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THE INCIDENCE OF *PYRENOCHAETA TERRESTRIS* IN ROOT OF DIFFERENT PLANT SPECIES IN SERBIA

ABSTRACT: Root samples of cereals (oats, wheat, barley, maize and sorghum), vegetables (garlic, onion, pepper, cucumber, pumpkin, carrot and tomato), industrial plant (soya bean) and weeds (Johnson grass, barnyard grass and green bristle-grass) collected in different agroecological conditions in Serbia were analysed for the presence of *Pyrenochaeta terrestris*. The fungus was found in 42 out of 51 samples (82.4%), while the incidence varied from 2.5 to 72.5%. The highest incidence was detected in cereals (average 30.3%), and then in weeds of the *Poaceae* family (average 14.2%). Considering single species, maize (up to 72.5% in root) and Johnson grass (up to 37.5%) were mostly attacked by this fungus. The lowest incidence of the fungus was determined in vegetable crops (average 6.7%). Red to reddish discoloration of root was correlated with the incidence of the fungus. Obtained data indicate that *P. terrestris* is widespread in Serbia and conditions for its development are favourable.

KEYWORDS: *Pyrenochaeta terrestris*, root, incidence, cereals, soya bean, vegetables, weeds

INTRODUCTION

Pyrenochaeta terrestris (Hansen) Gorenz, Walker & Larson (syn. *Phoma terrestris* Hansen), a common soil inhabitant, affects root of many plants, particularly monocots, including maize, cereals, grasses, sorghum and sugar cane (Walker, 1952). It has also been reported in cucumber, carrot, tomato, pepper and other plant species (Walker, 1952). This pathogen has been identified most frequently in onion (Yassin et al., 1982) and in maize (Mao et al., 1998). Rotting results in total plant collapse. It appears in the initial growing stages, but most commonly occurs in root of nearly mature plants.

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Since the 1950s there have been a few published papers on the appearance of the fungus in other plants than onion or maize.

The first identification of *P. terrestris* in Serbia was in root of three types of *Allium* species (onion, garlic and leek) (Aleksić et al., 1989), and then in root of maize and other hosts (Petrović and Lević, 1999). Recent studies indicate that this fungus is common in root of maize in Serbia (Lević et al., 2011, 2012a, 2012b), while there is no data on its incidence in root of other hosts. Therefore, we have collected root samples different plant species belonging to the group of cereals, vegetables, soya bean and weeds in maize crops. The incidence of *P. terrestris* in Serbia was determined on the basis of the mycological analysis of collected samples.

MATERIAL AND METHODS

Sample collection. Root samples were collected from 16 plant species: cereals (oats, wheat, barley, maize and sorghum), vegetables (garlic, onion, pepper, cucumber, pumpkin, carrot and tomato), weeds (Johnson grass, barnyard millet and green bristle-grass) and soya bean. These samples have been collected from 27 localities in Serbia, mostly from the Province of Vojvodina (Fig. 1). Samples of maize leaf sheaths have also been collected from these localities. Symptoms of root rot in the collected samples have been described and the fungus has been isolated on potato dextrose agar (PDA).

Isolation and incidence of the fungus. Roots of individual plants were washed gently in running tap water to remove soil, and then they were excised. A small section (3-4 mm) was usually cut from the margin of lesions developed in root. Then, the sections were washed for 2 h in running tap water, sterilised in 1% sodium hypochlorite (NaOCl) for 3 min, rinsed three times in sterile distilled water, and blotted dry between two layers of soft paper. Forty root sections of each plant were arranged in five Petri dishes on PDA and incubated for seven days under laboratory conditions. This procedure was also applied to the samples of maize leaf sheaths. After incubation, root and leaf sheath sections were examined for a fungal growth under a stereomicroscope (x15-25).

Identification and maintenance of isolates. In order to reliably identify the fungus, the fragments of colonies developed on root and leaf sheath sections were transferred to PDA and carnation leaf agar (CLA). The fungus was incubated on PDA at 25° C in the dark and on CLA at 25° C with alternating 12h periods of darkness and combined fluorescent and near ultraviolet light. The composition and the preparation of these media were described by Burgess et al. (1994).

The identification of *P. terrestris* was performed according to the description by Punithalingam and Holliday (1973), Zitter et al. (1996), Gorenz et al. (1948) and Ferreira et al. (1991), and on the basis of our experience gained during studying this fungus (Petrović and Lević, 1999; Lević et al., 2011, 2012a, 2012b).

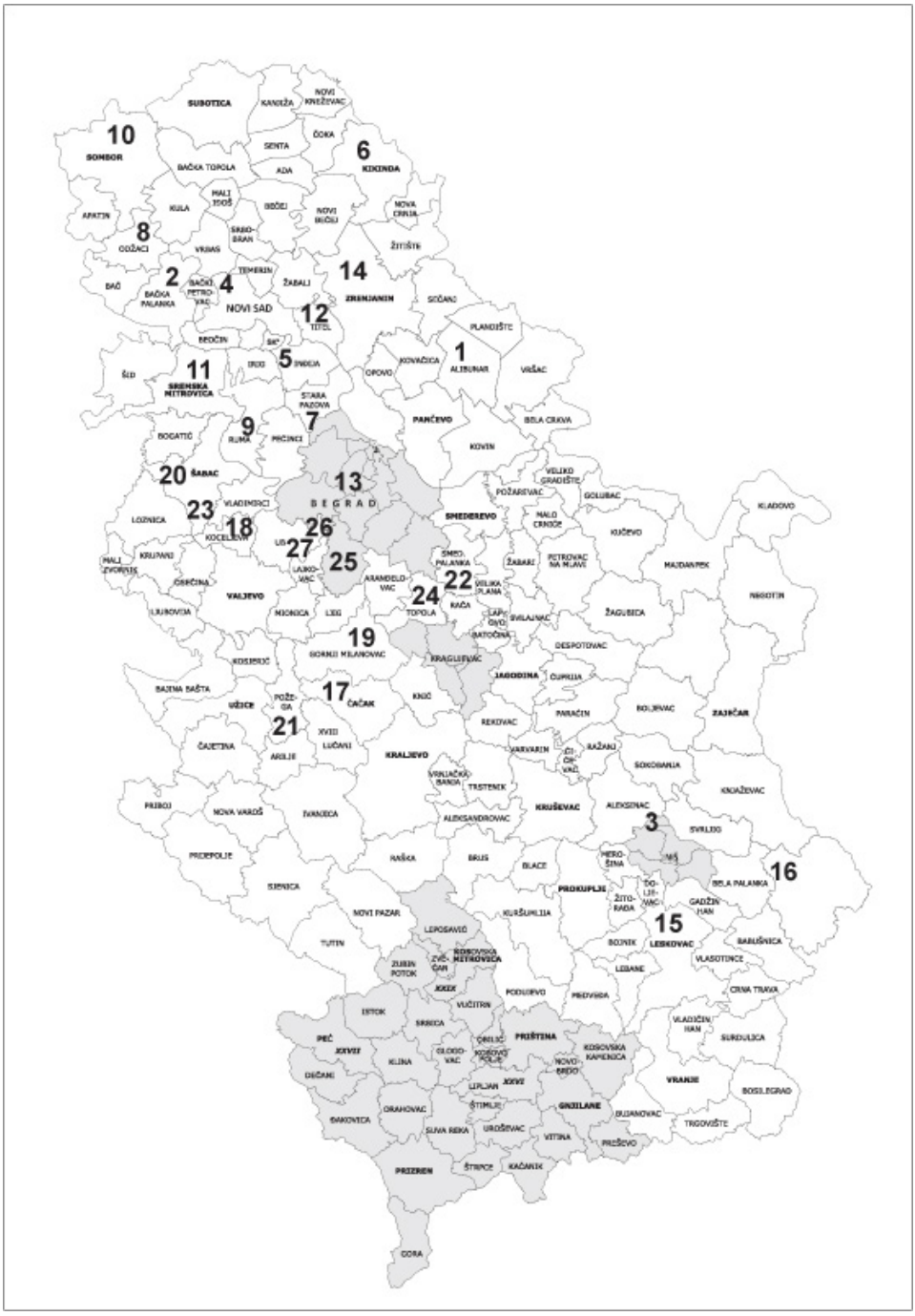

Figure 1. Map of Serbia and marked localites in which plant samples were collected.

P. terrestris cultures, isolated in the course of this study and designated as MRIZP, were stored on agar (PDA and CLA) slants in 5 ml vials within the collection of the Maize Research Institute, Zemun Polje.

Statistical analysis. The incidence (I) was calculated from the number of sections in a surface sterilised root sample colonised by *P. terrestris* as follows (Lević et al., 2011): I = [Number of root sections in which *P. terrestris* was detected/Total number of root sections] x 100.

RESULTS

The prevalent symptom in root samples on the majority of studied plant species was a combination of red (pinkish, reddish, red, purple) and brown discoloration (Table 1). Moreover, brown discoloration without redness was determined in root samples of pepper (*Capsicum annum* L), cucumber (*Cucumis sativus* L.) and squash (*Cucurbita pepo* L). Pycnidia formed below the root epidermis were observed only in the case of tomato (*Lycopersicon esculentum* Mill.). Fusiform lesions were formed in root of Johnson grass (*Sorghum halepense* L.) and maize (*Zea mays* L.).

Table 1. Description of symptoms on root samples of tested plant species

No.	Plant species	Symptoms on root samples
	Allium cepa L.	Pinkish, reddish brown & pale brown discoloration
	Allium sativum L.	Pinkish, reddish brown & brown discoloration
	Avena sativa L.	Reddish brown & brown discoloration
	Capsicum annuum L.	Brown discoloration
	Cucumis sativus L.	Brown discoloration
	Cucurbita pepo L.	Brown discoloration
	Daucus carota L.	Brown & reddish grey discoloration
	Echinochloa crus-galli (L.) P. Beauv.	Purple red & brown discoloration
	Glycine max (L.) Merr.	Reddish brown & brown discoloration
	Hordeum vulgare L.	Reddish brown & brown discoloration
	Lycopersicon esculentum Mill.	Brown reddish root & formed pycnidia under the epidermis
	Setaria viridis (L.) P.B.	Brown & reddish brown discoloration
	Sorghum bicolor (L.) Moench.	Pinkish, reddish brown, brown & scab root
	Sorghum halepense Pers.	Red, reddish brown & scab root, formed fusiform spots on root
	Triticum aestivum L.	Reddish brown & brown discoloration
	Zea mays L.	Reddish, purple red & brown root, formed fusiform spots on root

P. terrestris was determined in 42 out of 51 root samples or in 12 out of the 16 plant species. The incidence of this fungus varied depending on plant species and agroecological conditions under which these species were culti-

vated or grew in the wild (Table 2). On the average, the highest incidence of *P. terrestris* was noticed in cereals (30.3%), then in weeds (14.2%) and less in root of vegetables (6.7%). The incidence of *P. terrestris* varied from 2.5% to 75% in cereals, from 0% to 22% in vegetables and from 2.5% to 37% iin root of weeds.

Table 2. Incidence of *P. terrestris* on roots of different plant species

No.	Location (No. on map)	Plant species	*P. terrestris* incidence (%)
		Cereals	
	Zemun Polje (13)	*Avena sativa* L.	50.0
	Krnješevci (7)	*Hordeum vulgare* L.	5.0
	Zemun Polje (13)	*Sorghum bicolor* (L.) Moench.	14.2
	Inđija (5)	*Triticum aestivum* L.	2.5
	Ruma (9)	*Triticum aestivum* L.	2.5
	Zemun Polje (13)	*Triticum aestivum* L.	25.0
	Badince (15)	*Zea mays* L.	22.5
	Blato (16)	*Zea mays* L.	47.5
	Družetić (18)	*Zea mays* L.	37.5
	Gornji Milanovac (19)	*Zea mays* L.	72.5
	Inđija (5)	*Zea mays* L.	10.0
	Krnješevci (7)	*Zea mays* L.	37.5
	Krnješevci (7)	*Zea mays* L.	22.5
	Požega (21)	*Zea mays* L.	42.5
	Smederevska Palanka (22)	*Zea mays* L.	45.0
	Titel (12)	*Zea mays* L.	22.5
	Zemun Polje (13)	*Zea mays* L.	55.5
	Average		30.3
		Vegetables	
	Alibunar (1)	*Allium cepa* L.	5.0
	Bajevac (27)	*Allium cepa* L.	5.0
	Šabac (23)	*Allium cepa* L.	15.0
	Veliki Crljeni (25)	*Allium cepa* L.	0.0
	Zemun Polje (13)	*Allium cepa* L.	20.0
	Alibunar (1)	*Allium sativum* L.	22.5
	Smederevska Palanka (22)	*Allium sativum* L.	12.5
	Topola (24)	*Allium sativum* L.	2.5
	Veliki Crljeni (25)	*Allium sativum* L.	0.0
	Badince (15)	*Capsicum annuum* L.	0.0
	Veliki Crljeni (25)	*Capsicum annuum* L.	0.0
	Veliki Crljeni (25)	*Cucumis sativus* L.	0.0
	Veliki Crljeni (25)	*Cucurbita pepo* L.	0.0
	Badince (15)	*Daucus carota* L.	0.0
	Veliki Crljeni (25)	*Daucus carota* L.	0.0

Badince (15)	*Lycopersicon esculentum* Mill.	20.8
Sremska Mitrovica (11)	*Lycopersicon esculentum* Mill.	17.5
Veliki Crljeni (25)	*Lycopersicon esculentum* Mill.	0.0
Average		6.7
	Industrial plants	
Zemun Polje (13)	*Glycine max* (L.) Merr.	27.5
	Weeds	
Družetić (18)	*Echinochloa crus-galli* (L.) P.Beauv.	20.0
Zemun Polje (13)	*Echinochloa crus-galli* (L.) P.Beauv.	30.0
Zemun Polje (13)	*Setaria viridis* (L.) P.B.	15.0
Bačka Palanka (29	*Sorghum halepense* Pers.	7.5
Čačak (17)	*Sorghum halepense* Pers.	7.5
Donja Toponica (3)	*Sorghum halepense* Pers.	20.0
Futog (4)	*Sorghum halepense* Pers.	7.5
Inđija (5)	*Sorghum halepense* Pers.	5.0
Jelenča (20)	*Sorghum halepense* Pers.	15.0
Kikinda (6)	*Sorghum halepense* Pers.	2.5
Odžaci (8)	*Sorghum halepense* Pers.	20.0
Sombor (10)	*Sorghum halepense* Pers.	5.0
Zemun Polje (13)	*Sorghum halepense* Pers.	37.5
Zrenjanin (14)	*Sorghum halepense* Pers.	15.0
Zvečka (26)	*Sorghum halepense* Pers.	5.0
Average		14.2

The incidence of *P. terrestris* in small grain cereals was 50% in root of oats (*Avena sativa* L.), 25% in wheat (*Triticum aestivum* L.) and 5% in barley (*Hordeum vulgare* L.) at Zemun Polje, and 2.5% in wheat root in two localities (Inđija and Ruma).

The fungal incidence in maize root varied, depending in the locations, from 10% (Inđija) to 72.5% (Gornji Milanovac). High incidence of *P. terrestris* in maize root was determined at the locations of Zemun Polje (55.5%), Blato (47.5%), Smederevska Palanka (45%) and Požega (42.5%).

In some *P. terestris* was isolated from maize leaf sheaths (Titel and Zemun Polje) localilies. From the leaf sheath with purple red symptoms the fungus was isolated in 16.7% samples, and from the bluish tissue it was 27.5% (data not presented in table).

P. terrestris was found in only half of the collected samples of vegetables, and with the incidence ranging from 2.5% to 22%. The incidence of the fungus in positive samples varied from 5% (Alibunar and Bajevac) to 20% (Zemun Polje) in onion root (*Allium cepa* L.), from 2.5% (Topola) to 22.5% (Alibunar) in garlic (*Allium sativus* L.) and from 17.5% (Sremska Mitrovica) to 20.8% (Badince) in tomato (*Lycopersicon esculentum* Mill.). The fungus was not found in root of pepper, cucumber, squash and carrot.

As far as industrial plants are concerned, only soya bean root was analysed and 27.5% of analysed sample showed the presence of the fungus.

The root samples of all weed species were infected with *P. terrestris*. The fungal incidence in Johnson grass varied from 2.5% (Kikinda) to 37.5% (Zemun Polje). The moderate incidence (≤20%) was determined in two localities (Donja Toponica and Odžaci), while low incidence (5-15%) was observed in eight localities. Root of barnyard grass (*Echinochloa crus-galli* L. P. Beauv.) was infected with the fungus in the amount of 20% (Družetić) and 30% (Zemun Polje), while 15% of green bristle-grass root (*Setaria viridis* L. P. B.) was infected.

DISCUSSION

The obtained results indicate that *P. terrestris* is a widespread pathogen in Serbia. This pathogen was determined in 25 out of 27 localities or in 12 of the 16 plant species. It is known that *P. terrestris* attacks root of numerous crop plants, such as onion, soya bean, oats, barley, wheat, maize, cucumber, tomato, pepper, carrot and other plant species (Kreutzer, 1941). In the present research, positive results were obtained for these species as well, with the exception of cucumber and carrot.

The analysis of symptoms and the detection of the fungus in root of certain plant species showed their interrelationship. In fact, *P. terrestris* was determined in all plant species with a symptom of reddish discoloration of root. On the other hand, the fungus was not present in plant species only with brown root discoloration, such as in cases of pepper, cucumber, squash and carrot. *P. terrestris* differs from other species of *Pyrenochaeta*, not only in morphological characteristics, but also as it causes red root rot (Watanabe and Imamura, 1995; Zitter et al., 1996; Lević et al., 2011, 2012a, 2012b), while other species cause black (Ramsey, 1990) or brown root rot (Ball, 1979).

The highest fungal incidence was determined in maize root (up to 72.5%), then in oats (50.0%) and Johnson grass (37.5%). In previous studies we have found that this fungus occurred early in the season and intensively developed in root of maize (Lević et al., 2012a, 2012b), which is in accordance with results obtained in this study. The relatively high incidence of the fungus in root of Johnson grass can be explained by the fact that this plant species belongs to the family *Poaceae* in which the fungus often appears (Sprague, 1944). Furthermore, perennial species of the *Poaceae* family, including Johnson grass, are widespread weeds in maize crops in Serbia (Stefanovic and Simic, 2006; Simić and Stefanović, 2006).

The incidence of *P. terrestris* in root of different plant species was affected by agroecological conditions in some localities. The effects of local agroecological conditions on the fungal incidence was most obvious in Veliki Crljeni because all the samples collected in this locality were negative for the presence of this fungus. On the other hand, the fungus was found in all samples collected in the locality of Zemun Polje and its incidence varied from 14.2% to 55.5% (average, 28.1%). The highest incidence was recorded in maize sample from Gornji Milanovac (72.5%).

In conclusion, the data show that *P. terrestris* is a very important root pathogen of maize, oats and weeds (Johnson grass and barnyard grass), which are often associated with maize crops in Serbia. These data are in accordance with the literature data indicating that this fungus usually occurs on root of plants belonging to the family of *Poaeceae*. Furthermore, the fungus was rarely and with a lower incidence determined in root of vegetable crops, and even in root of *Allium* species that are worldwide known as common hosts of *P. terrestris*.

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УЧЕСТАЛОСТ ПОЈАВЕ *PYRENOCHAETA TERRESTRIS* НА КОРЕНУ РАЗЛИЧИТИХ БИЉНИХ ВРСТА У СРБИЈИ

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РЕЗИМЕ: Узорци корена жита (овас, пшеница, јечам, кукуруз и питоми сирак), поврћа (бели лук, црни лук, паприка, краставац, бундева, шаргарепа и парадајз), индустријског биља (соја) и корова (дивљи сирак, коровски просо и зелени мухар), који су прикупљени у различитим агроеколошким условима у Србији, анализирани су на присуство *Pyrenochaeta terrestris*. Гљива је утврђена у 42 од 51 узорка (82,4%), а степен напада је варирао од 2,5% до 72,5%. Генерално, највећи степен напада гљиве утврђен је на корену жита (просек 30,3%), а затим на корену корова (просек 14,2%) из породице *Poaceae*. Међу појединачним врстама, гљива је у највећем степену утврђена на корену кукуруза (до 72,5%) и дивљег сирка (до 37,5%). Насупрот томе, гљива је ређе утврђена на корену повртарских култура (просек 6,7%). Црвенило (ружичаста, црвенкаста, црвена и љубичаста

боја) корена било је у корелацији са учесталошћу појаве гљиве. Добијени подаци указују на то да је *P. terrestris* широко распрострањена у Србији и да су повољни услови за њен развој.

КЉУЧНЕ РЕЧИ: *Pyrenochaeta terrestris*, корен, учесталост, жита, соја, поврће, коров