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## VIRULENCE OF *FUSARIUM* SPECIES TO ALFALFA SEEDLINGS\*

**ABSTRACT:** In *in vitro* conditions, virulence of 91 isolates of species *Fusarium* genus (*F. oxysporum*, *F. solani*, *F. acuminatum*, *F. equiseti*, *F. arthrosporioides*, *F. proliferatum*, *F. avenaceum*, *F. semitectum*, *F. tricinctum*, *F. sporotrichioides* and *F. graminearum*) towards alfalfa seedlings was investigated. Isolates of investigated species originated from diseased alfalfa plants collected on four locations in Serbia based on symptoms of wilting caused by fusarium and root rotting. Pathogenicity and virulence of investigated isolates of *Fusarium* spp. were determined by visual evaluation of inoculated seedlings of cultivar K28 in laboratory conditions. All isolated of investigated species had pathogenic effect on alfalfa seedlings, which expressed symptoms such as necrosis of root, moist rotting and „melting of seedlings”. Colour of necrotic root tissue varied from light brown, brown, lipstick red to explicit black, depending on the *Fusarium* species. Strong virulence was established in 48 isolates, medium virulence in 31 and weak virulence in 12 isolates.

**KEY WORDS:** alfalfa (*Medicago sativa* L.), seedlings, *Fusarium* spp., virulence

### INTRODUCTION

Longevity of alfalfa crops is conditioned by condition of root system, primarily in the root neck and small roots in the most active zone of the root. Therefore, rotting of root and root neck, since frequent in case of alfalfa, is one of the most important factors which reduces the longevity of alfalfa crops, yield and quality of alfalfa. *Fusarium* species isolated from diseased alfalfa roots, especially root neck, were more frequent than any other type of fungus. Except type of rot, symptoms of disease are manifested in form of chlorosis of leaves and lower plants.

\* The paper was presented at the first scientific meeting MYCOLOGY, MYCOTOXICOLOGY AND MYCOSES held from 20—22 April 2005 in Novi Sad.

*Fusarium oxysporum* Schlect, *F. solani* (Mart.) Appel & Wollenw. Emend. Snyder & Hansen and *F. roseum* Lk. ex Fr. Emend. Snyd. & Hans. are species constantly isolated from alfalfa root (O'Rourke and Millar, 1966; Graham et al., 1979). According to data from literature regarding the etiology of root rot, other *Fusarium* species are also important, such as *F. avenaceum* (Fr.) Sacc., *F. arthrosporioides* Sherb., *F. culmorum* (W. G. Smith) Sacc., *F. scirpi* Lamb, et Fautr. var. *acuminatum*, *F. poae* (Peck) Wr., *F. sambucinum* Fuckel and *F. tricinctum* (Corda) Sacc. (Erwin, 1954; Chi et al., 1964; Nedelnik, 1988; Hwang et al., 1989).

On territory of Serbia, from alfalfa plants demonstrating symptoms of wilting and rotting of root and root neck, most frequently isolated were numerous *Fusarium* species (Milijić et al., 1984, 1986, Vico et al., 1996; Krnjaja and Ivanović, 2001; Krnjaja et al., 2002). Damage caused by nematodes (*Pratylenchus penetrans* Cobb) enables more intensive development of *Fusarium* wilt (*Fusarium oxysporum* var. *medicaginis*) on alfalfa root (Grujičić et al., 1984). *Fusarium* species were isolated also from alfalfa seed (Krnjaja et al., 2003), which could be source of further spreading of pathogens on vegetative parts of the plant and cause problem in establishing of alfalfa crops.

Considering how frequent incidences of fuzariozing wilting and root rotting are in alfalfa crops on the territory of Serbia as well as great number of isolated *Fusarium* species, objective of this research was to investigate virulence of different types of *Fusarium* species to alfalfa seedlings.

## MATERIAL AND METHODS

Applying standard phyto-pathological methods, isolates of *Fusarium* spp. were separated from alfalfa plants, collected in the vicinity of Belgrade (Zemun, Padinska Skela), Novi Sad (Rimski Šančevi) and Kruševac, with symptoms of *Fusarium* wilt and root rot. According to morphological characteristics described by Nelson et al. (1983) and Burgess et al. (1994), investigated isolates belong to following species: *F. oxysporum*, *F. solani*, *F. acuminatum*, *F. equiseti*, *F. arthrosporioides*, *F. proliferatum*, *F. avenaceum*, *F. semitectum*, *F. tricinctum*, *F. sporotrichioides* and *F. graminearum*.

In *in vitro* conditions, inoculation of seedlings of cultivar K28 was carried out according to method described by Chi et al. (1964). Alfalfa seed was disinfected in 95% ethanol for 10 seconds, subsequently in 7% sodium hypo chlorite (NaOCl) for 10 minutes, rinsed in sterile water and dried on room temperature. Section of the colony of investigated isolates 4–5 mm<sup>2</sup> in diameter and five days old was placed in centre of Petri dish with 1,7% potato dextrose agar. Around the section of colony, on distance of 2 cm in diameter, 15 seeds of alfalfa were placed. Petri dishes were incubated on room temperature. After two days, primary roots were placed so that their tips were touching the rim of the fungus colony in the centre of Petri dish.

After 10 day incubation, degree of pathogenicity (virulence) of isolates was evaluated by visual inspection of necrotic areas according to following scale: 0 = no virulence (no necrotic areas on the root), 1 = weak virulence

(necrosis on the tip of the root), 2 = medium virulence (root and low part of the stem — stem butt, but necrosis or fungus mycelium didn't spread on leaves and upper section of stem) and 3 = strong virulence (necrosis or fungus mycelium have spread entirely over root, stems and leaves, and in some cases even „melting” of seedlings occurred).

## RESULTS OF INVESTIGATION

By inoculation of alfalfa seedlings in laboratory conditions it was established that all 11 isolates of *Fusarium* species were pathogenic. Two days subsequent to contact between root and fungus colony necrosis appeared in all investigated isolates. Necrosis spread vertically and after 10 days of incubation isolates of strong virulence were completely spread over root, stems and leaves of seedlings, causing in some cases so called „melting” of seedlings (Fig. 1).

Necrotic tissue of the root was rotten and decayed. In case of isolates which haven't caused spreading of necrosis further from the root, herbaceous parts of seedlings which weren't necrotic tore easily when pulled from disintegrated and softened root tissue. Colour of necrotic root parts was light brown, brown, red brown, and lipstick red to black (Fig. 1). Roots of control seedlings were without necrosis, healthy and with stable structure.

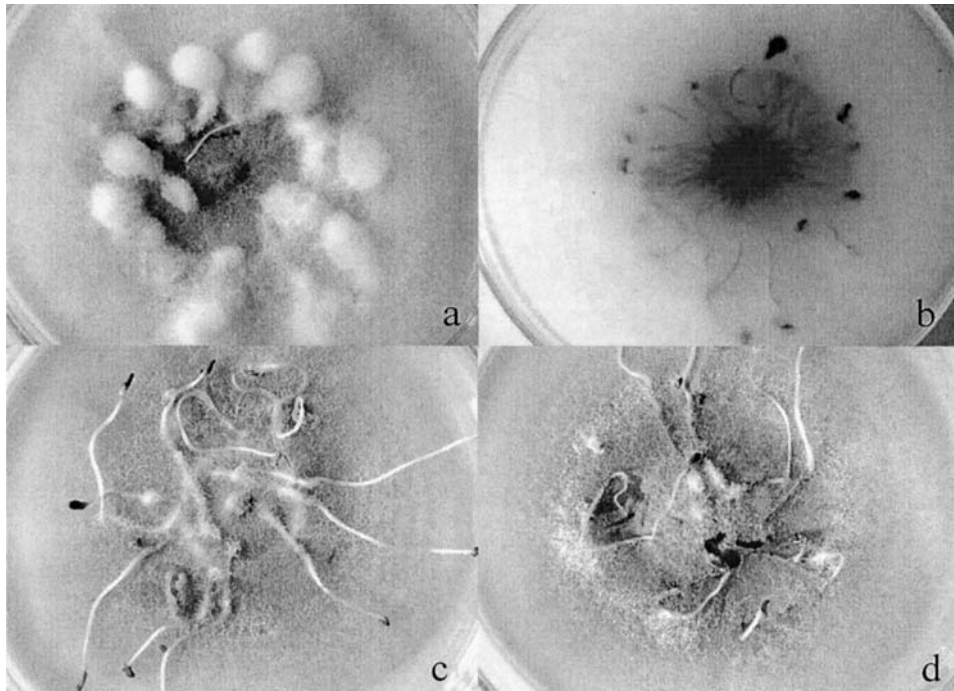


Figure 1. Appearance of necrotic alfalfa seedlings. „Melting” of seedlings in air (a) and substrate (b) section of the colony of the isolate LU32ZE; necrosis of root in the colonies of the isolates LU24ZE (c) and LU33ZE (d).

Among investigated isolates *Fusarium* spp. 48 demonstrated strong virulence, 31 isolates medium virulence, and weak virulence 12 isolates (tab. 1). Among investigated isolates of *F. oxysporum* 16 isolates demonstrated strong virulence (grade 3), 14 isolates demonstrated medium virulence (grade 2), and 4 isolates demonstrated weak virulence (grade 1). Among investigated isolates of *F. solani* 16 isolates demonstrated strong virulence, and one isolate demonstrated medium virulence. Seven isolates of *F. acuminatum* demonstrated strong, and two weak virulence. All investigated isolates of *F. equiseti* demonstrated weak virulence. Six isolates of *F. arthrosporioides* demonstrated medium virulence and one isolate strong virulence. All investigated isolates of *F. proliferatum* demonstrated strong virulence. One isolate of *F. avenaceum* demonstrated strong, and two isolates medium virulence. All investigated isolates of species *F. semitectum* and *F. sporotrichioides* demonstrated medium virulence. One isolate of *F. tricinctum* demonstrated strong, and one medium virulence. One investigated isolate of *F. graminearum* demonstrated strong virulence (tab. 1).

Table 1. Degree of pathogenicity (virulence\*) of isolates of *Fusarium* species to alfalfa seedlings in *in vitro* conditions

Isolate	Virulence	Isolate	Virulence	Isolate	Virulence
<i>F. oxysporum</i>					
LU4ZE	2	LU20ZE	3	LU6RS	2
LU5ZE	2	LU21ZE	1	LU10RS	2
LU6ZE	2	LU22ZE	2	LU22RS	2
LU7ZE	2	LU23ZE	2	LU3KS	3
LU8ZE	3	LU30ZE	2	LU5KS	3
LU9ZE	1	LU32ZE	3	LU15KS	3
LU10ZE	1	LU43ZE	3	LU16KS	3
LU11ZE	2	LU44ZE	3	LU17KS	3
LU12ZE	2	LU45ZE	3	LU1PS	3
LU14ZE	1	LU47ZE	3	LU4PS	3
LU16ZE	2	LU49ZE	3		
LU17ZE	2	LU1RS	3		
<i>F. solani</i>					
LU24ZE	3	LU33ZE	3	LU40ZE	3
LU25ZE	3	LU34ZE	3	LU41ZE	3
LU26ZE	3	LU36ZE	3	LU42ZE	3
LU28ZE	3	LU37ZE	3	LU46ZE	3
LU29ZE	3	LU38ZE	3	LU48ZE	2
LU31ZE	3	LU39ZE	3		
<i>F. acuminatum</i>					
LU6PS	3	LU10KS	3	LU16RS	3
LU4KS	2	LU7RS	3	LU19RS	3
LU9KS/1	2	LU8RS	3	LU24RS	3

<i>F. equiseti</i>					
LU2KS	1	LU3RS	1	LU25RS	1
LU6KS	1	LU5RS	1	LU26RS	1
LU8KS	1	LU12RS	1		
<i>F. arthrosporioides</i>			<i>F. proliferation</i>		
LU1ZE	3	LU17RS	2	LU2PS	3
LU2ZE	2	LU18RS	2	LU13KS	3
LU3PS	2			LU14KS	3
LU9KS/2	2			LU20RS	3
LU2RS	2			LU21RS	3
<i>F. avenaceum</i>		<i>F. semitectum</i>		<i>F. tricinctum</i>	
LU9RS	3	LU3ZE	2	LU7KS	2
LU27ZE	2	LU5PS	2	LU11KS	3
LU35ZE	2	LU13RS	2		
<i>F. sporotrichioides</i>		<i>F. graminearum</i>		Kontrola	
LU1KS	2	LU18ZE	3	—	0
LU23RS	3				

\* 0 = no virulence, 1 = weak virulence, 2 = medium virulence, 3 = strong virulence

## DISCUSSION

In the test for control of pathogenicity of *Fusarium* species to alfalfa seedlings pathogenicity of isolates of all investigated *Fusarium* spp. was established, as well as high sensitivity of alfalfa in pheno — stage of seedlings. Similar results were confirmed in previous investigations (Weimer, 1927, 1928, loc. cit. Schmittenner, 1964; Chi et al., 1964; Hancock, 1983, 1985) when it was proved that *Fusarium* species can infect alfalfa seedlings. Weimer (1927, loc. cit. Schmittenner, 1964) has established that *Fusarium* spp. and *Rhizoctonia* spp., isolated from rotten root neck and root of alfalfa cause moist rotting of seedlings. *F. oxysporum* f. sp. *medicaginis* is also pathogenic to alfalfa seedlings (Weimer, 1928 loc. cit. Schmittenner, 1964). Isolates of *Rhizoctonia* spp. and *F. oxysporum* f. sp. *medicaginis* originating from alfalfa have demonstrated strong pathogenicity not only to alfalfa seedlings but also to seedlings of bird's foot trefoil, red and white clover (Vico, 1997). Histological researches have shown that penetration and further development of *F. avenaceum*, *F. oxysporum* and *F. solani* are similar in case of alfalfa and red clover seedlings (Chi et al., 1964). Results obtained by these authors indicate that all three *Fusarium* species have penetrated into uninjured epidermal root cells, seed coat and cotyledons by direct penetration without formation of apresoria. Penetration was intercellular and intracellular. Most frequently, pathogens penetrated the meristematic tissue, although regions of cell magnification and differentiation were also affected. Pathogens colonize completely cortex of the alfalfa root. All three species colonize xylem, and *F. solani* is most limited when developing in epidermal and cortical tissue.

es. Tips of roots are affected by pathogens in two-day old seedlings. Seed coat was colonized quickly by all three fungus species. Lot of hyphae were found in cotyledons, leaf primordia and young stems. No difference was established between plants which became diseased naturally and artificially inoculated plants in regard to development of fungus (Chi et al., 1964).

## CONCLUSION

Investigations of pathogenicity and virulence of *Fusarium* species *in vitro* have lead to following conclusions:

— *F. oxysporum*, *F. solani*, *F. acuminatum*, *F. equiseti*, *F. arthrosporioides*, *F. proliferatum*, *F. avenaceum*, *F. semitectum*, *F. tricinctum*, *F. sporotrichioides* and *F. graminearum* are pathogen to seedlings of K28 alfalfa;

— Main symptoms of disease are change of colour from brown to black depending on the investigated species, necrosis of root, moist rotting and „melting of seedlings”;

— Most of the isolates demonstrated strong virulence (48) to medium virulence (31), and only 12 weak virulence, none of the isolates were no virulence;

— *F. solani* and *F. acuminatum* demonstrated mostly strong virulence, *F. arthrosporioides* medium virulence, whereas virulence of *F. oxysporum* varied from weak to strong;

— All isolated of *F. proliferatum* demonstrated strong virulence, and of *F. equiseti* weak virulence;

— Less present species on alfalfa root, such as *F. avenaceum*, *F. tricinctum* and *F. graminearum*, demonstrated medium to strong virulence, and *F. semitectum* and *F. sporotrichioides* medium virulence.

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## ВИРУЛЕНТНОСТ ВРСТА РОДА *FUSARIUM* ПРЕМА КЛИЈАНЦИМА ЛУЦЕРКЕ

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### Резиме

У *in vitro* условима проучена је вирулентност 91-ог изолата врста рода *Fusarium* (*F. oxysporum*, *F. solani*, *F. acuminatum*, *F. equiseti*, *F. arthrosporioides*, *F. proliferatum*, *F. avenaceum*, *F. semitectum*, *F. tricinctum*, *F. sporotrichioides* и *F. graminearum*) према клијанцима луцерке. Изолати испитиваних врста пореклом су из оболелих биљака луцерке које су прикупљене из четири локалитета у Србији на основу симптома фузариозног увенућа и трулежи корена. Патогеност и вирулентност испитиваних изолата *Fusarium* spp. утврђени су визуелним оцењивањем инокулисаних клијанаца сорте К28 у лабораторијским условима. Сви изолати испитиваних врста патогени су према клијанцима луцерке, који су испољили симптоме у виду некрозе корена, влажне трулежи и „топљења клијанаца”. Боја некротираног ткива корена варира од светло смеђе, смеђе, црвено-смеђе, кармин црвене до изразито црне, зависно од врсте рода *Fusarium*. Јаку вирулентност испољило је 48 изолата, средњу 31 изолат, а слабу 12 изолата.