Morphological and molecular identification of *Diaporthe helianthi* from *Xanthium italicum*

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ABSTRACT

Up to the present time, *Diaporthe helianthi* has not been reported outside the genus *Helianthus* in Croatia. This pathogen has been recently recovered on *Xanthium italicum* (cocklebur) in Slavonia and Baranja County. Isolates of *Diaporthe* sp. originating from *X. italicum* were studied and compared with *D. helianthi* isolates from sunflower. Phylogenetic analysis of nuclear ribosomal DNA internal transcribed spacer sequences (ITS1 and ITS2) showed that *X. italicum* is a new host for *D. helianthi*.

Key words: *Diaporthe helianthi* – identification – *Xanthium italicum*.

INTRODUCTION

The cultivated sunflower (*Helianthus annuus* L.) is the most important annual crop grown for edible oil in Croatia. Stem canker caused by *Diaporthe helianthi* Munt.-Cvet. et al. (anamorph *Phomopsis helianthi* Munt.-Cvet. et al.) is one of the most important sunflower diseases. The pathogen was identified in former Yugoslavia for the first time in 1980 (Mihaljcevic et al., 1980; Muntanola-Cvetkovic et al., 1981) and nowadays this disease has spread to many other countries. The fungus can cause yield losses up to 40% (Demazure, 1995) and reduce oil content (Franco and Morales, 1997) in the case of environmental conditions being favourable for infection.

The occurrence of fungi from *Diaporthe*/*Phomopsis* genera on weeds has been studied over a long period. Weeds as alternative hosts of *Diaporthe*/*Phomopsis* have a very important role as a potential source of inoculum for cultivated plants (Roy et al., 1997; Mengistu and Reddy, 2005; Vrandecic et al., 2006). Mihaljcevic and Muntanola-Cvetkovic (1985) reported *Phomopsis* spp. on 15 plant species, among which were *Xanthium strumarium* L. and *X. italicum* Moretti. Nikandorow et al. (1990) determined *X. spinosum* L., *X. orientale* L. and *X. occidentale* L. to be hosts of *Phomopsis* species. Muntanola-Cvetkovic et al. (1996) identified two *Diaporthe/Phomopsis* species on *X. italicum*. Until recently, sunflower was the only known host for *D. helianthi*. Piven' et al. (2000) stated that *Cyclachaena xanthiifolia* (family *Astraceae*) are potential alternative hosts for *Phomopsis arctii* (Lasch) Nitschke (*Diaporthe arctii*) and *P. helianthi*. This paper presents results of the study on morphological, cultural and biomolecular characterization to identify *Diaporthe/Phomopsis* isolate obtained from *X. italicum*.

MATERIALS AND METHODS

Isolates used in this study (Table 1) were obtained from naturally infected living plants or overwintered residues of *X. italicum* and sunflower plants from location in Slavonia and Baranja County (Croatia). *X. italicum* plant tissues with symptoms of infection with *Diaporthe*/*Phomopsis* were disinfected and small tissue pieces were placed in Petri dishes on moist filter paper or directly on potato dextrose agar (PDA). Petri dishes were kept at 25°C with 12 h light/dark regime. Isolation of *D. helianthi* isolates was performed by transferring mycelia or pycnidia with conidia exudates on PDA. In order to examine microscopic features and cultural characteristics, pure cultures were kept in a thermostat (25°C, 12 h light/dark). Morphological and cultural characteristics of *Diaporthe/Phomopsis* isolates from *X. italicum* were compared with isolates of *D. helianthi* from sunflower.

DNA extraction was made following Cenis (1992). The standard PCR conditions for ITS475 primers are described in White et al. (1990). Purified PCR products were sequenced in both directions using primers ITS4 and ITS5 (Gene Lab – ENEA, Roma). Sequences were aligned by CLUSTAL W (Thompson et al., 1994) and manually adjusted by Chromas (version 1.45). Additional *Diaporthe/Phomopsis* sequences were obtained from GenBank (http://www.ncbi.nlm.nih.gov) and added to alignment. The *Colletotrichum coccodes* (Wallr.) Hughes and *Colletotrichum dematium* (Pers.) Grove were included as an outgroup. Alignment gaps were treated as missing data. Phylogenetic analysis was

conducted by UPGMA (Kimura 2-parameter model) methods using MEGA version 3.1. (Kumar et al. 2004). Bootstrap analysis for 1000 replicates was done to evaluate tree topologies.

Table 1. Isolates used in this study

Isolate	Species	Host	Origin	Reference	GenBank numbers
CBS592.81	*D. helianthi*	Sunflower	ex Yugoslavia	Rekab et al. (2004)	AY705842
IMI318865	*D. helianthi*	Sunflower	ex Yugoslavia	Rekab et al. (2004)	AJ312363
Dh95004	*D. helianthi*	Sunflower	France	Say-Lesage et al. (2002)	AF358438
Dh95016	*D. helianthi*	Sunflower	France	Say-Lesage et al. (2002)	AF358435
F1	*D. helianthi*	Sunflower	France	Rekab et al. (2004)	AJ312350
A3	*D. helianthi*	Sunflower	Argentina	Rekab et al. (2004)	AJ312364
Xa3	*D. helianthi*	*X. italicum*	Croatia	This study	
Xa5	*D. helianthi*	*X. italicum*	Croatia	This study	
Su5/04	*D. helianthi*	Sunflower	Croatia	This study	
Su12/05	*D. helianthi*	Sunflower	Croatia	This study	
978	*C. coccodes*	Pepper	Italy		AM422215
AR3563	*C. dematium*	*Lirope muscarii*	Mexico	Farr et al. (2006)	DQ286154

RESULTS AND DISCUSSION

After 8 days, colonies of *Diaporthe/Phomopsis* from *X. italicum* on PDA formed less abundant white mycelium, the aerial part plenty of it, sometimes with narrow greenish-yellow areas. Reverse of culture was whitish to beige color and had in the beginning light brown scattered spots, which later turned dark brown. The pycnidia formed in simple stromatic structures usually aggregate, rarely solitary, measuring 240-450 x 230-380 µm. Conidia only of ß-type, 24.4 x 1.8 µm. After 30-40 days isolates from *X. italicum* (Xa3 and Xa5) and isolate Su5/04 from sunflower formed spare globose perithecia. Biometrical values of perithecia (isolates from *X. italicum*) were 290 x 280 µm. Asci hyaline, elongated-elliptical, 8-spored, 37.1-59.8 x 5.8-10.5 µm (av.= 47.3 x 7.8), ascospores irregularly biseriate, subelliptical, slightly constricted at the septum, 1-septate, 9.2-16.2 x 2.2-5.5 µm (av.= 12.5 x 3.2). Comparing cultures characteristics and biometrical values of *Diaporthe/Phomopsis* from *X. italicum* and *D. helianthi* from sunflower, no differences were determined.

Comparing our isolates from Croatia with the *Phomopsis* sp. isolates from *X. italicum* (XIT-2) described by Muntanola-Cvetković et al. (1996), similarities are established in symptoms, pycnidia formation and biometrical values of ß conidia, as well as in development and the characteristics of teleomorphic stage. Our isolates did not form pycnidia containing α conidia either in natural environment or in laboratory. Muntanola-Cvetković et al. (1996) found this type of conidia in the majority of cultures, although always in a small number.

The sequence analyses of rDNA-ITS *Diaporthe/Phomopsis* isolates using UPGMA methods revealed that our isolates from *X. italicum* (Xa3 and Xa5) group together (100% bootstrap support) with the isolates of *D. helianthi* from sunflower (Su5/04 and Su12/05) and with *D. helianthi* isolates from former Yugoslavia (AJ312363 and AY705842), France (AF358438, AJ312350 and AF358435) and one from Argentina (AJ312364) which Rekab et al. (2004) marked as *D. helianthi s. str*. All isolates from France and former Yugoslavia originated from countries where severe epiphytotics of sunflower stem canker were reported. On the basis of morphological and molecular characteristics, the fungi isolated from *X. italicum* were identified as *D. helianthi*.

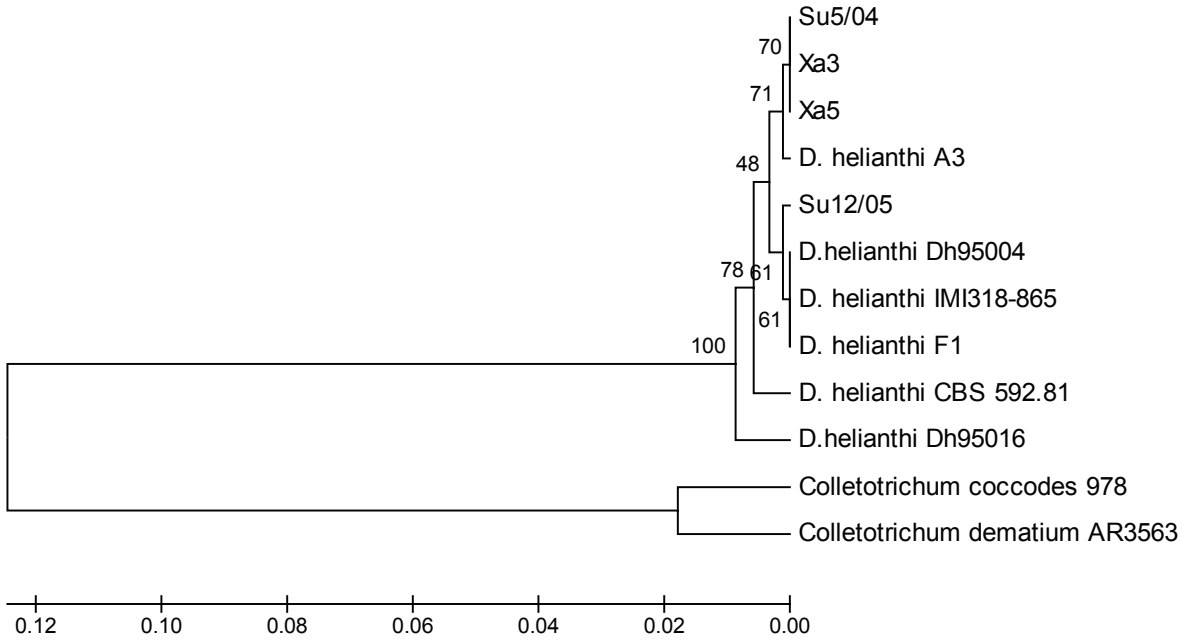

Fig. 1. Molecular phylogenetic tree based on ITS1-5.8S gene-ITS2 sequences using UPGMA -Kimura 2-parameter model. Numbers above each branch represent percentages of 1000 bootstrap repetitions. *C. coccodes* (AM422215) and *C. dematium* (DQ286154) were used as an outgroup. The scale bar shows the number of substitutions per site.

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