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DIFFERENT SYMPTOMS IN MAIZE ROOT CAUSED BY *PYRENOCHAETA TERRESTRIS* AND THE FUNGAL COLONY PROPERTIES

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ABSTRACT

Rot symptoms that *Pyrenochaeta terrestris* causes on the root of each internode of maize hybrids belonging to different maturity groups, as well as the impact of growing conditions (substrate, temperature and light) on the properties of the colony and the pycnidial formation of this fungus were studied. The large number of symptoms was obtained by various combinations of tissue colour changes (red, brown, yellow, blue and lighter or shades of these colours), changes in a particular part of the root (root top, part of the epidermis, the entire epidermis, tissue under the epidermis or the whole root) and the form of spots and streaks (shape and size). Pinkish red symptoms prevailed on younger roots, particularly from the 5th to the 7th internode. When *P. terrestris* was grown on potato dextrose agar (PDA) in the dark at 25ºC, the variability of the fungus was expressed in relation to the colour of the aerial (white, pink, grey, yellow and brown) and the substrate mycelium (purple, pink, grey, green and yellowish) and to the pycnidial formation (present or absent). The interrelationship between types of symptoms and properties of *P. terrestris* colonies was not determined on PDA. The fungus always produced the red purple pigment and mostly pycnidia on carnation leaf agar (CLA) at 25ºC and under the alternating 12 h combined light (fluorescent and near ultra violet (NUV) light)/dark conditions. These conditions are suitable for the reliable identification of the fungus.

Key words: *Pyrenochaeta terrestris*, maize, red root rot, symptom types, colony properties.

INTRODUCTION

Pyrenochaeta terrestris (Hansen) Gorenz, Walker & Larson (syn. *Phoma terrestris* Hansen) in a complex of organisms causing red rot root of maize is indicated as a primary pathogen (Mao et al., 1998). The disease occurs during the entire growing period, infecting the root and the basal part of the maize stalk, primarily under the ground (Whitney and Mortimore, 1961). A week after root infection, the pathogen penetrates the layer between the epidermis and the vascular tissue, and it sometimes penetrates into the vascular tissue (Carvajal, 1945). Shallow dark brown and stained spots are formed on the reddish surface of the infected tissue. The root of infected plants is usually brittle. A pink mycelium and the rudiments of olive dark pycnidia, developing in the epidermis layer within the endodermal cells, are visible in the root.

In North Carolina in July 2006, symptoms of the red root rot (*P. terrestris*) developed on leaves of sweet maize in a 5-8-day interval, a week or two after silking, included leaf drying and a weak ear development (Koenning et al., 2007). Three weeks after pollination, when sweet maize was harvested, the crown and the first internode above the ground of the infected plants were rotten and reddish, but the root seemed to be normal. Olive black pycnidia with long setae around ostioles were immersed in the stalk close to the first internode above the ground.

In Serbia, occurrence of red root rot caused by *P. terrestris* was described in different plants (Petrović and Lević, 1999), and different maize hybrids (Lević et al., 2011). The objective of this study was to examine in detail the development of symptoms in different root internodes during

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phenol-phases of the intensive vegetative development. Furthermore, this research was also performed to determine whether there is any connection between morphological traits of different isolates of the fungus and symptoms that this fungus causes on the maize root.

MATERIAL AND METHODS

Collection of maize samples and fungus isolation

The root samples of the four maize genotypes were collected on the 70th days after maize sowing. Maize genotypes, belonging to different maturity groups, were used as a material in this study: FAO-400 – medium early maturity hybrid (H-1), FAO-500 – medium late maturity hybrid (H-2) and FAO-700 – late maturity hybrids (H-3 and H-4).

A method previously described by Lević et al. (2011) was used for the isolation of the fungus. In short, roots of five plants per replicate of each genotype were thoroughly rinsed under running tap water. After drying, root parts were separated according to procedure described by Guingo and Hébert (1997). A four-replicated trial was set up. All changes in individual parts of each root internode were observed visually and described, and 10-15 mm long fragments were excised at the border between the infected and the healthy tissue. Each fragment of a sample was rinsed under running tap water for four hours, sterilised for five minutes in 1% hypochlorite and rinsed three times with distilled water. Individual sterilised samples were dried between two layers of soft paper and then were cut with a sterile scalpel into 2-3 mm long fragments. Eight fragments of each individually sterilised root part were placed in Petri dishes with potato dextrose agar (PDA) to which 300 mg of streptomycin sulphate was added.

Identification and maintenance of isolates

A part of the colony, which was developed around a root fragment, which was assumed to be of *P. terrestris*, was transferred after 6-7 days to PDA and carnation leaf agar (CLA). The composition and the preparation of these media were described by Burgess et al. (1994) in their Laboratory Manual for *Fusarium* Research.

The fungus was grown on PDA at 25ºC in the dark, and on CLA in the alternating 12 h combined light (fluorescent and near ultra violet (NUV) light)/dark conditions. The appearance and the colour of colonies were described after the 7-10-day development of isolates on PDA, while the colour and the production of pycnidia with sets and pycnospores on CLA were described mainly after 2 weeks.

The frequency (F) of symptoms occurrence (%) caused by *Pyrenochaeta terrestris* were estimated as follows: F (%) = [Number of root samples in which a species occurred/Total number of root samples] x 100 (Ghiasian et al., 2004).

The *Pyrenochaeta terrestris* cultures, isolated in the course of this study and designated MRIZP, were stored on agar slants (PDA, CLA and SNA) in 5-ml vials within the collection of the Maize Research Institute, Zemun Polje. Synthetic nutrient-poor agar (SNA – Spezieler Nährstoffarmer Agar) was prepared after Nirenberg (1976).

RESULTS

Pyrenochaeta terrestris was present in all root parts of all four hybrids on the 70th day after maize sowing. Tables 1-4 present symptoms whose individual frequency was above 10%.

The main symptoms in the medium early maturity hybrid H-1 were as follows: pink tissue under the root epidermis on the 5th internode (66.7%), pinkish brown tissue of the 1st internode (50.0%), root tip necrosis on the 6th internode (50.0%), and yellowish to brown spots in the primary root (46.9%) (Table 1).

Pyrenochaeta terrestris was isolated from 38.1% of symptomless root samples on the 4th internode of the hybrid H-1.

Of the 41 symptoms on the roots caused by the fungus *P. terrestris*, the largest number was determined on roots of medium late hybrid H-2 (Table 2). The most frequent

symptoms in this hybrid were red tissue (100%), necrosis (58.3%), pink tissue (55.6%) and yellowish to yellowish brown tissue (42.8%) in the root on the 7th internode, primary root, in the root on the 1st and 5th internode, respectively. The remaining types of symptoms that were not individually presented in Table 2 encompassed 23.6% in the root on the 2nd internode and 22.9% in the root on the 5th internode. Symptoms in the form of spots were developed in the roots on the 2nd (20.4%) and the 4th internode (10.0-15.0%), but they were more significant and intensive on the 1st internode (44.4%). *Pyrenochaeta terrestres* was isolated from insect-damaged roots of the 3rd internode up to 15% (Table 2). Considering the distribution of certain symptoms, the soft epidermis tissue and the red tissue under the epidermis were the most frequent types of symptoms (100%) in the root on the 6th internode of the late maturity hybrid H-3, followed by brown to yellowish brown spots in the root on the 2nd and the 3rd internode (57.8% and 41.8%, respectively), and the reddish yellow tissue under the epidermis in the root on 6th internode (50.0%) (Table 3). The pink to dark purple tissue was the most intensive on the 1st internode (44.4%), and reddish brown tissue on the 4th internode (33.3%). The fungus was isolated in the amount of 10.9% and 23.8% from insect-damaged roots on the 3rd and 4th internode, respectively.

The symptom in a form of the pinkish brown and necrotic tissue was the most frequent in the root on the 5th internode of the late maturity hybrid H-4 (100.0%), as well as, brown to yellowish brown spots and streaks on the 1st internode (88.9%) (Table 4). Up to 32.0% of *P. terrestris* were isolated from insect-damaged roots. High frequency of symptoms (40.0%) presenting both swollen tissue and cracked epidermis, was noted on the root in the 4th internode.

Table 1. The frequency (%) of disease symptoms caused by *Pyrenochaeta terrestris* in roots of the H-1 hybrid on the 70th days after sowing

Symptom in roots	Primary root	Root on internodes						
		1st	2nd	3rd	4th	5th	6th	7th
Pale brown to yellowish brown tissue				18.8				
Pink to purplish violet tissue							25.0	
Pinkish brown tissue		50.0						
Pale pinkish yellow to yellow tissue				15.2				
Pink tissue under the epidermis						66.7		
Red tissue		10.0					25.0	
Yellow to yellowish brown tissue			13.5					
Yellow to yellowish red tissue				28.1				
Necrosis and dark brown tissue	36.8	10.0	17.7		18.2	33.3		
Root tip necrosis							50.0	
Dark brown to yellowish brown spots and strips		10.0	14.9	25.0	13.6			
Dark brown fusiform spots and streaks	12.2		17.3					
Yellowish to brown spots	46.9							
Sum	95.9	80.0	63.2	87.1	31.3	100.0	100.0	0.0
Other symptoms	4.1	20.0	36.8	12.9	30.6	0.0	0.0	0.0
Symptomless					38.1			

Table 2. The frequency (%) of disease symptoms caused by *Pyrenochaeta terrestris* in roots of the H-2 hybrid on the 70th days after sowing

Symptom in roots	Primary root	Root on internodes						
		1st	2nd	3rd	4th	5th	6th	7th
Pale brown to brown tissue					25.0	42.3		
Pale brown to yellowish brown tissue				15.0				
Necrosis	58.3						28.6	
Pink to purplish violet tissue			26.2					
Pink tissue		55.6						
Pinkish brown tissue	12.5						28.6	
Red tissue								100
Reddish yellow to yellowish red tissue				25.0				
Pale reddish brown to pale brown tissue				20.0				
Pale reddish to reddish brown tissue					20.0	11.8		
Yellowish to brownish yellow tissue	12.5							
Pale yellowish brown to yellow tissue						11.5		
Yellowish to yellowish brown tissue					25.0		42.8	
Glossy black spots						11.5		
Dark brown fusiform spots and streaks		44.4						
Brown to yellowish brown spots and strips			20.4		15.0			
Pale pink spots					10.0			
Under-grown root	12.5							
Cracked epidermis			29.8	10.0				
Insect-damaged tissue				15.0				
Sum	95.8	100	76.4	85.0	95.0	77.1	100	100
Other symptoms	4.2	0.0	23.6	15.0	5.0	22.9	0.0	0.0

Table 3. The frequency (%) of disease symptoms caused by *Pyrenochaeta terrestris* in roots of the H-3 hybrid on the 70th days after sowing

Symptom in roots	Primary root	Root on internodes						
		1st	2nd	3rd	4th	5th	6th	7th
Pale brown to greyish brown tissue			10.1	30.9				
Necrosis and brown tissue	22.6							
Pink to dark purple tissue		44.4						
Pinkish yellow to brownish yellow tissue				10.9				
Reddish brown tissue		22.3			33.3			
Reddish lateral root	11.1							
Reddish yellow tissue under the epidermis						50.0		
Soft epidermis and red tissue under the epidermis							100	
Yellow to brownish yellow tissue	25.9		14.4					
Brown to yellowish brown spots	25.6		57.8	41.8				
Dark brown fusiform spots and streaks	11.1							
Cracked epidermis					23.8			
Insect-damaged tissue				10.9	23.8			
Sum	96.7	66.7	82.3	94.5	80.9	50.0	100	0.0
Other symptoms	3.3	33.3	17.7	5.5	19.1	50.0	0.0	0.0

Table 4. The frequency (%) of disease symptoms caused by *Pyrenochaeta terrestris* in roots of the H-4 hybrid on the 70th days after sowing

Symptom in roots	Primary root	Root on internodes						
		1st	2nd	3rd	4th	5th	6th	7th
Pale brown to greyish brown tissue			27.2					
Dark brown swollen tissue				12.0				
Brownish yellow to moderate brown tissue				16.0				
Pinkish brown and necrotic tissue						100		
Reddish brown tissue		11.1						
Necrosis of root and lateral roots	42.1			16.0				
Dry tissue	21.1							
Brown to greyish brown spots and streaks	26.3							
Brown to yellowish brown spots and stripes		88.9						
Yellow bands	10.5							
Swollen tissue					40.0			
Cracked epidermis			22.7		40.0			
Insect-damaged tissue			31.6	32.0	20.0			
Sum	100	100	81.5	76.0	100	100	0.0	0.0
Other symptoms	0	0.0	18.5	24.0	0.0	0.0	0.0	0.0

The fungus was also isolated from tissues with different types of symptoms than described in Tables 1-4, but their frequency was below 10%. The group of symptoms with the intensity below 10% encompassed necrosis (4.8-9.6%), swellings (4.3-9.5%), lentil-like brown spots (1.2-9.4%), dents in the root (9.4%), swollen and red root tip (9.1%), dark pith (7.7%), root purple edges (5.0%), red to pale reddish brown tissue under the epidermis (7.6%), pale reddish root (6.3%), cracked epidermis (4.1-6.1%), yellow root tip (5.8%), greyish brown tissue (2.9-5.5%), dark brown, swollen root (4.9%), brown spots with black corpuscles (1.2%) and root drying (1.2%). On the whole, these symptoms appeared up to 50%, 36.8%, 24.0% and 23.6% on the root of hybrids H-3, H-1, H-4 and H-2, respectively (Tables 1-4).

P. terrestris cultures, isolated from different parts of the root and with various symptoms, were most often floccose, compact, grew slowly, with lightly elevated smooth mycelia and with differently coloured aerial and substrate mycelia on nutrient-rich agar, such as potato dextrose agar (Table 5). Macroscopically different colonies were isolated from samples with similar symptoms, but also, similar colonies of this fungus were isolated from samples with different symptoms.

The aerial mycelium of *P. terrestris* was usually white or whitish, then purplish brown, reddish pink, violet, grey or greyish green, greyish brown, brown, dark brown and yellow on PDA. On the other hand, the substrate mycelium was pink, yellowish, greyish brown, greyish green, purple and purplish brown. In cases when colonies were white or grey, the reddish violet pigment was the most often produced in the medium at one end of the colony. A very low number of isolates produced pycnidia with pycnospores on PDA.

P. terrestris isolates produced a scarce mycelium on nutrient-poor agar such as CLA, while the pigment varied from red to purple red. The majority of isolates produced pycnidia with pycnospores on this medium. Similar results were obtained when the fungus was grown under laboratory conditions, 25±2°C and daylight, instead of the alternating combined light/dark conditions as described above.

The fungus also formed red to purple red pigment on paper on SNA medium with a sterile piece of filter paper 20 x 30 mm, but pycnidia were less often formed than on the CLA.

Table 5. Properties of *Pyrenochaeta terrestris* colonies originating from infected root samples of four maize hybrids

Description of *P. terrestris* colonies on potato dextrose agar	Description of symptoms on maize root
Floccose and abundant mycelium, with scarce pycnidia; scanty mycelium with purple pigmentation of agar; floccose, fast-growing, white mycelium; floccose, pink aerial and substrate mycelium.	Reddish tissue under the epidermis.
Whitish aerial mycelium and pink substrate mycelium.	Reddish tissue of the epidermis.
Purple aerial and substrate mycelium.	Dark brown root, cracked epidermis; yellowish brown spots; swellings on root.
Floccose, whitish pink mycelia, abundant pycnidia; floccose, abundant whitish pink aerial mycelium and purple substrate mycelium, abundant pycnidia; grey mycelium.	Dark brown spots and stripes.
Dark brown aerial mycelium and dark brownish purple substrate mycelium; purplish brown aerial and substrate mycelium; floccose, abundant, very smooth, grey aerial mycelium and greyish green substrate mycelium.	Dark brown elongated spots, root tip necrosis, cracked epidermis.
Floccose, dark brown mycelium.	Necrotic root tip.
Purple substrate mycelium; greyish brown aerial and substrate mycelium.	Necrosis of lateral roots
Floccose, compact, whitish aerial mycelium and yellowish substrate mycelium.	Necrosis of root surface layer.
Floccose, cirrhous or smooth aerial mycelium.	Root tip necrosis, dark brown root, cracked epidermis; fusiform smaller spots and swollen brown root.
Greyish olive green mycelium.	Cracked epidermis of the root tip, necrosis.
Compact, pale pink aerial mycelium and greyish substrate mycelium.	Small dented spots, insect-damaged tissue; yellowish surface, reddish pith.
Smooth, white aerial mycelium and greyish brown substrate mycelium; half a colony pink, the other half dark purple.	Moderate brown tissue, cracked epidermis; insect-damaged tissue.
Floccose, greyish purple mycelium, abundant pycnidia.	Dented lentil-like brown spots; dark brown spots; dark brown dented spots.
Floccose, yellow mycelium, abundant pycnidia.	Greater dark brown dented spots; insect-damaged tissue; pale brown root.
Floccose, white aerial mycelium; reddish pink with olive white mycelium in the middle of colony.	Yellow root or dark brown streaks.
Floccose, compact, whitish aerial mycelium and yellowish substrate mycelium; floccose, white aerial mycelium and pink annulate substrate mycelium.	Yellowish brown root; insect-damaged root.

DISSCUTION

A total of 41 different symptoms of disease were described in samples from which *P. terrestris* was isolated. The fungus was also isolated from insect-damaged (up to 23.8%) and symptomless roots (38.1%). The large number of symptoms was obtained by various combinations of tissue colour changes (red, brown, yellow, blue and lighter or darker combination of these colours), changes in a particular part of the root (root top, part of the epidermis, the entire epidermis, tissue under the epidermis or the whole root) and the form of spots and streaks (shape and size).

The symptoms frequency ranged from 1.2% to 100% depending on the hybrid response and part of the infected root. The most common symptoms were red, reddish, pink and pinkish coloured root tissue. These prevailing symptoms, as implied by the name of the disease caused by *P. terrestris*, occur not only in maize, but also in some other plant species (Newby et al., 1997). Gunasekaran and Weber (1981) identified cynodontin as red pigments in the liquid culture of the fungus.

According to studies carried out by Hornby and Ullstrup (1967), symptoms of the disease caused by *P. terrestris* in the maize roots differed in time of their incidence and

invasion. Mao et al. (1998) established that *P. terrestris*, causing the occurrence of red spots in roots in the mid-growing season, caused 40-55% of red root rot in susceptible hybrids at the end of the growing season. These authors stated that root damages caused by environmental stress and/or by soil pests and nematodes or secondary attackers affected the incidence of this fungus in the maize root.

The basic properties of pure cultures produced by *P. terrestris* on PDA at 25°C and dark established by the present studies had different growth, mainly slow, a compact mycelium, floccose, abundant mycelium with different colours of the aerial and substrate mycelium. The colour of colonies varied not only over isolates, but also within a single isolate. The colour of the aerial mycelium varied from white, over pink, grey, olive to yellow and brown. On the other hand, the colour of the substrate mycelium varied from purple, pink, grey, greyish green to yellowish. In previous studies, it was shown that the isolates of *P. terrestris* produced reddish purple colonies, while others had a floccose greyish centre with a pale greenish or brownish lower surface of the colony, or the upper and the lower surfaces of the colony were dark red or purple and olive green towards the periphery (Petrović and Lević, 1999).

According to literature data, *P. terrestris* colonies on PDA were described as purple, followed by red and black (Lascaris, 1986; Ferreira et al., 1991; Koenning et al., 2007). Zitter et al. (1996) stated that *P. terrestris* on PDA produced rounded, pale grey to olive brown colonies with a smooth margin that could be interspersed with a purple, fluffy aerial mycelium. The fungus coloured the medium in dark purple in the middle of the colony, with margins hatched pink, although it could vary and be with greyish patches, depending on the medium thickness, temperatures and the culture age.

Obtained results showed that *P. terrestris* rarely produced pycnidia on PDA. Ferreira et al. (1991) stated that this fungus produced on PDA pycnidia with or without setae. Kulik and Tims (1960) established that isolates had produced pycnidia, pycnidium-like bodies (absence of setae and pycnospores, less than real pycnidia) or that they had not produced pycnidia, pycnidium-like bodies. No isolate simultaneously produced pycnidia and pycnidium-like bodies. Due to frequent absence of pycnidia and pycnospores in pure cultures, Schneider (1965) stated that *P. terrestris* was a sterile red fungus. The fungus produced intercalary and terminal chlamydos-pores on PDA (Koenning et al., 2007).

Awuah and Lorbeer (1988) cultured *P. terrestris*, originating from onion roots, on the medium of maize flour with the addition of 500 ppm chloramphenicol and established that all isolates grew slowly and produced pink rounded colonies with smooth margins.

According to results obtained in this study, the medium and the light had a significant effect on the expression of cultural and morphological characteristics of *P. terrestris*, which provided a reliable identification of the fungus. On CLA, the medium with sterile carnation leaf fragments, under the alternating 12 h combined light (fluorescent and near ultra violet light)/dark conditions, the fungus always formed a specific red purple pigment and very often formed pycnidia with pycnospores.

Other authors also found that a medium with organic plant fragments or filter paper, as well as, the temperature and the light affected the traits of the fungus that provided a reliable identification of the fungus. Under laboratory conditions, *P. terrestres* produced the red pigment on nutrient-poor agar (20 g agar, 3 g $NaNO_3$, 1 g $MgSO_4$ and 1 L water) with fragments of wheat straw (Watson, 1961). *P. terrestres* was also successfully isolated from fragments of plants of the genera *Chloris*, *Dichantium* and *Echinochloa*, which were, instead of wheat straw fragments, placed into Petri dishes with Watson's agar (Alvardo and Polanco, 1984). In the identification procedure of *P. terrestres* not producing pycnidia in the culture, Coleman et al. (1997) relied upon symptoms in the host plant, colony morphology and the production of the pink pigment after a week on a medium

with 2% agar and wheat straw fragments. *P. terrestres* is the only known fungus that produces the red pigment on wheat straw and therefore the presence of the colour is a positive test for this fungus identification (Watson, 1961). Furthermore, on the medium with sterile wheat straw, *P. terrestris* produces pycnidia with setae and pycnospores in the course of 12 days, but some isolates abundantly sporulate after 3-4 days, while others produce 2-3 pycnidia with setae (Hess et al., 1964). In order to identify this fungus, Schwartz and Mohan (1995) used the medium whose surface was covered with sterile cheesecloth on which the fungus produced the characteristic red pigment. Camargo and Kimati (1991) established that *P. terrestris* more abundantly produced pycnidia and pycnospores if grown on the oatmeal agar covered with a sterile filter paper and if it was permanently exposed to the light.

The red pigment on certain media provides easy identification of the *P. terrestris* even without the presence of pycnidia and pycnospores. According to Zitter et al. (1996) the fungal isolation on water agar at 27-30ºC and permanent fluorescence light was a useful procedure for the *P. terrestris* identification, as well as the isolation on the V-8 agar, as the fungus sporulated 3-4 weeks later. Schneider (1965) established that the fungus produced pycnidia under near ultra violet light.

Properties of pycnidia and pycnospores established in the present study correspond to the descriptions made by Gorenz et al. (1948) and Ferreira et al. (1991). *P. terrestris* pycnidia vary in their size, shape, length and the number of ostioles, number and arrangement of setae. Pycnidia were globose to subglobose, single, immersed in the mycelium with a distinct ostiole and exuded conidial exudation, warty to mildly beaked, dark brown to black, strongly pigmented around the ostioles. Setae were dark brown, with 1-5 septata, abundant close to the ostiole or scattered over the pycnidium. Pycnospores were hyaline, oblong to oval with two guttulae at the end of the rounded tops. They escaped as gelatinous cirrus through the ostiole or the damaged pycnidium. Gorenz et al. (1948) found out that some *P. terrestris* isolates, after two subsequent sub-culturing, produced pycnidia with less setae, of irregular shape and of a smaller size in comparison with the original isolate. According to Hess et al. (1964) pycnidia produced in agar usual did not have setae.

CONCLUSIONS

Based on the obtained results, it can be concluded that symptoms observed in the root from which *P. terrestres* was isolated, were very different and depended on the maize genotype, the time of the fungal development in the root and the root senescence on certain internodes. It is better to use CLA for the identification of this fungus, as the fungus forms a characteristic red to red purple pigment and very often forms pycnidia on this medium. In contrast to CLA, the fungus forms very variable colonies and rarely forms pycnidia on PDA. There was no interdependence of cultural properties of fungi on PDA and symptoms caused by the fungus on the roots of maize.

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