BIOLOGICAL TREATMENT WITH DEBARYOMYCES HANSENII REDUCES THE CONTENT OF DEOXYNIVALENOL AND CULMORIN IN DURUM WHEAT GRAIN Urszula Wachowska¹, Marian Wiwart¹, Elżbieta Suchowilska¹, Michael Sulok², Wolfgang Kandler², Rudolf Krska², Dariusz Gontarz³

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Durum wheat (Triticum turgidum ssp. durum) is extremely susceptible to Fusarium head blight (FHB), and breeding new varieties with enhanced FHB resistance are rare in the primary gene pool of tetraploid wheats. Fungi of the genus Fusarium cause

Fusarium head blight (FHB) and produce numerous secondary metabolites, thus decreasing the quality of durum wheat grain. Trichothecenes, including deoxynivalenol (DON), are the most commonly occurring and the most toxicologically important mycotoxins. Culmorin (CUL, a tricyclic sesquiterpene diol), which remains weakly researched, enhances the phytotoxic effects of DON on wheat seedlings. The control of FHB with the use of fungicides is difficult due to their limited effectiveness. Biocontrol agents such as yeasts, including *Debaryomyces hansenii*, applied during the growing season to suppress *F. graminearum*, could pose a viable alternative to fungicides. **The aim** of this study was to evaluate the concentration of DON and CUL in *Triticum turgidum* ssp. *durum* grain during infection with *F. graminearum* after biocontrol treatment.

Methods

Field experiments were conducted to evaluate the applicability of a *Debaryomyces hansenii* isolate (NCBI GenBank accession number KX444668) for reducing the concentrations of DON and CUL in the grain of 5 spring cultivars of durum wheat grown at two locations in Poland (Bałcyny near Olsztyn and Niedrzwica Kościelna near Lublin). *D. hansenii* cells were applied two times during the flowering stage of wheat. Flowering spikes were inoculated with an aqueous suspension of *F. graminearum* spores DON genotype (Fig. 4) . The concentration of fungal cells in the suspension was 10⁴ cells in 1 cm³ of water. Unprotected plants were the control. Durum wheat spikes (Fig. 1,3) were inoculated with a spore suspension of *F. graminearum* DON genotype (Fig. 4) in a field, 24 hours after the application of the biocontrol agent (Fig. 2, 4). The analysis of the mycotosin profile by LC-MS / MS was performed by IFA-Tulln.

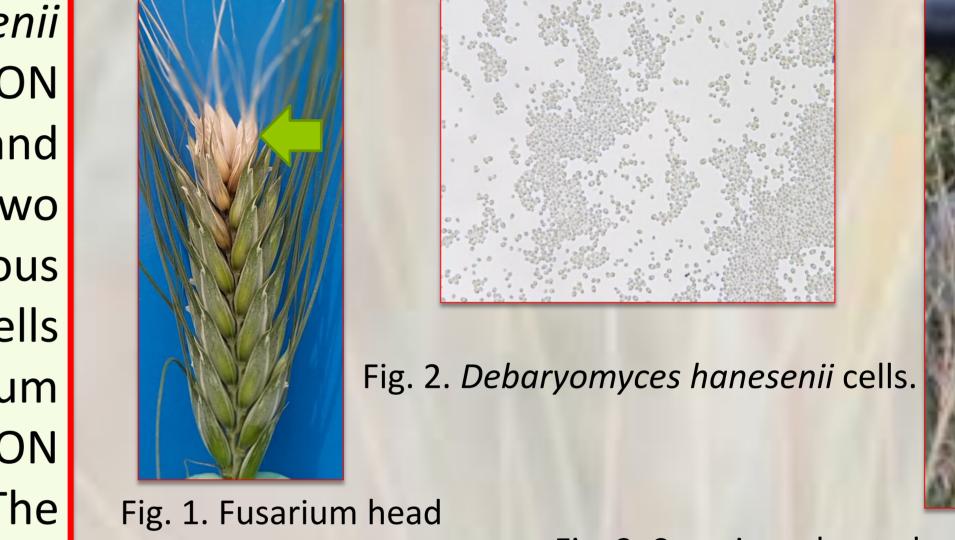


Fig. 3. Spraying wheat plants with *D. hansenii* suspension

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Table 1. Characteristics of spring durum wheat cultivars

Cultivar	DON accumulation rate	Breeding	FHB resistance (9-point scale)
Duragold	medium	Slovakia	6.8
Duranegra	medium	Slovakia	6.4
Durasol	high	Germany	4
Floradur	medium	Germany	6
Tamadur	medium	Germany	6

Fg1 Fg2 Fg3 Fg4 Fg5 Fg6 Fg7 Fg8 Fg9 Fg10 Fg11 Fg12

blight (FHB) symptoms.

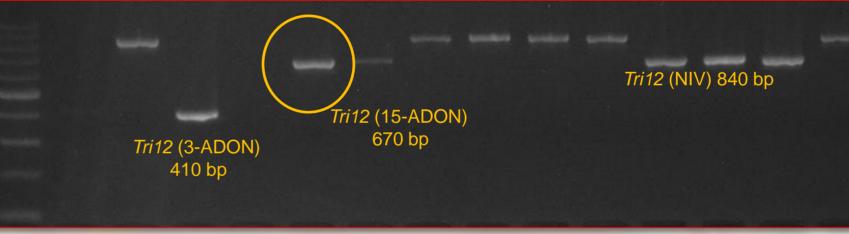


Fig. 4. Genotypes of*F. graminearum*. PCR products:M-marker 100-1000bp, lines3,4,6-12 *F. graminearum* isolates.



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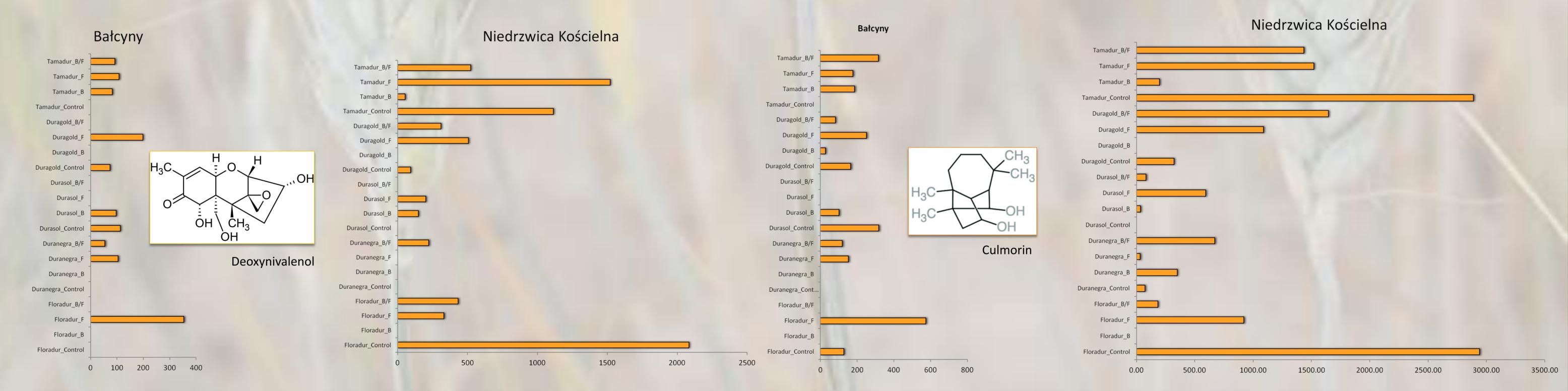


Fig. 5. Concentration DON (μ g/kg) in durum wheat grains of biological treatment (B) and inoculated *F. graminearum* (F).

Fig.6. Concentration DON (μ g/kg) in durum wheat grains of biological treatment (B) and inoculated *F. graminearum* (F).

 DON and DON-3-glucoside (D3G) were detected in 26 out of the 40 examined samples. The DON content of grain was particularly high in the control samples of cv. Floradur and Tamadur in Niedrzwica Kościelna (2081 and 1113 μg/kg, respectively). In most cases, the inoculation of wheat spikes with *F. graminearum* increased the DON content of grain up to 1519 μg/kg.

 Biological treatment involving a cell suspension of D. hansenii isolate decreased DON concentrations in grain, compared with control samples and grain samples from spikes inoculated with F. graminearum (Fig.5).

The CUL content of grain was usually higher than DON content. A clear positive correlation was found between CUL and DON concentrations.
CUL, 15-hydroxyculmorin and 5-hydroxyculmorin were detected in 35 grain samples, and their content was high (up to 2887.87 µg/kg) in cv.
Tamadur and Duragol in Niedrzwica Kościelna. At this location, the biological treatment exerted a particularly high inhibitory effect on CUL production by *Fusarium* spp. (Fig.6).