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PATHOGENICITY OF *FUSARIUM* SPP. ISOLATED FROM WEEDS AND PLANT DEBRIS IN EASTERN CROATIA TO WHEAT AND MAIZE

Jelena Ilić, Jasenka Ćosić, Draženka Jurković, Karolina Vrandečić

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SUMMARY

Pathogenicity of thirty isolates representing 14 *Fusarium* species isolated from weeds and plant debris in eastern Croatia was investigated in the laboratory. Pathogenicity tests were performed on wheat and maize seedlings. The most pathogenic *Fusarium* spp. was *F. graminearum* isolated from *Amaranthus retroflexus*, *Abutilon theophrasti* and *Chenopodium album*. There was a noticeable inter- and intraspecies variability in pathogenicity towards wheat and maize. Isolates of *F. solani* from *Sonchus arvensis* and *F. verticillioides* from *C. album* were highly pathogenic to wheat seedlings and a pathogenic to maize seedlings. Isolates of *F. venenatum* were very pathogenic to wheat and maize being the first report about pathogenicity of this species. This experiment proves that weeds and plant debris can serve as alternate hosts and source of inoculum of plant pathogens.

Key-words: pathogenicity, *Fusarium* spp., weeds, plant debris

INTRODUCTION

Genus *Fusarium* belongs to class *Hyphomycetes*, order *Hyphales* including over 1000 species, both saprophytic and phytopathogenic. *Fusarium* species are present in all parts of the world, some of them are ubiquitous, while other can live only in specific climate conditions. They were isolated from eternal ice on Arctic and sand of Sahara desert (Booth 1971). Pathogenic *Fusarium* species cause diseases, mycosis of plants and mycotoxicosis of animals and humans. Many consider this fungal genus as the one of the most important with species that can significantly influence yield and quality of cultivated plants. It is supposed that the number of *Fusarium* species is much higher than currently reported.

Depending on the plant species and the stage of plant development *Fusarium* spp. can cause various plant diseases: seed rot, seedlings blight, root rot, bulb rot, rot of lower stem part, head blight, ear rot, tracheo-fusariosis (Lević, 2008).

Fusarium species belong to the most commonly isolated plant pathogens and, as many other fungi, can survive on different substrates. For example they were found in conserved food, stored chemicals and fuel tanks (Gaylarde et al., 1999). Numerous weed species can serve as alternate hosts to fungal plant pathogens

and several authors have proved that fungal isolates from symptomless weed can be pathogenic to cultivated plants (Helbig and Carroll, 1984; Jenkinson and Parry 1994; Inch and Gilbert 2003).

Among other pathogenic fungal species, *Fusarium* spp. can also often be found on plant debris (Parry et al., 1995; Vrandečić et al., 2006; Postić et al., 2012). In Croatia, several authors have investigated this issue and determined the significant presence of maize, wheat, sunflower and soybean pathogens and their pathogenicity to cultivated plants (Vrandečić et al., 2003; Svitlica et al., 2008; Ćosić et al., 2009).

Plant debris can also serve as hosts for plant pathogens survival during unfavourable conditions. Pereyra et al. (2004) investigated survival and inoculum production of *Gibberella zeae* (Schw.) Petch on wheat debris in the field and determined that fungi can survive for more than two years after the harvest.

Cotten and Munkvold (1998) investigated survival of *F. moniliforme* Sheld., *F. proliferatum* (Matsushima) Nirenberg and *F. subglutinans* (Wollenw. & Rein.) Nelson,

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Tousson & Marasas on maize debris in the field and determined that they can survive for at least 630 days and in such way serve as a long term inoculum source for infection of cultivated plants.

In previous research we isolated *Fusarium* spp. from plant debris and symptomless weeds from the field. The objective of this research was to investigate pathogenicity of selected *Fusarium* spp. isolated from weeds and plant debris to wheat and maize seedlings.

MATERIAL AND METHODS

In the laboratory trials we investigated the pathogenicity of 30 monosporic isolates of *Fusarium* spp. representing 14 *Fusarium* species: *Fusarium acuminatum* Ellis & Everhart, *Fusarium avenaceum* (Fries) Sacc., *Fusarium concolor* Reinking, *Fusarium cro-*

okwellense Burgess, Nelson & Tousson *Fusarium equiseti* (Corda) Saccardo, *Fusarium graminearum* Schw., *Fusarium oxysporum* Schlech. emend. Snyder & Hansen, *Fusarium proliferatum* (Matsushima), *Fusarium semitectum* Berk. & Rav., *Fusarium solani* (Martius) Appel & Wollenw. emend. Snyder & Hansen, *Fusarium sporotrichioides* Sherb., *Fusarium subglutinans* (Wollenw. & Rein.) Nelson, Tousson & Marasas, *Fusarium venenatum* Nirenberg and *Fusarium verticillioides* (Sacc.) Nirenberg. Isolates originated from the hosts *Abutilon theophrasti* Med., *Agrostemma githago* L., *Amaranthus retroflexus* L., *Ambrosia artemisiifolia* L., *Arctium lappa* L., *Capsella bursa pastoris* L. Medik., *Chenopodium album* L., *Sonchus arvensis* L., *Sorghum halepense* (L.) Pers. and maize debris (Table 1) were collected from agricultural fields on 12 locations in Eastern Croatia.

Table 1. *Fusarium* spp. used for pathogenicity tests and their hosts

Tablica 1. *Fusarium* spp. primijenjene za test patogenosti i njihovi domaćini

<i>Fusarium</i> spp.	Host - Domaćin	<i>Fusarium</i> spp.	Host - Domaćin
<i>F. acuminatum</i> 88	<i>A. theophrasti</i>	<i>F. proliferatum</i> 101	<i>C. album</i>
<i>F. acuminatum</i> 265	<i>C. bursa pastoris</i>	<i>F. semitectum</i> 125	<i>A. theophrasti</i>
<i>F. avenaceum</i> 312	<i>A. githago</i>	<i>F. solani</i> 112	<i>C. album</i>
<i>F. concolor</i> 282	maize debris	<i>F. solani</i> 149	<i>S. arvensis</i>
<i>F. crookwellense</i> 271	<i>A. lappa</i>	<i>F. solani</i> 185	<i>A. theophrasti</i>
<i>F. equiseti</i> 50	<i>A. theophrasti</i>	<i>F. sporotrichioides</i> 1	maize debris
<i>F. graminearum</i> 92	<i>A. theophrasti</i>	<i>F. sporotrichioides</i> 4	maize debris
<i>F. graminearum</i> 249	<i>A. retroflexus</i>	<i>F. subglutinans</i> 91	<i>A. theophrasti</i>
<i>F. graminearum</i> 277	<i>C. album</i>	<i>F. subglutinans</i> 111	<i>C. album</i>
<i>F. oxysporum</i> 61	<i>A. theophrasti</i>	<i>F. venenatum</i> 21	<i>A. theophrasti</i>
<i>F. oxysporum</i> 87	<i>A. theophrasti</i>	<i>F. venenatum</i> 24	maize debris
<i>F. oxysporum</i> 99	<i>A. artemisiifolia</i>	<i>F. venenatum</i> 193	maize debris
<i>F. oxysporum</i> 119	<i>A. artemisiifolia</i>	<i>F. verticillioides</i> 68	<i>A. retroflexus</i>
<i>F. oxysporum</i> 175	<i>S. halepense</i>	<i>F. verticillioides</i> 102	<i>C. album</i>
<i>F. proliferatum</i> 86	<i>A. theophrasti</i>	<i>F. verticillioides</i> 305	<i>A. githago</i>

Pathogenicity of selected *Fusarium* spp. were tested on wheat seedlings with the method described by Mesterhazy (1978). Pure 7-day old cultures of *Fusarium* spp. were homogenized by adding 40 ml of distilled water to each Petri dish. Ten wheat grains, previously disinfected in ethanol, were placed in each Petri dish, covered with 10 ml of mycelial suspension and incubated for 24 hours (12 hours light/12 hours dark regime) at 20°C. As a control 10 grains were covered with the equal amount of distilled water. Inoculated grains were planted into sterile sand (3 hours at 100°C), at room temperature and watered when needed. Grading of healthy and diseased seedlings was done after 15 days at scale 0 to 5 (0 – healthy seedling, 5 – rot seedling, non-germinating). Based on these grades we determined Disease Index by McKinney (1923).

$$I = \frac{\sum(n_x k)}{N_x K} \times 100$$

n – number of plants by categories

k – number of categories

N – number of all investigated plants

K – number of adopted categories

We also determined the pathogenicity of chosen *Fusarium* species to maize seedlings using the method described by Molot-Simone (1967): selected *Fusarium* spp. were grown on PDA (Potato Dextrose Agar) media and kept in a growth chamber at 22°C, with 12 hours day/12 hours night regime. After 7 days mycelial

suspension was prepared from and maize grains were inoculated in Petri dishes. The control was covered with the equal amount of distilled water. Petri dishes were kept for 48 hours at 22°C in the dark and for 72 hours at 10°C. We planted inoculated grains into pots with sand. Pots with inoculated maize grains were placed in growing chamber at 22°C and 12/12 light regime and watered with distilled water. At the stage of three leaves we removed the plants from the pots and graded root and hypocotile rot by the scale 0 to 5 (0 – healthy seedling, 5 – rot seedling, non-germinating). Based on

these grade scale the Disease Index was determined using McKinney formula (1923).

RESULTS AND DISCUSSION

All tested isolates of *Fusarium* spp. were pathogenic to wheat seedlings and the disease index was statistically significantly higher than the disease index of the control (5.5). The most pathogenic isolates were *F. graminearum* isolated from *A. retroflexus* and *A. theophrasti* and *F. solani* from *S. arvensis* with disease index 100.0. (Table 2).

Table 2. Pathogenicity of *Fusarium* isolates for wheat seedlings

Tablica 2. Patogenost *Fusarium* izolata za klijance pšenice

<i>Fusarium</i> spp.	Disease index Indeks bolesti	<i>Fusarium</i> spp.	Disease index Indeks bolesti
<i>F. graminearum</i> 249	100.0 ^A	<i>F. oxysporum</i> 87	70.0 ^{DEF}
<i>F. graminearum</i> 92	100.0 ^A	<i>F. concolor</i> 282	67.5 ^{DEFG}
<i>F. solani</i> 149	100.0 ^A	<i>F. semitectum</i> 125	66.0 ^{DEFG}
<i>F. crookwellense</i> 271	95.0 ^{AB}	<i>F. avenaceum</i> 312	60.0 ^{EFGH}
<i>F. venenatum</i> 193	90.0 ^{ABCD}	<i>F. solani</i> 112	57.5 ^{FGHI}
<i>F. venenatum</i> 21	90.0 ^{ABCD}	<i>F. acuminatum</i> 88	56.5 ^{FGHI}
<i>F. acuminatum</i> 265	82.5 ^{ABCD}	<i>F. equiseti</i> 50	51.5 ^{FGHI}
<i>F. verticillioides</i> 305	82.5 ^{ABCD}	<i>F. venenatum</i> 24	51.25 ^{FGHI}
<i>F. subglutinans</i> 91	81.5 ^{ABCD}	<i>F. oxysporum</i> 175	50.0 ^{GHI}
<i>F. proliferatum</i> 101	80.0 ^{BCD}	<i>F. proliferatum</i> 86	47.5 ^{HJJ}
<i>F. sporotrichioides</i> 1	80.0 ^{BCD}	<i>F. verticillioides</i> 68	45.0 ^{HJJ}
<i>F. graminearum</i> 277	77.5 ^{BCD}	<i>F. solani</i> 185	42.5 ^{HJJ}
<i>F. sporotrichioides</i> 4	76.0 ^{BCD}	<i>F. oxysporum</i> 99	40.0 ^{HJJ}
<i>F. oxysporum</i> 119	72.5 ^{CDE}	<i>F. oxysporum</i> 61	27.5 ^J
<i>F. subglutinans</i> 111	72.5 ^{CDE}	Control	5.5 ^K
<i>F. verticillioides</i> 102	72.5 ^{CDE}		

*values in columns marked with the same letter do not have significant difference according to LSD test ($P < 0.05$) - *vrijednosti u stupcima označene istim slovom ne razlikuju se značajno prema LSD testu ($P < 0,05$)

Regarding the pathogenicity of selected *Fusarium* isolates to maize seedlings, the most pathogenic was the isolate *F. graminearum* from *A. theophrasti* with disease index 96 (Table 3).

Table 3. Disease index of artificial infection of maize seedlings with *Fusarium* spp.

Tablica 3. Indeks bolesti umjetne infekcije klijanaca kukuruza s *Fusarium* spp.

<i>Fusarium</i> spp.	Disease index Indeks bolesti	<i>Fusarium</i> spp.	Disease index Indeks bolesti
<i>F. graminearum</i> 92	96 ^A	<i>F. proliferatum</i> 86	24.5 ^{EFGH}
<i>F. avenaceum</i> 312	95.5 ^A	<i>F. semitectum</i> 125	24 ^{EFGH}
<i>F. graminearum</i> 249	88.5 ^A	<i>F. acuminatum</i> 88	22 ^{FGHI}
<i>F. subglutinans</i> 111	86 ^A	<i>F. oxysporum</i> 175	20 ^{FGHI}
<i>F. crookwellense</i> 271	84.5 ^A	<i>F. solani</i> 112	18.5 ^{FGHI}
<i>F. graminearum</i> 277	75.5 ^{AB}	<i>F. sporotrichioides</i> 4	17.5 ^{FGHI}
<i>F. subglutinans</i> 91	63 ^{BC}	<i>F. solani</i> 185	17 ^{FGHI}
<i>F. sporotrichioides</i> 1	49 ^{CD}	<i>F. equiseti</i> 50	12.5 ^{FGHI}
<i>F. venenatum</i> 193	46.5 ^{CD}	<i>F. oxysporum</i> 119	12.5 ^{FGHI}
<i>F. oxysporum</i> 87	45 ^{CDE}	<i>F. proliferatum</i> 101	10 ^{GHI}
<i>F. venenatum</i> 24	45 ^{CDE}	<i>F. verticillioides</i> 68	8.5 ^{GHI}
<i>F. verticillioides</i> 305	33.5 ^{DEF}	<i>F. oxysporum</i> 61	8 ^{GHI}
<i>F. venenatum</i> 21	32 ^{DEF}	<i>F. solani</i> 149	6.5 ^{HI}
<i>F. oxysporum</i> 99	28.5 ^{DEFG}	<i>F. verticillioides</i> 102	2.5 ^I
<i>F. acuminatum</i> 265	25 ^{EFGH}	Control	7.5 ^{GHI}
<i>F. concolor</i> 282	24.5 ^{EFGH}		

*values in columns marked with the same letter do not have significant difference according to LSD test ($P < 0,05$) - *vrijednosti u stupcima označene istim slovom ne razlikuju se značajno prema LSD testu ($P < 0,05$)

The most pathogenic *Fusarium* species in our research was *F. graminearum*. Isolates of *F. graminearum* from *A. theophrasti*, *A. retroflexus* and *C. album* were very pathogenic to wheat and maize. Ćosić et al. (2007) determined that the most dominant *Fusarium* species found on wheat and barley in Croatia is *F. graminearum*. Spanić et al. (2010) in their research determined that the most significant pathogens which cause Fusarium Head Blight in eastern Croatia are *F. graminearum*, *F. avenaceum* and *F. culmorum*. In our research we also determined pathogenicity of other *Fusarium* spp. and noticed inter- and intraspecies variability and specialisation towards different hosts and phases of plant development. *F. avenaceum* isolated from *A. githago* was pathogenic to wheat seedlings and very pathogenic to maize seedlings. *F. crookwellense* isolated from *A. lappa* was very pathogenic to wheat and maize seedlings. Chehri et al. (2011) proved that *F. crookwellense* is pathogenic to wheat seedlings. *F. oxysporum* isolated from *A. artemisiifolia* caused very high disease index of wheat seedlings. On the other hand it was not pathogenic to maize seedlings. *F. oxysporum* isolated from *A. theophrasti* was pathogenic to wheat and maize seedlings. *F. semitectum* from *A. theophrasti* was very pathogenic to wheat seedlings and moderately pathogenic to maize seedlings. Tancić et al. (2009) determined moderate pathogenicity of *F. semitectum* to maize seeds.

F. solani isolated from *S. arvensis* had very high disease index of wheat seedlings (100 out of 100), whereas on the other hand very low disease index of maize seedlings. *F. solani* isolate from *C. album* was pathogenic to wheat seedlings and apathogenic to maize seedlings. *F. sporotrichioides* 1 and 4 isolated from maize debris had very high disease index of wheat seedlings, isolate 1 was pathogenic and isolate 4 apathogenic to maize seedlings. *F. subglutinans* isolated from *A. theophrasti* was very pathogenic to wheat and maize seedlings. *F. subglutinans* from *C. album* was pathogenic to wheat and maize seedlings. In our research *F. venenatum* proved to be highly pathogenic to wheat and maize and according to available literature we determined that this is the first report about the pathogenicity of *F. venenatum*. Isolates *F. verticillioides* 305 and 102 from *A. githago* and *C. album* were very pathogenic to wheat seedlings. On the other hand isolate 102 was apathogenic to maize seedlings and had lower disease index than the control, showing positive influence of *F. verticillioides* on maize seedlings germination and growth. Svitlica et al. (2008) investigated pathogenicity of *Fusarium* spp. to maize cobs and *F. graminearum* isolated from weed *S. halepense* was the most pathogenic. Jenkinson and Parry (1994) tested *Fusarium* isolates from weed and, out of 77 isolates, 75 were pathogenic to wheat seedlings. The most pathogenic isolates in their research were *F. avenaceum*, *F. culmorum* and *F. graminearum*.

Pathogenicity of isolates which belong to the same species can be significantly different. Manka (1989) investigated pathogenicity of 30 isolates of *F.*

culmorum to wheat seedlings and determined that their pathogenicity varied from apathogenic to very pathogenic. Similar results were obtained by Chelkowski et al. (1995) for isolates of *F. verticillioides*. Different pathogenicity of six isolates of *F. graminearum* was studied by Asran and Buchenauer (2003), and determined statistically significant differences in pathogenicity of different isolates. It is in accordance with results of Nagy and Bagiu (2000), Ledencan et al. (2001) and Jurković et al. (2002). Tancić et al. (2009) examined differences in pathogenicity of *Fusarium* spp. originating from maize kernels and wheat grains and determined inter- and intraspecies variability regarding the effects on maize seed germination. Some authors have proven that plant pathogens isolated from weeds are often more pathogenic compared to ones isolated from cultivated plants. Akinsanmi et al. (2007) determined that passage through alternative hosts changes the fitness of *F. graminearum* and *F. pseudograminearum*. They concluded that one passage through alternative hosts reduces pathogenicity, but at the same time provides better ability for colonisation of primary host. This could explain the lack of symptoms on alternative hosts. It is important to emphasize that our samples were surface sterilized and that weed did not exhibit disease symptoms, which could mean that isolated *Fusarium* species live inside these plants as endophytes (Barrow et al. 2008.).

CONCLUSION

In this research we have determined pathogenicity of 30 *Fusarium* spp. isolated from weed and plant debris to wheat and maize seedlings. The most pathogenic *Fusarium* species in all pathogenicity tests was *F. graminearum*, isolated from *A. retroflexus*, *A. theophrasti* and *C. album*. In conclusion we can say that *Fusarium* spp. isolated from weed and plant debris from agricultural fields can be pathogenic to wheat and maize and that there is inter- and intraspecies variability in their pathogenicity.

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PATOGENOST *FUSARIUM* SPP. IZOLIRANIH S KOROVA I BILJNIH OSTATAKA U ISTOČNOJ HRVATSKOJ ZA PŠENICU I KUKURUZ

SAŽETAK

U laboratoriju je ispitana patogenost trideset izolata 14 Fusarium vrsta izoliranih s korova i biljnih ostataka u istočnoj Hrvatskoj. Testovi patogenosti provedeni su na klijancima pšenice i kukuruza. Najpatogenija Fusarium vrsta bila je F. graminearum izolirana s Amaranthus retroflexus, Abutilon theophrasti i Chenopodium album. Primijetili smo značajnu varijabilnost u patogenosti za pšenicu i kukuruz između i unutar vrsta. Izolati F. solani s Sonchus arvensis i F. verticillioides s Chenopodium album bili su vrlo patogeni za pšenicu, a nepatogeni za kukuruz. Izolati F. venenatum bili su vrlo patogeni za pšenicu i kukuruz, što je prvo izvješće o patogenosti te vrste. Ovo istraživanje potvrđuje da korovi i biljni ostaci mogu poslužiti kao alternativni domaćini i kao izvor biljnih patogena.

Ključne riječi: patogenost, Fusarium spp., korovi, biljni ostaci

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