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Occurrence of *Fusarium* species isolated from winter wheat and barley grains in Croatia

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Abstract

Fusarium head blight of small grain cereals is an important disease occurring across a broad range of environmental conditions. Several *Fusarium* species may be associated with the disease, including species that are potentially toxigenic. During the harvest, shriveled infected grains that are light enough to be eliminated from the combine harvester cause direct yield loss. The grains that are less severely infected are lighter than uninfected grains and affect the «thousand kernel weight». The objectives of our study were to estimate occurrence of *Fusarium* species on wheat and barley grains and evaluate the influence of *Fusarium* species on yield and thousand kernel weight. A total of 1,352 winter wheat samples and 494 barley samples were collected and analyzed from 1998 to 2005. Eight *Fusarium* species were isolated from wheat grains and seven from barley grains. The most frequent species on wheat and barley grains was *F. graminearum* Schw. (52.66 % and 39.87 % respectively). According to its occurrence on wheat grains, *F. graminearum* was followed by *F. moniliforme* Sheld. (16.35 %), *F. subglutinans* (Wollenw. & Reink.) Nelson, Toussoun & Marasas (13.68 %), *F. avenaceum* (Fr.) Sacc. (6.88 %), *F. culmorum* (W.G. Smith) Sacc. (3.85 %), *F. poae* (Peck) Wollenw. (3.40 %), *F.*

sporotrichioides Sherb. (2.00 %) and *F. oxysporum* Schlecht emend. Snyder & Hans. (1.18 %). On barley grains except *F. graminearum* the following *Fusarium* species were isolated: *F. moniliforme* (16.35 %), *F. subglutinans* (17.00 %), *F. avenaceum* (11.34 %), *F. poae* (4.25 %), *F. oxysporum* (3.85 %) and *F. sporotrichioides* (3.04 %). Isolates of *F. graminearum* were the most pathogenic to wheat and barley grains. Depending on isolates, percentage of infected seeds was between 63.50 and 87.75 %. Out of a total of infected seeds, only 30.05 to 49.77 % germinated. Weight of 1,000 seeds was reduced from 35.05 to 55.00 %, if compared to control.

Key words: *Fusarium*, wheat, barley, grain

Introduction

Fusarium head blight (FHB) is an important disease of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) occurring across a broad range of environmental conditions. Large level of precipitation and air temperature between 22 and 26 °C from the flowering stage to the end of vegetation favour infection of wheat and barley by *Fusarium* species (Teich 1989). Fungal infection of grain in the field can result in yield loss

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and quality reduction (Nijs et al., 1996; Mesterhazy et al., 2003). Several *Fusarium* species may be associated with the disease, including species which can produce secondary metabolites some of which can unfavorably affect human and animal health (Marasas, 1984). The most important pathogen of FHB of wheat and barley in Croatia is *Fusarium graminearum* Schw., a very important producer of B-type trichothecene mycotoxins, particularly deoxynivalenol, in harvested grains. The most obvious symptoms of FHB are premature bleaching and blighting of wheat spikelets. During the harvest, shrivled infected grains that are light enough to be eliminated from the combine harvester cause direct yield loss. The grains that are less severely infected are lighter than uninfected grains and affect the «thousand kernel weight».

The objectives of our study were to estimate occurrence of *Fusarium* species on wheat and barley grains and evaluate the influence of *Fusarium* species on yield and thousand kernel weight.

Material and methods

Wheat and barley grains were collected during an eight-year period (1998-2005) in ten sites of Croatia (Vukovar, Zupanja, Vinkovci, Djakovo, Osijek, Beli Manastir, Nasice, Slavonski Brod, Kutjevo, Donji Miholjac).

Wheat and barley grains were disinfected, put in moist chambers and incubated in thermostat at 20 ± 1 °C, in regime of 12 hours light / 12 hours dark. After five days, developed mycelium with its spores was examined with stereomicroscope, and direct microscopic observations were done with light microscope. The mycelia and spores identified as *Fusarium* spp. were used for production of the basic cultures on potato-dextrose agar (PDA; Difco Laboratories, Detroit, MI). Parts of developed mycelium with and without conidia, which were assumed to belong to *Fusarium* species, were transferred to PDA, carnation leaf agar (CLA) and water agar. Identification of *Fusarium* species was based on colony and conidial morphology, using dissecting

and compound microscopes and a standard identification key.

A total of 38 isolates of *Fusarium* species (10 of *F. graminearum*, 7 of *F. avenaceum* (Fr.) Sacc., 5 of *F. moniliforme* Sheld. and *F. subglutinans* (Wollenw. & Reink.) Nelson, Toussoun & Marasas, 4 of *F. poae* (Peck) Wollenw., 3 of *F. oxysporum* Schlecht emend. Snyder & Hans., and 2 isolates of *F. sporotrichioides* Sherb. and *F. culmorum* (W.G. Smith) Sacc.) were used in testing their pathogenicity to wheat and barley ears. The wheat and barley heads were sprayed at mid-anthesis with a hand sprayer, ensuring that all spikelets were exposed to the inoculum. The ears of control plants were sprayed with distilled water. For each isolate 4x50 heads were sprayed with 10 ml of suspension and then covered with PVC bags for 24 hours to ensure high relative humidity. At the stage of full ripening, 40 ears were taken per each repetition, they were manually harvested, thousand kernel weight (TKW) was determined, as well as percentage of infected seeds and their germination.

Results

A total of 1,352 winter wheat samples and 494 barley samples were collected and analyzed from 1998 to 2005. Eight *Fusarium* species were isolated from wheat grains and seven from barley grains.

The most frequent species on wheat and barley grains was *F. graminearum* (52.66 % and 39.87 % respectively). According to its occurrence on wheat grains, *F. graminearum* was followed by *F. moniliforme* (16.35 %), *F. subglutinans* (13.68 %), *F. avenaceum* (6.88 %), *F. culmorum* (3.85 %), *F. poae* (3.40 %), *F. sporotrichioides* (2.00 %) and *F. oxysporum* (1.18 %). On barley grains except *F. graminearum* the following *Fusarium* species were isolated: *F. moniliforme* (16.35 %), *F. subglutinans* (17.00 %), *F. avenaceum* (11.34 %), *F. poae* (4.25 %), *F. oxysporum* (3.85 %) and *F. sporotrichioides* (3.04 %).

Isolates of *F. graminearum* were the most

pathogenic to wheat and barley grains. Depending on isolates, percentage of infected seeds was between 63.50 and 87.75 %. Out of a total of infected seeds, only 30.05 to 49.77 % germinated. TKW was reduced from 35.05 to 55.00 %, if compared to control. Table 1 presents

Table 1. Pathogenicity of *Fusarium* spp. to wheat and barley ears.

<i>Fusarium</i> species	Isolate	Pathogenicity to wheat			Pathogenicity to barley		
		Infected seed (%)	Germinated seed % (of infected ones)	Weight 1000 seed (% of control)	Infected seed (%)	Germinated seed % (of infected ones)	Weight 1000 seed (% of control)
<i>Fusarium graminearum</i>	FGW1	78.00	40.80	45.90	75.25	43.00	46.25
	FGW2	66.25	43.85	53.80	60.75	44.50	49.55
	FGW3	78.50	46.00	55.78	79.00	42.50	52.35
	FGW4	68.00	39.25	49.66	64.25	38.75	53.55
	FGW5	67.50	41.00	53.95	60.50	39.25	48.65
	FGB1	75.50	35.75	50.50	77.50	38.25	55.37
	FGB2	81.75	39.25	52.60	80.50	41.50	54.00
	FGB3	89.25	33.25	52.75	90.25	35.50	50.15
	FGB4	74.00	35.00	50.55	70.75	37.75	47.68
	FGB5	80.75	35.75	49.25	82.50	39.00	51.39
<i>Fusarium subglutinans</i>	FSW1	55.25	69.00	90.55	52.50	65.25	88.00
	FSW2	61.50	72.50	88.45	59.75	74.50	86.32
	FSW3	58.00	65.00	86.53	56.00	69.25	89.98
	FSB1	63.25	70.00	90.25	59.00	72.50	86.35
	FSB2	57.75	66.50	86.00	59.25	69.00	91.20
<i>Fusarium avenaceum</i>	FAW1	75.25	56.75	85.21	79.75	59.75	89.25
	FAW2	70.50	60.00	89.45	68.50	57.25	83.55
	FAW3	76.75	50.50	82.30	73.50	55.25	87.66
	FAW4	80.2	65.50	89.00	78.75	66.00	86.10
	FAW5	69.50	57.25	87.68	66.25	60.25	85.80
	FAB1	60.50	57.00	84.22	64.50	63.25	81.60
	FAB2	59.75	61.50	87.50	61.25	58.50	89.44
<i>Fusarium moniliforme</i>	FMW1	55.50	77.00	86.10	53.50	82.25	88.54
	FMW2	47.75	81.50	90.95	49.25	78.25	92.15
	FMB1	45.25	83.50	85.36	46.75	76.75	84.95
	FMB2	49.00	75.25	91.10	51.50	73.25	89.86
	FMB3	52.50	77.75	87.26	54.75	74.25	90.05
<i>Fusarium poeae</i>	FPW1	50.25	91.25	93.28	48.75	93.50	95.65
	FPW2	44.75	90.75	95.55	45.75	92.50	92.54
	FPB1	40.00	85.75	96.00	50.25	88.50	95.53
	FPB2	46.00	87.50	93.78	49.25	85.7	90.83
<i>Fusarium culmorum</i>	FCW1	70.75	45.75	65.78	78.25	49.25	62.50
	FCW2	77.75	47.25	70.00	73.50	46.00	68.78
<i>Fusarium sporotrichioides</i>	FSPW1	55.25	58.00	84.70	59.75	65.00	82.35
	FSPB1	58.75	62.50	89.25	55.00	61.75	88.70
<i>Fusarium oxysporum</i>	FOW1	55.50	73.75	84.38	53.75	70.25	86.17
	FOB1	61.25	67.50	83.25	60.75	71.75	80.97
	FOB2	57.75	70.00	89.00	55.25	75.25	90.26

results of pathogenicity of *Fusarium* spp. isolates to wheat and barley.

Discussion

Fusarium head blight is a fungal disease that infects cereal crops world-wide in a variety of climatic regions and is responsible for significant reductions in yield and quality (Ireta and Gilchrist, 1994). Grain contaminated with *Fusarium* species which produce mycotoxins may be completely unusable for feed, food and malting purposes. Several studies have shown that up to 17 species of *Fusarium* can be readily isolated from grain cereals (Parry et al., 1995). *F. graminearum* is one of the most frequently found *Fusarium* species on European cereals where it is more common in wet and warm climate of Central and Southern Europe. Common species in cooler regions are *F. culmorum* and *F. avenaceum*. Large number of wheat and barley grain samples provided an exhaustive picture of the variety of the *Fusarium* spp. that naturally infect cereals. The *Fusarium* species predominantly found in Croatian cereal samples are *F. graminearum*, *F. moniliforme* and *F. subglutinans*. Yield reductions in small grain cereals following naturally occurring epidemics of FHB have been observed in many countries with estimated losses in the order of 15-50 % (Snijders 1989, McMullen et al., 1997). More precise data relating to the effects of FHB on wheat and barley yield have been obtained from inoculated trials (Mesterhazy, 1978, Häni, 1981). After artificial inoculating wheat ears with *F. culmorum* and *M. nivale*, Häni (1981) observed grain yield reductions of 15 % and 60 %, respectively. Artificial infection of wheat ears with *F. graminearum* reduced TKW up to 36 % (Wong et al., 1992).

Wheat and barley grains infected by *Fusarium* species, specially by *F. graminearum* and *F. culmorum*, is associated with reduced germination. In the work reported in this study germination of grains infected by *F. graminearum* as the most pathogenic species was reduced from 54.00 to 66.75 % if compared to control.

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