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# ABILITIY OF *Fusarium graminearum* ISOLATES TO PRODUCE DEOXYNIVALENOL

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## ABSTRACT
*F. graminearum*, predominant *Fusarium* species on wheat in Croatia, produce several mycotoxins of which deoxynivalenol (DON) is most frequantly found in wheat grains and flour. Food and feed contaminated by mycotoxins represent health risks for humans and animals. Fourteen isolates of *Fusarium graminearum* were investigated for their pathogenicity on wheat ears as well as for deoxynivalenol production. DON was detected in all 14 samples at concentrations ranging from 2.51 to 12.14 mg $kg^{-1}$.

**Key words:** *F. graminearum*, wheat, DON

## INTRODUCTION
Fusarium head blight of wheat result in reduction in crop yield and seed and grain quality, especially through the production toxic secondary metabolites (mycotoxins) such as trichothecenes, moniliformin and zearalenone (Chelkowski 1998, Nicholson et al. 2003). Presence of mycotoxins in feeds and foods is often associated with acute or chronic mycotoxicoses in livestock and could threaten human health (Marasas et al. 1984). The disease is caused by several *Fusarium species* but the principal pathogen associated with Fusarium head blight in Croatia is *Fusarium graminearum* Schw. This species is a very important producer of B-type trichothecene mycotoxins, particularly deoxynivalenol, in harvested grains.
The objectives of this study were to assess the pathogenicity of *F. graminearum* isolates isolated from wheat ears and determine their potential to produce DON.

## MATERIAL AND METHODS

### *Artificial inoculation*
A total of 14 isolates of *F. graminearum* originated from wheat were used in testing their pathogenicity and ability to produce DON. Isolates were collected from ten sites of Croatia (Vukovar, Županja, Vinkovci, Đakovo, Osijek, Beli Manastir, Našice, Slavonski Brod, Kutjevo, Donji Miholjac).
The wheat heads (4x50 ears, cv. Monika) were sprayed at mid-anthesis (Zadoks' growth stage 65) with a hand sprayer, ensuring that all spikelets were exposed to the

inoculum. Inoculated heads were covered with PVC bags for 24 hours to ensure high relative humidity. The heads of control plants were sprayed with distilled water. At the stage of full ripening ears were manually harvested, thousand kernel weight (TKW) and percent of Fusarium demaged kernel (FDK) were determined, as well as DON content.

***Mycotoxin analyses***

All solvents used for the DON extraction from cereals samples, as well as for the mobile phase preparation were of HPLC grade. All chemicals used in the investigation were of reagent grade. Solutions were prepared in doubly deionized water except when stated otherwise. Two measurements for each isolate were done.

DON (Biopure, Tulln, Austria) was purchased as an analytical standard. Calibrant solution was prepared in ethyl acetate-methanol (19:1, v/v) at the concentration of 85.05 μg/ml from crystalline substance according to AOAC method 986.17. Stock solution containing DON at 17.01 μg/ml was prepared by measuring 2.00 ml calibrant solution of DON into a 10 ml volumetric flask and diluting to volume with ethyl acetate-methanol (19:1, v/v). Working calibrant solutions were prepared by evaporation of the appropriate volume of the stock solution and dilution with the appropriate volume of methanol. Standard solutions were stored at 4 °C.

Multifunctional columns MycoSep™ 225 (Romer Labs, Inc., Washington, USA) column was used for purification, and in this case 25.0 g of the sample were extracted with 100 ml of acetonitrile-water (84:16, v/v) and shaken on a magnetic stirrer for 60 minutes. After filtration through Advantec filter paper, 6.0 ml of the extract were applied to the MycoSep 225 column. The pushing speed for the solution was 2 $cm^3$/min. 3.0 $cm^3$ of the cleaned-up extract was evaporated to dryness, dissolved in 3 ml of ethyl acetate and quantitatively transferred to an evaporation vessel by triple washing with 1.5 ml ethyl acetate. The eluate was evaporated just to dryness. Evaporated residue was redissolved in 300 μl methanol, and a 15 μl aliquot of the solution was injected into the LC system.

The equipment consisted of an LC system – HP 1090 Liquid Chromatograph (Hewlett Packard, Palo Alto, CA, USA) with a DAD detector (Hewlett Packard, Palo Alto, CA, USA) and a column Hypersil ODS (100 x 4.6 mm i.d., particle size 5 μm, Agilent Technologies, USA). LC analysis of DON was performed after evaporation, the residue was redissolved in 300 μl methanol, and a 15 μl aliquot of the solution was injected into the LC system. A mobile phase consisting of a mixture of acetonitrile-water (16:84, v/v) was used at 0.6 ml/min. UV detection was performed at 220 nm. The mobile phase was filtered through a 0.45 μm membrane (Aura industries, TFM, Hewlett Packard).

Statistical analyses

The obtained data were analyzed with Statistica for Windows v. 6.0.

## RESULTS AND DISSCUSION

After wheat head inoculation with 14 *F. graminearum* isolates the results of yield reductions for each isolate are presented in Table 1.

All investigated isolates reduced TKW and produced DON. TKW was reduced from 30.62 to 53.24%, if compared to control. DON contamination level varied from isolate

to isolate and was in range from 2.51 to 12.14 mg $kg^{-1}$. This results are in agreement with previous data indicating that isolates of one fungi species could produce unequal amount of mycotoxin (Mirocha et al. 1989, Windels et al. 1989, Bagi et al. 2000, Ferreira Geraldo et al. 2006).

*Table 1. Influence of F. graminearum isolates on TKW, percentage of FDK and DON content in wheat grain samples*

| Isolate | FDK (%) | TKW (% of control) | DON (mg $kg^{-1}$) |
|---|---|---|---|
| Control | 0.56 | 100.00 | not detected |
| FGwg1 | 55.25 | 46.76 | 10.18 |
| FGwg2 | 56.70 | 53.33 | 8.59 |
| FGwg3 | 52.15 | 65.35 | 3.96 |
| FGwg4 | 50.97 | 64.29 | 2.51 |
| FGwg5 | 51.35 | 58.05 | 2.74 |
| FGwg6 | 53.05 | 58.17 | 4.51 |
| FGwg7 | 57.29 | 50.95 | 12.04 |
| FGwg8 | 57.65 | 53.26 | 12.14 |
| FGwg9 | 52.40 | 55.21 | 8.67 |
| FGwg10 | 52.30 | 54.95 | 5.55 |
| FGwg11 | 55.75 | 60.16 | 3.72 |
| FGwg12 | 53.15 | 56.34 | 3.40 |
| FGwg12 | 50.86 | 61.95 | 3.26 |
| FGwg14 | 51.35 | 69.38 | 2.64 |

Small grain cereals, including wheat, are susceptible to head infection under wet, warm conditions from fead emergence onwards. Early infection results in light, shrivelled grains (*Fusarium* damaged or scabby kernels) covered with white or pink mycelium. Our investigation showed that percentage of FDK depending from isolate to isolate and was between 50.86 and 57.65.

For investigated *F. graminearum* isolates percentage of FDK correlated negatively ($r = -0.67$, $P<0.05$) with TKW and correlated positively ($r = 0.81$, $P<0.05$) with DON content. Similar observations were reported by Wong et al. (1995), Muthomi et al. (2000) and Wanyoike et al. (2002).

## CONCLUSIONS

In this study we investigate pathogenicity and ability to produce DON of 14 *F. graminearum* isolates. All isolates were pathogenic to wheat ears and produce DON in range from 2.51 to 12.14 mg $kg^{-1}$. In the next year we will carry out the same investigation with *F. graminearum* isolates from maize and weeds.

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