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PATHOGENICITY OF *FUSARIUM* SPECIES IN SOYBEAN*

ABSTRACT: The paper describes the symptoms of the *Fusarium* wilt and necrosis of root and lower stem of soybean, which include leaf chlorosis, wilt of the apical portion of the plant, necrosis of the root and lower stem, and wilting of the whole plant. The pods are often poorly developed. The seeds may be smaller and lighter in the weight and infected, as well.

Isolated from diseased soybean plants were the species *Fusarium avenaceum, F. equiseti, F. oxysporum* and *F. poae.* Pathogenicity tests under artificial infection conditions showed *F. oxysporum* (isolate S/1) to be the most pathogenic among of the four investigated species. The other species proved much less pathogenic.

KEY WORDS: soybean, *Fusarium avenaceum, F. equiseti, F. oxysporum, F. poae*, pathogenicity

INTRODUCTION

The *Fusarium* wilt and necrosis of root and lower of soybean (abbreviated to FWNS) is an important disease in many countries. It can cause great damage, as it may reduce the average yield of soybean by up to 59% (Sinclair and Backman, 1989). The fusariosis of soybean was first recorded in 1917 in the U.S. (Cronwell, 1917) and has since been reported in many parts of the world (Sinclair and Backman, 1989). In our country, the disease was first observed and described in 1964 by Aćimović (1988), and later by Tošić et al. (1986) as well. These authors identified *Fusarium* sp. as the causal agent of the disease without specifying which of the species in particular were responsible for causing it.

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In Serbia and Montenegro, the FWNS occurs sporadically and with varying intensity. The severity of attack mainly depends on weather conditions during the growing season. There are no data available on the specific species that cause soybean fusariosis in the country and their importance and pathogenicity to soybean.

Because of this, the objective of our study was to identify the species of the genus *Fusarium* that cause the FWNS and to investigate their pathogenicity.

MATERIALS AND METHODS

The FWNS was observed on soybeans at some locations in the province of Vojvodina. Samples of soybean plants showing symptoms of the disease were collected in order to determine and identify the causal organism responsible. The randomly selected diseased plants were used to isolate the fungi causing the disease.

Isolation of fungi

Using a scalpel, pieces of tissue 0.5 x 0.5 cm in size were cut out of the marginal zone between the healthy and diseased stem tissue. The pieces were then immersed in a sublimate ($HgCl_2$) for about half a minute for external desinfection purposes, after which they were rinsed with sterile water. The sterilized stem pieces were then placed in Petri dishes filled with a potato dextrose agar (PDA) medium and used for isolation of fungi. The Petri dishes were kept in a thermostate at 25°C. After the development of colony the usual phytopathological methods were applied to obtain pure, monosporous culture of isolates and their determination.

Determination of species from genus Fusarium

The monosporous cultures of *Fusarium* fungi were transferred onto a medium consisting of water, agar and carnation leaves, known as the CLA medium (Fisher et al., 1982). The isolates were grown at room temperature under artificial lighting with ultraviolet light added. The source of light were three 40W neon tubes and a black tube emitting the so-called black light (Philips TLD 36W/08). Growing fungi from genus *Fusarium* on the CLA medium in the above manner promotes their sporulation and pigmentation.

Ten to 14 days after the incubation, the isolates of monosporous cultures were used for further study of morphological characteristics, species determination and pathogenicity tests.

Taxonomic characteristics were determined based on the appearance of the colony of fungi on the PDA medium and the formation of conidia, conidiophores and chlamydospores on the CLA medium (Nelson et al., 1983; Burgess et al., 1988; Burgess et al., 1994).

Pathogenicity tests

The pathogenicity of the isolates of fungi was tested in several ways:
— by soybean seed inoculation on filter paper;
— by sowing inoculated seeds in sterile soil;
— by sowing uninfected seeds in artificially infected soil.

The following *Fusarium* isolates were used for pathogenicity tests: S/5, S/8, S/1, S/2 and S/10. They differed from each other in color and colony appearance and were all obtained from diseased stems except the isolate S/2, which was obtained from a wilted soybean seedling.

The conidia suspension for inoculation was prepared by pouring 50 ml of sterile water into each of the Petri dishes containing 14-day-old *Fusarium* isolates, stirring the mixture with a sterile glass stick, and pouring it into a glass. The concentration of conidia in the suspension was determined using Türk-Bürger's plate for spore count. It was set to $1x10^6$ conidia/ml.

Test on filter paper

Seeds of the soybean cultivar Ravnica were sterilized for three minutes with a 1% solution of sodium hypochlorite, rinsed twice with sterile water, and then dipped in the conidia suspension of the each of investigated isolate for a period of five hours. After that, the seeds were placed on wet filter paper in four Petri dishes, each representing one replicate, with 15 seeds per dish. The seeds were germinated in a thermostate at 25°C. Soybean seeds dipped in sterile water for five hours were used as the control. The number of germinated and rotted seeds was determined after seven days, while the number of deformed (diseased) seeds and the total number of healthy seeds were determined after ten days. All the data were statistically processed by the analysis of variance and by determining the significance threshold using Duncan's test.

Planting of infected seeds in sterile soil

For this test, the preparation of conidia suspension and seed inoculation were carried out identically as in the previous one. Inoculated seeds were sown in pots containing sterile soil (10 seeds per pot). The trial included four replicates, so each isolate was used to inoculate 40 seeds in total. The pots were kept in a greenhouse at 22—24°C and watered according to their need. Used as the control treatment were the seeds dipped in sterile water and then planted in sterile soil. The number of emerged plants was recorded ten days after planting.

Test in artificially infected soil

Healthy soybean seeds were planted in pots filled with sterile soil, after which the soil was artificially infected by pouring 50 ml of the conidia suspen-

sion into each pot. Ten seeds per pot were planted. The trial had four replicates, so 40 seeds were inoculated with each isolate overall. The pots were kept in a greenhouse at 22—24°C and watered according to their need. Sterilized soil watered with 50 ml of sterile water per pot was used as the control. Ten days after planting, the number of emerged plants was counted. The number of plants that wiled after emergence was recorded after 14 days.

RESULTS

Disease symptoms

During cool and wet springs, wet rotting and damping off of soybean seedlings was observed in inspected soybean fields. The seedlings often died before emerging, still in the soil. The diseased seedlings necrotize and rot in the soil. The emerged seedlings are stunted in growth. The cotyledons are chlorotic, later become necrotic and decay. The diseased seedlings wilt and dry up. This type of symptoms occurs rarely in Serbia and Montenegro. If the diseased seedlings do not decay, they produce plants that have poor development, have stunted growth and form pods with smaller and curved seeds. The seeds of such plants are often infected.

The symptoms may also appear in older plants during mid-growing season under warm weather conditions. One of the typical signs of the disease is leaf chlorosis. The diseased leaves wilt and dry up. Dropping and wilting of the stem tip is another characteristic symptom. The diseased plants may wilt down and dry up completely. Their roots are necrotic and rotten, and the necrosis will often spread to the lower stem. A cross-section of the stem will reveal necrosis of the vessels. The diseased plants develop fewer pods, which contain smaller seeds.

Most frequently isolated from such plants were fungi of the genus *Fusarium.*

Isolation of fungi

The following species were isolated first from the diseased seedlings and then from the infected stems as well: *F. avenaceum, F. equiseti, F. oxysporum* and *F. poae*.

Determination of Fusarium *species*

On the potato-dextrose medium, isolate S/5 formed colonies light yellow to reddish in color. The color of the colony in the medium was pinkish red or brown. On the monophialides conidiophores in the carnation medium, the isolate developed long and slender macroconidia, whose walls were parallel on a portion of the conidium. Microconidia formed very rarely, while chlamydospores did not form at all. Based on morphological characteristics, the isolate S/5 was determined to belong to the species *F. avenaceum.*

Colonies of the isolate S/8 were initially white but became darker with time and turned light brown in the end. In the medium, the colonies were brown as well. On the CLA medium, extremely curved, sickle-shaped macroconidia developed with their characteristic foot-shaped apical cells. Macroconidia did form on the monophialides, while microconidia did not. Chlamydospores formed in chains or clusters. The isolate S/8 was found to be the species *F. equiseti*.

Isolates S/1 and S/2 formed colonies ranging from white to dark purple in color depending on the isolate. On the carnation medium, macroconidia formed orange-colored sporodochia on the monophialides. Most often, they were short and had three septa and a pointed end. They formed a large number of microconidia, clustered into so-called false heads. The microconidia were unicellular and either elyptical or kidney-shaped. The colonies contained numerous chlamydospores. All these characteristics pointed to the isolates being of the species *F. oxysporum*.

On the potato medium, colonies of the isolate S/10 were white in the beginning but turned purple to brown with age. On the carnation medium, few macroconidia developed. This isolate was definitively identified as being of the species *F. poae* because of its characteristic round or lemon-shaped microconidia with a prominent papilla. The isolate did not form chlamydospores.

Pathogenicity test of Fusarium species

The test results are shown in Table 1. The table shows the average values of the four replicates.

Tab. 1. Pathogenicity of *Fusarium* species on soybean

Species	Filter paper test			Test with sterile soil		Inoculated soil	
	Average no. of germinated seeds	Average no. of rotted seeds	Average no. of malformed seedlings	Average no. of healthy seeds	Average no. of emerged plants	Average no. of emerged plants	Average no. of wilted plants
Fusarium avenaceum S/5	14.25	1.75	0.75	12.50**	4.75**	8.25	0.50
Fusarium equiseti S/8	15.00	0.25	2.50**	12.25**	6.50	8.00	0.25
Fusarium oxysporum S/1	12.50**	3.75**	1.50	10.00**	3.75**	9.25	1.75
Fusarium oxysporum S/2	14.00	1.75	2.50**	10.75**	6.00	8.50	0.25
Fusarium poae S/10	15.00	1.75	3.00**	10.25**	7.25	9.50	0.25
Control	15.00	0.25	0.50	14.25	7.25	8.50	0.00
LSD 0.01	1.76	2.95	1.98	1.91	2.43	2.57	2.30
0.05	1.09	1.75	1.48	1.43	1.80	1.86	3.61

Test on filter paper

According to the results of seed inoculation on filter paper shown in Table 1, all of the isolates of fungi from genus *Fusarium* exhibited a greater or lesser degree of pathogenicity, as they all significantly reduced the average number of healthy seeds (10—12.5) relative to the uninfected control treatment (14.25). *F. oxysporum* isolate S/1 proved to be the most aggressive one, as it had the most significant negative effect on seed germination. The rest of the *Fusarium* fungi had no significant influence on germination (Tab. 1).

Planting of infected seeds in sterile soil

As shown in Table 1, *F. avenaceum* (isolate S/5) and *F. oxysporum* (isolate S/1) were highly pathogenic, since they highly significantly reduced the germination of artificially infected soybean seeds, i.e. the number of plants emerged. *F. equiseti* had less negative influence on germination.

Test in artificially infected soil

In this case, the *Fusarium* isolates had no significant influence on seed germination. All of them, however, caused plant wilting after emergence. The average number of wilted plants ranged between 0.25 and 1.75, depending on the species. S/1 was the isolate with the highest level of pathogenicity in this particular test. However, no statistically significant differences in the average number of wilted plants were found related to the uninfected control.

Our results showed that *F. oxysporum*, isolate S/1 had the highest level of pathogenicity in all of the tests. The pathogenicity of the rest of the species (*F. avenaceum* (S/5), *F. equiseti* (S/8), *F. poae* (S/10) and S/2 *F. oxysporum*) varied.

DISCUSSION

In Serbia and Montenegro, the *Fusarium* wilt and necrosis of root and lower stem of soybean is present in some years to a greater or lesser extent. The FWNS was first observed in our country 1964 (Aćimović, 1988) and the causal organism was identified as *Fusarium* sp. Ever since, the disease has failed to receive adequate attention, although it does occur from time to time. The causal agents of FWNS, fungi of the genus *Fusarium*, were not determined, nor was their pathogenicity established. After isolation from diseased soybean plants taken from various locations in the Vojvodina province, the species *F. avenaceum, F. equiseti, F. oxysporum* and *F. poae* were identified.

Around 30 species from genus *Fusarium* have been described worldwide as causal agents of soybean fusariosis (Sinclair and Dhingra, 1975). However, not all of them are of equal importance. Some are very widespread

and pathogenic, while others are less virulent and have no major economic importance.

According to the literature, *Fusarium solani* f. sp. *glycines* is among the most pathogenic species in North and South America (Nelson et al., 1997; Roy, 1997; Homma et al., 2002). This parasitic species causes a rapid death of soybean plants (Sudden Death Syndrome) and it is responsible for major economic damages. In 2002, for example, it caused an estimated damage of around 157 million USD in some states in the U.S. (Wrather et al., 2003). In the present study, this species was not isolated from the diseased soybean plants, although it has been reported in some European countries (Patkowska, 2001).

In our study, the highest level of pathogenicity in all the tests was exhibited by *F. oxysporum*. This species has been reported as a major pathogen in many other countries of the world (Yasem de Romero et al., 2002; Patkowska, 2001; Reynolds and Potter, 2001; Tenuta, 2004). It was interesting that there exists signicant difference beetwen two *F. oxysporum* isolates (S/1 and S/2). The isolate S/1 obtained from diseased stem was much more virulent than the isolate S/2 from wilted seedlings. The rest of the species we isolated exhibited a considerably lower degree of pathogenicity and probably have no major importance in the etiology of the disease. Some of them have also been isolated in other countries but have not exhibited a significant level of pathogenicity, which supports the findings of the present study (Warren and Kommendahl, 1971; Vardaniya, 1971).

Other species mentioned in the literature as important soybean pathogens are *F. semitectum, F pallidoroseum, F. tucumaniae* and *F. virgiliforme* in South America and India (Goulart, 2000; Gupta and Aneja, 2001; Skandiani et al., 2004; Aoki et al., 2004). None of them have been isolated in Serbia and Montenegro, as their development requires warm and humid weather.

Since dry and warm summers are becoming more and more common in our country, special attention has to be paid to the species *Fusarium oxysporum*, which exhibited a significant amount of pathogenicity to artificially infected soybeans. This species is known as a weakness parasite that attacks plants weakened by unfavorable environmental conditions.

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ПАТОГЕНОСТ *FUSARIUM* ВРСТА НА СОЈИ

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Резиме

Фузариозна увелост, некроза корена и приземног дела стабла соје појединих година се јавља и у нашој земљи у већој или мањој мери. Ово обољење се интензивније јавља у годинама са топлим и сувим летима, погодним за развој увелости соје, проузроковане врстама из рода *Fusarium*. Из узорака оболелих биљака са симптомима обољења су изоловане и детерминисане врсте *Fusarium avenaceum, F. equiseti, F. oxysporum* и *F. poae*. У огледима са вештачком инокулацијом соје највећу патогеност испољавао је изолат S/1 *F. oxysporum*. *F. oxysporum* (S/1) је значајно смањио клијавост и ницање биљака соје, а повећао број трулих зрна. Остале врсте испољиле су знатно слабију патогеност.

Проблему фузариозне увелости, некрозе корена и приземног дела стабла соје требало би посветити већу пажњу због могућности увећања значаја овог обољења, услед све чешће појаве топлих и сувих лета, повољних за развој болести.