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## MORPHOLOGICAL IDENTIFICATION OF *DIAPORTHE/PHOMOPSIS* SP. ISOLATED FROM *XANTHIUM ITALICUM*

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### SUMMARY

*Diaporthe/Phomopsis* sp. was isolated from *Xanthium italicum* (Italian cocklebur) for the first time in Eastern Croatia during 2004 year. As *Diaporthe/Phomopsis* species are known as pathogens of soybean, sunflower and other arable crops, it is important to study alternative hosts as a possible source of inoculum from an epidemiological point of view. This paper describes symptoms of infection on *X. italicum*, pathogen morphological and cultural characters on potato-dextrose agar (PDA), biometrical values of reproductive structures (pycnidia, beta conidia, perithecia, asci and ascospores) of naturally infected plants and of cultures grown on PDA.

Results obtained from undertaken studies indicate that our *Phomopsis* sp. isolates from *X. italicum* belong to the group of *P. helianthi*.

**Key- words:** *Diaporthe/Phomopsis*, *Xanthium italicum*, *Phomopsis helianthi*, characteristics

### INTRODUCTION

As a part of *Phomopsis* (Sacc.) Bubák genus, there are more than 800 saprophytic or pathogenic species, whose identification, for at least 70% of species, is based on a genus name or on a name selected for the host plant (Uecker, 1988). Some of pathogenic species cause diseases on grapevines, sunflower or soybean, which can result in great yield reduction.

In identification of *Phomopsis* sp., classic mycology focuses on morphological and cultural characteristics, as well as on biometrical values of anamorph and teleomorph structures. Delimitation of the species is uncertain because some *Phomopsis* sp. have pycnidia with both types of conidia (alpha and beta), and the other produce only one type of conidia. Moreover, teleomorph stage of *Diaporthe* Nitschke was not determined in all described *Phomopsis* species (Uecker, 1988). Important fact is that one species can infect several botanically different hosts, and that several *Phomopsis* sp. can be isolated from one plant species (Van Niekerk et al., 2005).

From an epidemiological point of view, weeds as alternative hosts of *Phomopsis* spp. are potential source of inoculum. Roy et al. (1997) isolated *Phomopsis longicolla* Hobbs. from leaves of *Ambrosia trifida*, *Xanthium strumarium*, *Euphorbia maculata* and *Rumex crispus*. *P. longicolla* from *Abutilon theophrasti* caused stem lesion of soybean and on velvetleaf stems (Li et al. 2001). Hepperly et al. (1980) isolated *Phomopsis sojae* Leh., *Colletotrichum dematium* (Pers. ex Fr.) Grove var. *truncata* (Schw.) Arx. and *C. gloeosporioides* (Penzig) Sacc from *A. theophrasti*. It was determined that *C. dematium* var. *truncata* and *P. sojae* from velvetleaf were more pathogenic for green bean and soybean seeds than the isolates of the same species originating from soybean. Jackson et al. (2002) determined that 12 species of weeds, including genera of *Convolvulus*, *Digitaria* and *Xanthium*, could be infected with *P. longicolla*, thus being source of inoculum for soybean. Mihaljčević et al. (1985) identified *Phomopsis* spp. on 15 plant species, among which were *X. strumarium* and *X. italicum*.

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From the *Xanthium* genus, Nikandrow et al. (1990) stated *X. spinosum*, *X. orientale* and *X. occidentale* to be hosts of *Phomopsis* species. In an earlier study of weeds mycopopulation in Eastern Croatia, Vrandečić et al. (2004, 2005, and 2006) isolated *P. sojae*, *P. longicolla* and *Diaporthe phaseolorum* (Cke. et Ell.) Sacc. var. *caulivora* Athow and Caldwell from *A. theophrasti*. Studied species had negative effect on soybean seed germination, germ length and percentage of germs with necrotic lesions.

*Diaporthe/Phomopsis* spp. were isolated from *X. italicum* for the first time in 2004 year. Their identification based on morphological and cultural characteristics is the aim of this study.

## MATERIAL AND METHODS

*X. italicum* plants exhibiting no symptoms, as well as plants with symptoms of infection with *Phomopsis* spp. were collected towards the end of September and in November 2004, and during March 2005 on four locations in Eastern Croatia. Collection sites of *X. italicum* were next to sunflower fields. Stems and side branches of *X. italicum* were washed in running water, cut into pieces of 15 mm and disinfected in 96% ethanol solution for 60 seconds. Plant material was rinsed three times in distilled water, dried with filter paper and placed in Petri dishes on moist filter paper. Petri dishes were kept in thermostat at 25 °C with 12 h light/12 h dark regime (NUV). Isolation of *Phomopsis* spp. was performed by transferring pycnidia and conidia exudates on potato-dextrose agar (PDA, Difco Laboratories, Detroit, MI). Pure cultures were grown in a thermostat for 50 days, over which period their growth and sporulation were constantly monitored. Sizes of 100 beta conidia, 100 ascospores, 50 asci, 20 pycnidia and 10 perithecia were measured by the Olympus microscope, in the Olympus DP Soft measurement software.

Ten *X. italicum* plants with 8-10 developed leaves were used for pathogenicity studies. Stems were injured with sterile scalpel and agar plugs with mycelium (5x5 mm) 7-day old cultures of *Phomopsis* spp. were placed on those injured tissue. Agar with mycelium were also placed onto the top of leaf (without cutting it). Inoculum was grown on PDA and taken from the edge of culture. Inoculation point was covered with moist cotton and aluminum foil in order to maintain moisture. Inoculated plant parts kept in the moist conditions for 4 days. After 20 days, the size of lesion on inoculated plants was measured. Control plants were inoculated with pure PDA.

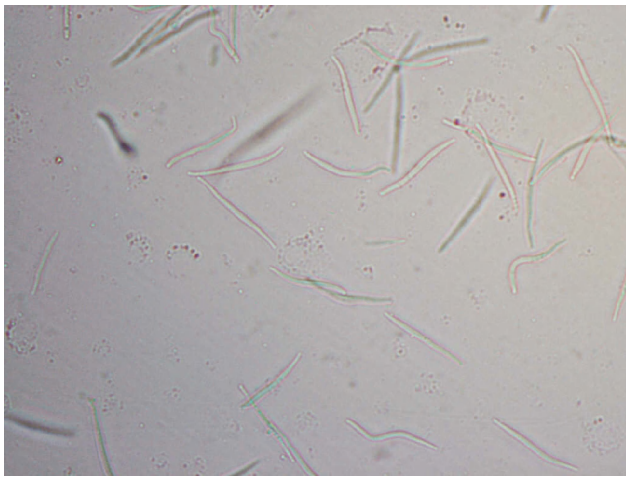
## RESULTS

Symptoms pointed out infection with *Diaporthe/Phomopsis* species were detected on main and side branches of *X. italicum* from the beginning of autumn (September) to the late autumn (November) of 2004. Grey pale lesions were found mainly around nodes. They had small black pycnidia immersed in epidermal tissue. Higher concentration of pycnidia was found around nodes and less on edges of lesion. The border between healthy and diseased tissue is diffused. Plants infected in such a manner were mostly found next to sunflower fields.

In 2-3 days after placing diseased plant parts (in length of 15 mm) in Petri dishes with wet filter paper, yellowish drops with many beta conidia appeared on pycnidia ostiolum.

In a pure culture (PDA, 25°C, 12 h light/12 h darkness), fungus formed less abundant white mycelium, which filled out a Petri dish (9 cm in diameter) in 8 days. Back side of culture was of whitish to beige color and had at the beginning light brown scattered spots, which turned later into dark brown. A 10-15-day old culture formed simple dark stroma with pycnidia on them. Yellowish spherical exudate contained only beta conidia. Pycnidia were spherical, measuring 240-450 x 230-380 µm. Conidia were filiform, straight or slightly curved at one end, measuring 24.4 x 1.8 µm (Photo 1).

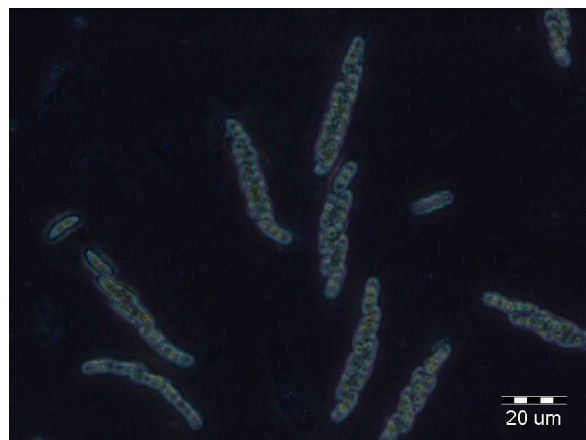
On the samples of *X. italicum*, which were collected in the end of November or in March after plants overwintered in fields, perithecia were formed in moist conditions. Fruiting body were completely placed in plant tissue, and necks being up to 1340 µm long were visible on the surface (Photo 2). Their width on the base was on the average 105.5 µm, and on top 52.5 µm. Diameter of perithecia was 280-430 µm. In a culture, perithecia were developed after 30 days on stomatal structures as pycnidia. Fruiting body were on the average smaller (290 x 280 µm) with necks (350-800 µm) shorter than of those in naturally infected plants. Asci were hyaline, elongated-elliptical in shape with eight two-celled ascospores in two rows (Photo 3).



**Photo 1. B conidia of *Phomopsis* sp. from *X. italicum* (orig.)**  
*Slika 1. B konidije Phomopsis sp. sa X. italicum (orig.)*



**Photo 2. Perithecia on stem *X. italicum* (orig.)**  
*Slika 2. Periteciji na stabljici X. italicum (orig.)*



**Photo 3. Asci and ascospore *Diaporthe* sp.**  
*Slika 3. Askusi i askospore Diaporthe sp.*

Biometrical values of reproductive structures were presented in Table 1 and compared with data of other authors for isolates of *Phomopsis* sp. from *X. italicum* and *P. helianthi* from *Helianthus annuus*. After 20 days of infection, symptoms on artificially infected plants of *X. italicum* were identical to those found on naturally infected plants. Zones of necrotic tissue spread above and below the infection point. Lesion length measured between 3-10 cm. Later on, pycnidia with beta conidia were formed within lesions. Side branches separated from diseased plant exhibited symptoms of wilting. On the infected leaf, necrosis was spreading along the main vein in shape of the letter V. In order to follow Koch's rules, pathogen was reisolated from artificially infected plants *X. italicum*.

## DISCUSSION

In Eastern Croatia, *Phomopsis* sp. was isolated from *X. italicum* for the first time in 2004 year. This occurrence was repeated in the following years, having identical symptoms. Infected plants were always found next to sunflower fields and sometimes on uncultivated area.

*X. italicum* is a common weed in arable crops and in ruderal habitat in Croatia and its numerousness on some locations can jeopardize the main crop.

Symptoms noticed on naturally infected plants in our studies do not correspond to those of Nikandrow et al. (1990). Symptoms occurred upon artificial infection of *X. italicum* with isolates of *Phomopsis* sp. (Carriere and Petrov 1990) matched symptoms common for infection of sunflower with *Diaporthe/Phomopsis helianthi*. Our findings contribute this allegation.

After comparing characteristics of our cultures of *Phomopsis* sp. from *X. italicum* with cultures of *P. helianthi* from sunflower (collection of the Chair for Phytopathology), no differences were determined.

Based on characteristics of *Phomopsis* species isolates from *X. italicum*, Muntanola-Cvetković et al. (1996) distinguished two groups, A and B. Comparing our isolates from Eastern Croatia with isolates of the B group (XIT-2), according to Muntanola-Cvetković et al. (1996) similarities are noticed in symptoms, formation and biometrical values of beta conidia, as well as in formation characteristic teleomorph stage. Over the whole period of our research, isolates from Eastern Croatia did not form pycnidia with alpha conidia either in natural environment or in laboratory. Muntanola-Cvetković et al. (1996) found alpha conidia in majority of cultures, although always in a small number. Mihaljčević and Vukojević (1994) reported on populations with either alpha or beta conidia, and on populations (mature plants of *X. italicum*) that had both types of conidia.

Isolates of *Phomopsis* sp. from *X. italicum* form perithecia on PDA (Muntanola-Cvetković et al. 1981), just like the isolates from Eastern Croatia. Yang et al. (1981) and Muntanola-Cvetković et al. (1981) reported that *P. helianthi* from sunflower on PDA also formed perithecia, while Carriere and Petrov (1990) found out that isolates of the same species from Vojvodina did not form perithecia on artificial substrate. They correlated isolates from *X. italicum* with the *Phomopsis* isolate determined by Yang (1984) and presupposed that *P. helianthi* could be adjusted form of *P. "xanthium"*, but on the new host – *Helianthus annuus*.

On the basis of our results obtained in studying symptoms, cultural and biometrical characteristics isolates of *Phomopsis* sp. from *X. italicum*, we consider that our isolates belong to *P. helianthi*, which need to be further proved in ongoing biomolecular researches.

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## MORFOLOŠKA IDENTIFIKACIJA *DIAPORTHE/PHOMOPSIS* SP. IZOLIRANOG *XANTHIUM ITALICUM*

### SAŽETAK

*Tijekom 2004. godine s Xanthium italicum (dikica vodena) prvi put je u istočnoj Hrvatskoj izoliran Diaporthe/Phomopsis sp. Kako su Diaporthe/Phomopsis vrste poznati paraziti soje, suncokreta i drugog uzgajanog bilja, značajno je, s epidemiološkog stajališta, proučiti krug alternativnih domaćina kao mogućeg izvora inokuluma. U ovom su radu opisani simptomi zaraze na X. italicum, proučene su karakteristike parazita uzgojem na krumpir dekstroznom agaru (PDA), utvrđene su biometrijske vrijednosti reproduktivnih struktura: pikhida, beta konidija, peritecija, askusa i askospora, s prirodno zaraženih biljaka i iz kultura razvijenih na PDA.*

*Na temelju dobivenih rezultata proučavanja Phomopsis sp. s X. italicum, držimo da naši izolati pripadaju vrsti P. helianthi.*

*Ključne riječi: Diaporthe/Phomopsis, Xanthium italicum, Phomopsis helianthi, karakteristike*

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**Table 1. Biometrical values of reproductive structures *Phomopsis* sp. and *P. helianthi* formed on PDA and in natural infection**

*Tablica 1. Biometrijske vrijednosti reproduktivnih organa Phomopsis sp. i P. helianthi formiranih na PDA i u prirodnoj infekciji*

Parasite <i>Parasite</i>	Host <i>Domaćin</i>	Author <i>Autor</i>	Biometrical values (µm) of reproductive structures Biometrijske vrijednosti (µm) reproduktivnih struktura					
			Pycnidia <i>Piknidi</i>	A conidia <i>A konidije</i>	B conidia <i>B konidije</i>	Perithecia <i>Periteciji</i>	Asci <i>Askusi</i>	Ascospores <i>Askospore</i>
<i>Phomopsis</i> sp. XIT-1 isolate-izolat A groups - <i>A grupa</i>	<i>X. italicum</i>	Muntanola-Cvetković et al. (1996) PDA	300-720 x 260-360	6.4-11.8 x 1.8- 2.5	present in most of cultures in less % - <i>prisutne</i> <i>u većini kultura</i> <i>u manjem %</i>	-	-	-
<i>Phomopsis</i> sp. XIT-2 isolate B groups - <i>B grupa</i>	<i>X. italicum</i>	Muntanola-Cvetković et al. (1996) PDA	+	present in most of cultures in less % - <i>prisutne u</i> <i>većini kultura</i> <i>u manjem %</i>	13.3-25.2 x 0.5- 1.5	+	+	+
<i>Phomopsis</i> sp.	<i>X. italicum</i>	Jurković et al. (2007) PDA	240-450 x 230-380	-	13.2-36.8 x 0.9- 2.2 (24.4 x 1.8)	200-450 x 200-350 (290 x 280)	37.1-59.8 x 5.8- 10.5 (47.3 x 7.8)	9.2-16.2 x 2.2- 5.5 (12.5 x 3.2)
<i>P. helianthi</i>	<i>Helianthus annuus</i>	Muntanola-Cvetković et al. (1981) PDA	170-320	-	22.0-32.0 x 0.5- 1.0	mostly 400 µm in diameter - <i>uglavnom 400</i> <i>µm u promjeru</i>	47.5-57.5 x 7.5- 12.0 (50 x 8)	12.5-14.5 x 3.25- 4.5
<i>P. helianthi</i>	<i>Helianthus annuus</i>	Fayret i Assemat (1987) natural infection- <i>prirodna</i> <i>infekcija</i>	125-288	-	28.4 x 1.7	350-480	39.0-56.0 x 6.6- 10.0 (50.0 x 8.5)	10.0-16.0 x 3.3- 5.5 (14.5 x 4.8)

+ (present) – *prisutni*; - (not determined) - *nisu utvrden*