of 20 cm. The sowing density amounted to 600 kernels/m^{-2}. The phytopathological estimation was performed twice during the wheat growth.

Germination of spikes (50 per a treatment), sampled at full ripeness, was tested and identifica-

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tion of *Fusarium* spp., was done after the method of Nelson et al. (1983).

Mycotoxicological tests were performed on wheat kernel samples. The following toxins were determined: zearalenone, fumonisins and deoxynivalenol. Zearalenone and deoxynivalenol were established by the HPLC technique after the APAC method. Fumonisins were established by a modified HPLC fluorimetric method, i.e. by an immunoaffinity column chromatography on the fluorimeter Vicam, Watertown, MA (Scott and Lawrence, 1995; Ware et al., 1994). The obtained values are the three-year trial results.

RESULTS AND DISCUSSION

Fusariosis is a disease that occurs in regions under wheat crop and significantly reduces yields and deteriorates technological quality. The disease is caused by many *Fusarium* species, but most often by *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium avenaceum*. As damage is done to spikes, seed germination is significantly reduced, grain is of poor quality with damaged starch granules and storage proteins (Bechtel et al., 1985). Infected kernels cause a seed disease (Manka, 1989). Deoxynivalenol and nivalenol are the most important trichothecenes synthesized by *Fusarium graminearum* (Ichinoe et al., 1983). Zearalenone, an estrogenic toxin, is mainly detected in cereals infected with *Fusarium graminearum* (Neish et al., 1983).

Trichothecenes and *Fusarium* toxins are mainly detected in cereals and their products. The symptoms of mycotoxicoses are the result of the interactions between mycotoxins and functional molecules and subcellular organelles in animal cells. According to Kiessling (1986), many mycotoxins are active at the DNA level disturbing the process of transcription and inhibiting the translation at the protein synthesis. Due to inhibition of DNA synthesis, mycotoxins are very cytotoxic.

Weinert and Wolf (1995) and Volke at al. (1996) assume that after infection, the fungi colonise the infected spikelet and spread to the spike spindle. Vascular bundles in the spike spindle are damaged or completely destroyed, and the upper part of the spike that is not supplied with water and nutritive substances starts wilting. The poorly filled kernels mean that the protein to starch ratio is disturbed in favour of the protein due to shortening of the grain development. Yield and infection of grain are for the most part directly correlated to spike diseases – therefore, a determination of infected spikes is sufficient for an fairly accurate estimation of fusarioses. The activity of the enzyme proteinase increases at germination, while trichothecenes obstruct the protein synthesis, hence wheat kernels infected with fungi have reduced germination.

Spikes were sampled in various phenophases of the wheat development and grain germination was determined and fungi of *Fusarium* species were identified. The obtained results indicate that *Fusarium graminearum* was a dominant species in the tested varieties. *Fusarium moniliforme* and *Fusarium proliferatum* occurred to a smaller extent (Table 1).

A high negative correlation (r = - 0.90) for wheat seed germination and the degree of spike infection was estimated by the regression analysis (Figure 1).

Vanco (1997) studied the effects of fungi *Fusarium graminearum* and *Fusarium culmorum* on the disease development in winter wheat and determined the correlation between 1000 kernels weight and kernel weight per spike (r = 0.78 and r = 0.66).

The trait 1000 kernels weight depends on the variety, climatic and edaphic factors, as well as on the level of applied cropping practices.

Table 1. The degree of spike infection (in %) of different winter wheat varieties caused by fungi of *Fusarium* spp.

Wheat variety (S)	Treatment (T)			$S_{\bar{x}}$
	Protection with fungicides	Artificial inoculation with fungi of *Fusarium* spp.	Spontaneous infection	
Jugoslavija	0.2	85	0.7	28.6
Francuska	0	65	0.3	21.8
PKB-Lepoklasa	0	100	1	33.7
$T_{\bar{x}}$	0.07	83.3	0.7	28.0

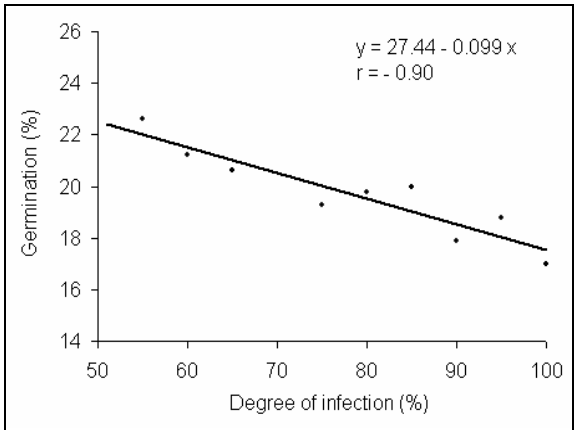

Figure 1. Regression analysis between seed germination and the degree of wheat spike infection

Regardless of the treatment in tested varieties, 1000 kernels weight ranged from 38.1 g (Francuska) to 44.2 g (Jugoslavija), and amounted to 41.0 g in the variety PKB-Lepoklasa (Protic, 2000). The regression analysis between 1000 kernels weight and the degree of infection of wheat spikes with fungi of *Fusarium* spp. expresses a weak correlation (r = 0.12, Figure 2).

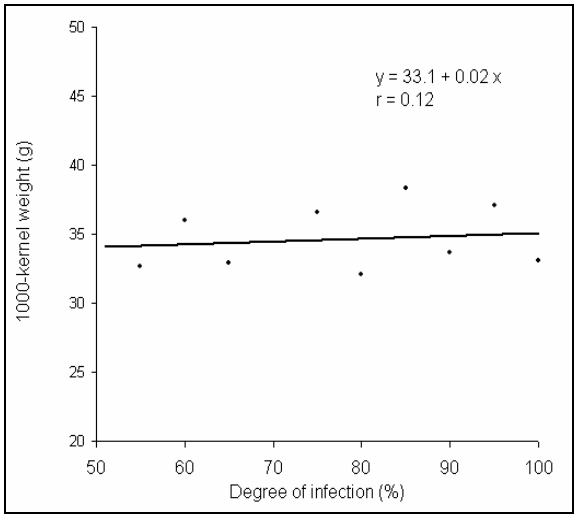

Figure 2. Regression analysis between 1000 kernels weight and the degree of wheat spike infection

During the three-year of experimentation, Golinski et al. (1997) determined that the degree of infection of wheat grain with fungi of *Fusarium* spp. ranged from 39.2 to 84.7% while 1000 kernels weight decreased between 80.5-92.5% in relation to the control.

According to results obtained by Balaš (1989), a high efficiency level in spike protection against pathogens of fusarioses was expressed by commercial preparations with two active ingredients (Impact-C) and mixture of two fungicides applied at the beginning of the wheat flowering stage. Increases of grain yield and 1000 kernels weight are correlated to the effect of wheat protection against diseases, hence a relative yield increase in relation to the control ranges from 2.1 to 14.5%. A correlation between the presence of fungi and their development in the plant in 15 resistant and susceptible winter wheat varieties inoculated with *Fusarium graminearum* and *Fusarium moniliforme* ranged from r = 0.91 to r = 0.99. Yields were in correlation with isolates (r = 0.84-0.97) (Klechkovskaya, 1997).

Šaric et al. (1984) determined 1000 kernels weight of 27.5 to 36.5 in Vojvodina, while the corresponding values for Belgrade ranged from 32.0 to 37.5 g (Šaric, 1993) and from 32.5 to 36.5 g (Mišic, 1989).

Mycotoxins synthesized by *Fusarium* spp. are stable during the production process and tend to accumulate in the products that are mainly used as food and feed. They are synthesized by fungi in cereals. Humid conditions during spring and summer favour zearalenone and deoxynivalenol, while dry conditions favour fumonisins. The molecular weight of mycotoxins is not adequately large for animal and human organisms to produce appropriate antibodies.

Zearalenone was detected only in kernels artificially inoculated with fungi of *Fusarium* spp. (Table 2). The coefficient of correlation (r = 0.46, Figure 3) was established by the regression analysis for the zearalenone concentration in wheat kernels and the degree of wheat spike infection.

In spite of its nonsteroidal structure, zearalenone expresses an oestrogenic activity against the uterus tissue stimulating the protein synthesis (Kawabata et al., 1982). Swines are very sensitive to zearalenone (Hagler et al., 1980).

Szuets et al. (1997) reported that the premature puberty of children in Hungary was attributable to zearalenone, which was proved in the se-

rum of patients. This phenomenon was also observed in Northern Italy (Fara et al., 1979). Fumonisins were detected in kernels artificially inoculated with fungi.

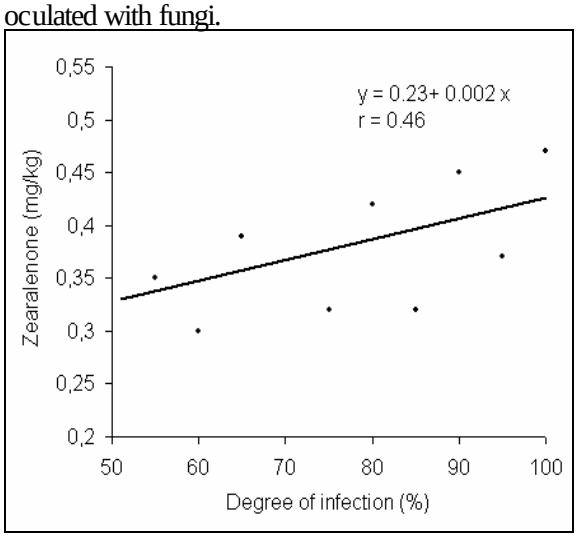

Figure 3. Regression analysis between zearalenone content in wheat kernels and the degree of wheat spike infection

The lowest fumonisin concentrations were determined in kernels of the variety Serbia and Montenegro. On the other hand, the highest fumonisin concentrations were detected in kernels of the variety Francuska and they amounted to 0.62 mg kg^{-1} on an average, while the highest values were observed in the third year of investigation (0.64 mg kg^{-1}) which was characterized by high relative air humidity and high temperatures (Table 2). A low negative correlation was estimated for fumonisin concentrations in wheat kernels and the degree of wheat spike infection with fungi (Figure 4).

Fusarium moniliforme and *Fusarium proliferatum* are fumonisin producers. Food and feed contamination with fumonisins is a constant risk, as *Fusarium moniliforme* is universally distributed in many economically important plants. *Fusarium moniliforme* is a main pathogen in maize under the tropics, humid and moderate regions. The temperature and humidity are the most important abiotic factors for toxigenesis.

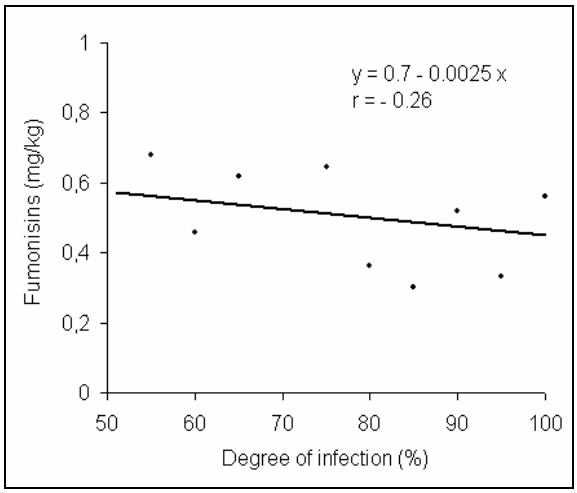

Figure 4. Regression analysis between fumonisin content in wheat kernels and the degree of wheat spike infection

Maize-based food, primarily maize flour and grits, in many countries often contains fumonisins (Pittet et al., 1992; Stock and Eppley, 1992). It is well known that fumonisin B1 causes LEM in horses, lung edema and hydrothorax in swine, liver cancer in rats, weight decrease in chickens, and also oesophagus cancer in humans (Kellerman et al., 1990; Gelderblom et al., 1991).

Deoxynivalenol was synthesized only in kernels of all varieties that had been artificially inoculated with fungi of *Fusarium* spp. (Table 2). A low correlation (r = 0.20) was found between the deoxynivalenol content in wheat kernels and the degree of wheat spike infection (Figure 5). In all animals, trichothecenes affect active cells division such as: epithelium, thymus, spleen, medulla oblongata, ovary, testicles and lymph nodes. The effect of trichothecenes depends on their concentration. Their high affinity to ribosome of eu-

Table 2. The content of mycotoxins (mg kg^{-1}) in wheat grain

Variety	Treatment	Mycotoxins		
		zearalenone	fumonisin	deoxynivalenol
Jugoslavija	Artificial inoculation with fungi of *Fusarium* spp.	0.32	0.30	0.010
	Kernels completely (100%) infected with fungi of *Fusarium* spp.	0.38	0.40	0.015
Francuska	Artificial inoculation with fungi of *Fusarium* spp.	0.39	0.62	0.015
	Kernels completely (100%) infected with fungi of *Fusarium* spp	0.62	0.80	0.018
PKB-	Artificial inoculation with fungi of *Fusa-*	0.47	0.56	0.017

caryotic cells, especially 60 S, in which peptidyl transferase activity proceeds, is the most important. The active centre can be masked by the components of a trichothecenes molecule.

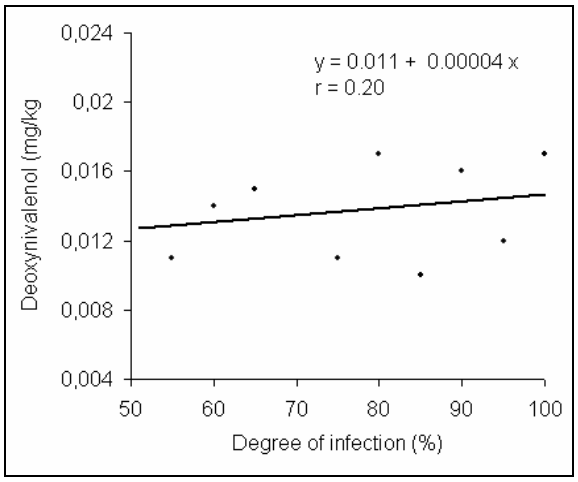

Figure 5. Regression analysis between deoxynivalenol content in wheat kernels and the degree of wheat spike infection

The acute and chronic toxicity of trichothecenes means disturbance of lymphatic tissue and changes of a immune response. A frequent introduction of trichothecenes enhances susceptibility of animals to infections with microorganisms (Ueno, 1980.) In relation to human health, it is necessary to find out if deoxynivalenol is unchanged, or, is, as a potentially toxic metabolite, included into the food chain of humans via animal products if animals were fed with deoxynivalenol.

Deoxynivalenol is phytotoxic due to the inhibition of eucaryotic protein synthesis (Casale and Hart, 1988). The concentration of 0.3 mg l^{1} inhibits a coleoptile growth of wheat varieties susceptible to fusariosis, while the 10-100 fold-higher concentration of this trichothecene is necessary for the growth of more resistant varieties. The deoxynivalenol concentration higher than 3 mg l^{1} inhibits the growth of wheat root (Shimada and Otani, 1990).

CONCLUSIONS

The following conclusions can be drawn on the basis of the results related to the effects of mycotoxins on germination and 1000 kernels weight:

- fungi *Fusarium graminearum* and *Fusarium moniliforme* were dominant in the selected winter wheat genotypes, while the fungus *Fusarium proliferatum* was detected to a smaller extent;
- the variety PKB-Lepoklasa was completely (100%) infected with fungi of *Fusarium* spp. under artificial inoculation treatment during all three years of investigation;
- the coefficient of correlation between germination and the degree of infection amounted to r = -0.90;
- the regression analysis between 1000 kernels weight and the degree of infection shows a low correlation (r = 0.12);
- mycotoxins were observed only in treatments with artificial inoculation with fungi of *Fusarium* spp. The highest zearalenone concentrations were determined in kernels of the variety PKB-Lepoklasa. The degree of plant infection and zearalenone concentrations were positively correlated (r = 0.46). On the other hand, high negative values of correlation coefficients were estimated for zearalenone concentrations and 1000 kernels weight (r = -0.83) and germination (r = -0.80);
- fumonisins were mainly detected in wheat kernels especially of the variety Francuska. Fumonisin concentrations were lowly correlated with the degree of infection (r = -0.26) and germination (r = -0.19) and highly but negatively correlated with 1000 kernels weight (r = -0.95) and germination (r = -0.60);
- concentrations of mycotoxins were higher in kernels that were completely (100%) infected with fungi of *Fusarium* spp. The content of trichothecene type B-deoxynivalenol was the highest in the variety PKB-Lepoklasa. Deoxynivalenol concentrations were not correlated with the degree of infection (r = 0.20).

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