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ORIGINAL PAPER

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MORPHOLOGICAL CHARACTERISTICS AND PATHOGENICITY OF *Diaporthe eres* ISOLATES TO THE FRUIT TREE SHOOTS

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ABSTRACT

This work is a continuation of research on *Diaporthe* genus isolates obtained in 2010−2012 from fruit trees in Poland, which on the basis of previously conducted molecular tests, have been identified as one species belonging to the *Diaporthe eres* species complex. The aim of this study was to determine the morphology and pathogenic abilities of tested *Diaporthe eres* isolates. The experiment included cross tests, in which the shoots of apple, pear, cherry and plum trees were inoculated with each of the 4 isolates derived from each mentioned host plants. As a result of experiment, the pathogenic nature of *D. eres* in relation to the shoots of fruit trees, was confirmed. The isolates were also characterized on the basis of the colony appearance and spore dimensions. Morphological features of studied *D. eres* cultures were very similar, regardless of the isolate and the host plant, from which they were obtained. All tested isolates formed alpha and beta conidia having the same range size.

Key words: macroscopic features, microscopic features, orchard plants, pathogenic ability, *Phomopsis*

INTRODUCTION

Genus *Diaporthe* Nitschke contains many important plant pathogenic species causing nonspecific symptoms on a wide range of host plants worldwide [Sutton 1980, Uecker 1988, Farr et al. 1999, 2002, Król 2002, Król and Kowalik 2010, 2011, Udayanga et al. 2011, 2014a, b, Gomes et al. 2013, Dissanayake et al. 2017, Guarnaccia et al. 2018]. Several species were also reported as pathogenic to fruit plants in Europe [Machowicz-Stefaniak 1993, Mostert et al. 2001, Karaoglanidis and Bardas 2006, Król 2006, 2007, Kirk et al. 2008, Udayanga et al. 2011, Gomes et al. 2013, Abramczyk and Król 2016, Michałecka et al. 2016, Guarnaccia and Crous 2017]. Species in this genus were characterized initially by host association and morphological characteristics, which resulted in a proliferation of names based on the hosts, from which they were isolated [Uecker 1988]. Recent studies have revealed that host affiliation is not a reliable character for species definition since the several species of *Diaporthe* have wide host ranges [Uddin et al. 1997, Mostert et al. 2001, van Niekerk et al. 2005, Santos and Phillips 2009, Diogo et al. 2010, Gomes et al. 2013, Udayanga et al. 2014a]. Additionally, the strains isolated from one host species are not necessarily closely related and may represent more than one taxon [Rehner and Uecker 1994, Farr et al. 2002].

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Recently, the redefinition of *Diaporthe* and *Phomopsis* species has been ongoing and there is a movement to provide all fungal species with a single name instead of the present practice of providing a teleomorph and anamorph name for different states of a species [Udayanga et al. 2011, 2014a, b, Gomes et al. 2013, Rossman et al. 2014, 2015]. Therefore, in the present study, name *Diaporthe* is adopted over *Phomopsis* for this group of fungi and all isolates of the fungus used in this study belonged to the conidial stage of the fungus.

The aim of the present study was to characterize isolates of *Diaporthe eres* based on morphological and pathological characteristics. Current studies are a continuation of the research on *Diaporthe* isolated from fruit plants, which were previously genetically identified by Abramczyk and Król [2016] as *Diaporthe eres* species complex.

MATERIAL AND METHODS

Selected isolates of *Diaporthe eres* obtained from apple (*Malus* Mill.), pear (*Pyrus* L.), cherry (*Cerasus* Mill.) and plum (*Prunus* L.) were characterized on the basis of size, structure and color of the colony, conidiomata formation and the rate of sporulation as well as on the appearance and size of alpha and beta conidia. The study included 40 randomly selected isolates of *D. eres*, 10 isolates from the shoots of each tree: apple, pear, cherry and plum. Cultures were cultured at 24°C in the dark on potato dextrose agar (PDA) and Czapek-Dox medium in Petri dishes. The inoculum was the 3 mm mycelial discs derived from sporulated single-spore colonies growing on PDA medium and placed mycelium in the center of the Petri dish. For each experimental combination, 4 dishes were considered the replications. Colony diameters were measured every 2 days by making two measurements per cross and they were used as a measure of the rate of growth. The appearance of colonies was described and photographed 14 days after medium inoculation. To analyze more accurate the formation of morphological structures, macro and microscopic observations were carried out every 2 days up to 40^{th} day of fungal culture incubation. Given the poor sporulation of most species of the genus *Diaporthe*, additionally the mycelium from studied isolates was transplanted onto poor PDA medium in 90 mm Petri dishes supplemented with carnation leaf fragments in order to stimulate the sporulation [Król 2005a]. Once the fungus sporulation has started, the microscopic preparations were prepared and the measurements of alpha and beta conidia were made using Images Plus 2.0 PL software. For each fungus isolate, the length and width of 100 alpha- and 50 beta-conidia were measured. For each plant species, the size of a total of 1000 alpha conidia (10 isolates × 100 conidia) and 500 beta conidia (10 isolates × 50 conidia) were analyzed. The resulting data allowed to calculate the range of size of both spore types and the range of their size in relation to the host plant, from which they were isolated.

During the study, the photographic documentation was carried out using scanning and light microscope combined with a Moticam 1SP digital camera.

Visually healthy one-year old shoots of apple, pear, cherry and plum as well as 4 isolates of *Diaporthe eres*: 269J, 292G, 322W, 359Ś derived from the same host plants were used for pathogenicity tests. In the laboratory, they were cut into approximately 20 cm sections without leaves and lateral shoots and washed under running water for 15 min. Then the shoots were subjected to surface disinfection by immersing for 30 s in 10% sodium hypochlorite, then rinsing three times for 3 min in sterile distilled water. Five shoots of a given plant species and the sterile fragment of the wet cotton wool were placed along the sterile humid chambers disinfected with 96% ethanol and properly signed (rectangular polystyrene trays having the following dimensions: 14 cm × 22.5 cm × 3 cm). In the middle part of the shoot, an epidermis was wounded using a sterile scalpel and the inoculum of the fungus was placed underneath. The inoculum was the disc of sporulating mycelium having a diameter of 3 mm. The experiment included cross tests, in which the shoots of apple, pear, cherry and plum trees were inoculated with the isolates derived from the same species of fruit trees. For each isolate and plant species, 15 shoots were examined (5 shoots in 3 replicates) and a single tray was considered a replicate. As a control, there were the shoots, on which a pure agar

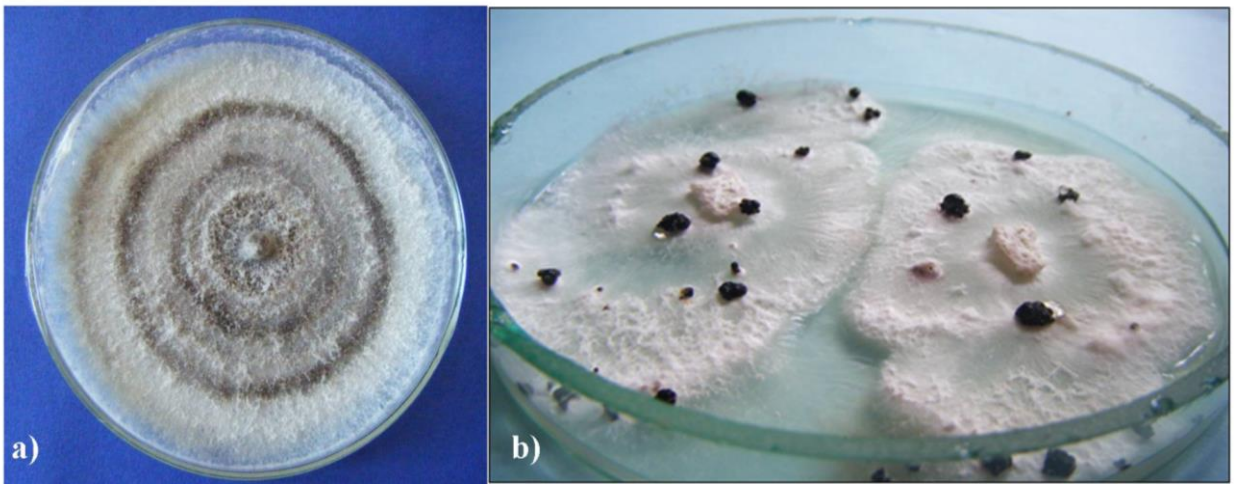
disc was placed. The trays were wrapped tightly with a cling film and kept at a constant temperature of 25°C. Every two days, 2 ml of distilled water was added to the cotton wool in order to maintain the optimum humidity conditions for fungal growth. The observations were taken every two days for a period of 24 days after inoculation. Each time the occurrence of the lesions in the inoculation place was observed, their length was measured and the pycnidia formation and sporulation of the fungus was recorded. The final step of the experiment was the re-isolation of the fungus performed in accordance with the Koch's postulates.

RESULTS

Results regarding the morphological characteristics showed that the mycelium growth rate and the colonies morphology of the tested *D. eres* isolates were very similar, regardless of the isolate and the host plant, from which they originated. Tested isolates growing on PDA medium at 24°C formed initially white-cream then cream-beige colonies with a characteristic zonation of more or less fluffy mycelium. Usually after 14 days of culture, the mycelium surpassed the entire surface of 90 mm Petri dish (Tab. 1, Phot. 1a).

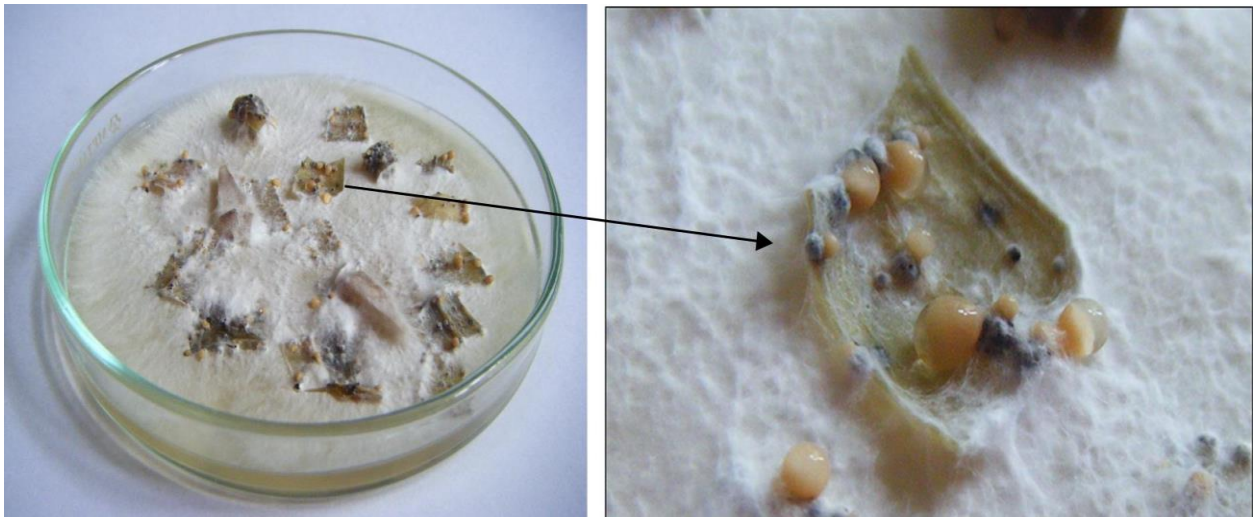
Table 1. Diameter of the colonies and dimensions of conidia of tested *Diaporthe eres* isolates growing on PDA medium (average values for 10 isolates)

Origin of isolates	Diameter of 14-day old cultures (mm)	Day of the first conidiomata formation	Day of sporulation appeared	Dimensions of alpha conidia (µm)	Dimensions of beta conidia (µm)
Diaporthe eres from apple	90	14–18	22–29	(5.4-)6.9–8.6(-9) × (1.9-) 2.2–3(-3.5)	25.9–35.9 × 1.2–2.4
Diaporthe eres from pear	90	16–19	24–32	(4.8-)6–7.9(-8.5) × (1.8-)2–3(-3.3)	19.5–31.4 × 1–2.3
Diaporthe eres from cherry	90	18–21	25–33	(4.9-)6.2–7.8(-8.8) × (1.8-)2.4–3.2(-3.5)	25.3–34.6 × 1,3 –2.4
Diaporthe eres from plum	90	15–20	21–31	(5.2-)6.5–8.5(-8.7) × (1.9-)2.2–2.8(-3.2)	19.4–34.5 × 1–2.2

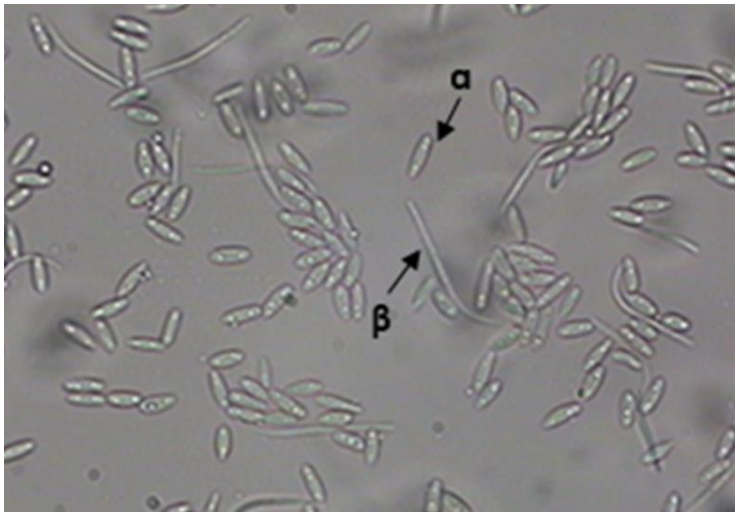


Phot. 1. Characteristic growth of *Diaporthe eres:* a) appearance of the colony b) conidiomata formation on PDA medium at 24°C, phot. B. Abramczyk

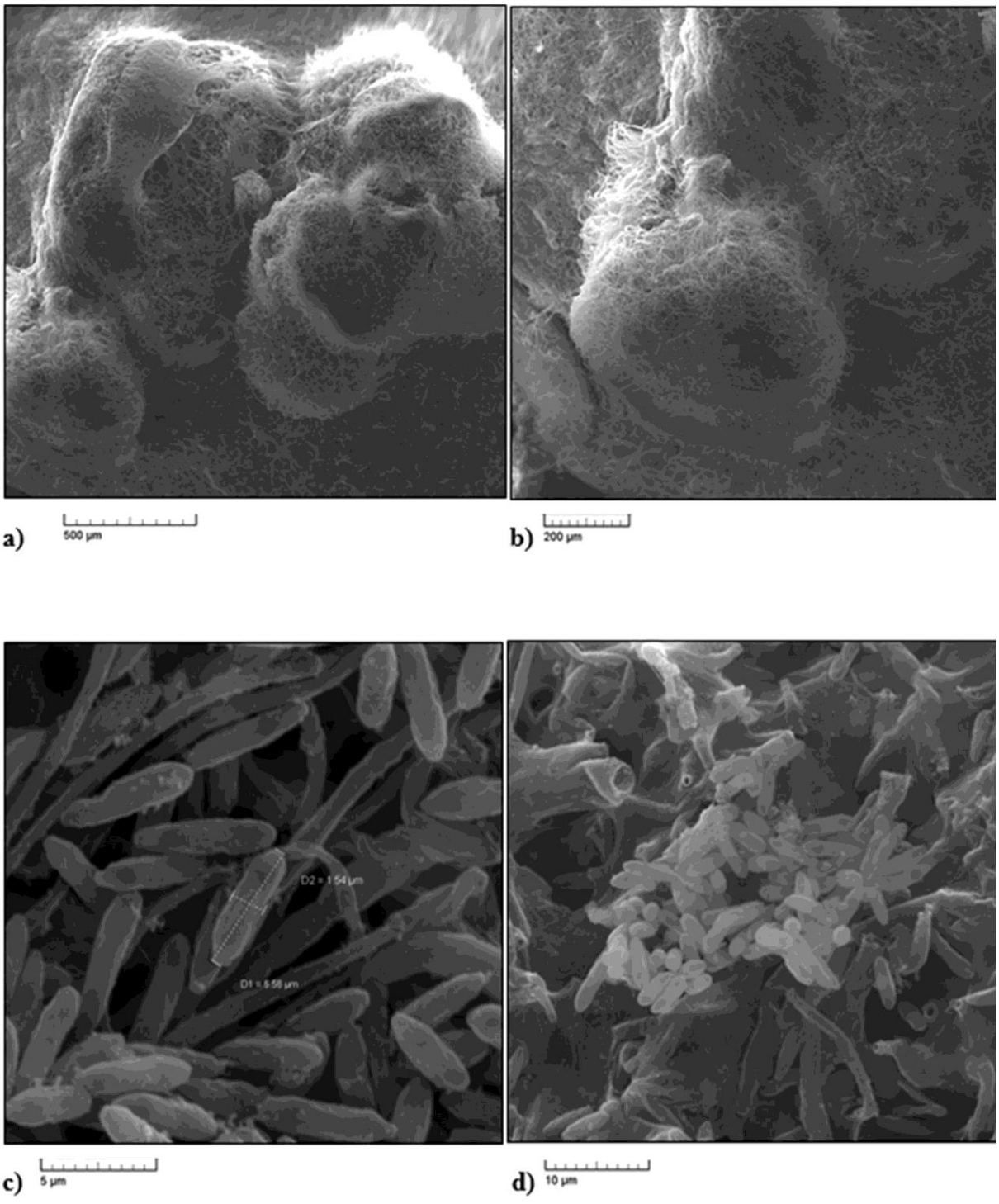
Meanwhile on Czapek-Dox, *D. eres* formed darker beige-brown colonies, but their growth rates were very similar. After 2–3 weeks of culture both on PDA and Czapek-Dox media, very few pycnidia appeared and they number increased after 4 weeks (Phot. 1b, 4a, b). The sporulation was observed usually after 7–10 days from the appearance of pycnidia, which was represented by creamy-yellow slimy drops seen on their surface. The reverse of the colonies was initially colorless, while at the time of sporulation – creamy-brown, and in the aging cultures – dark brown. Culture of selected *D. eres* isolates at poor PDA medium supplemented with carnation leaves allowed to obtain abundant sporulation after 2 weeks of incubation (Phot. 2).



Phot. 2. Signs of *Diaporthe eres* on PDA medium supplemented with carnation leaves at 24°C, phot. B. Abramczyk



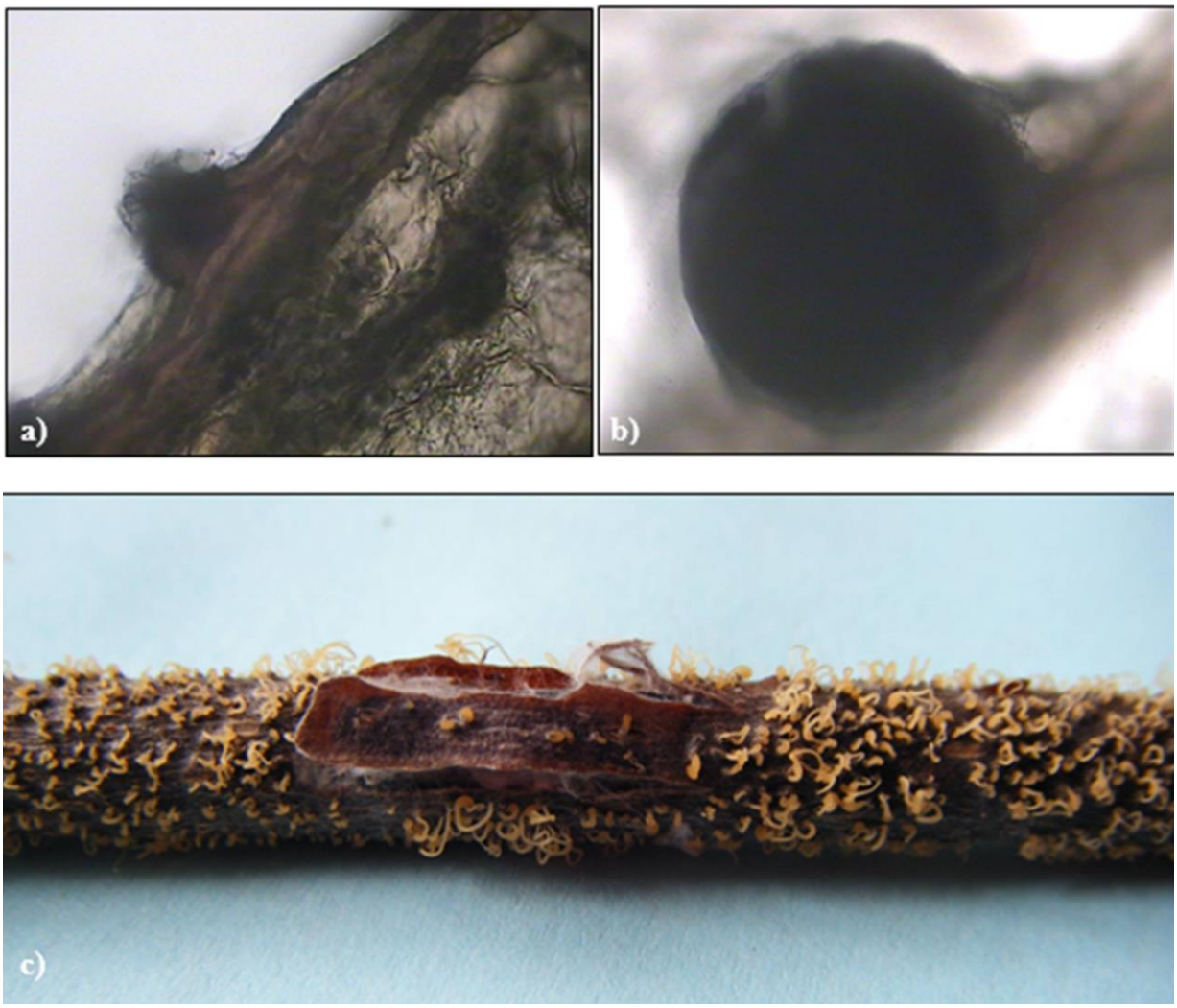
Phot. 3. Alpha (α) and beta (β) conidia of *Diaporthe eres* from fruit trees (zoom × 600), phot. B. Abramczyk



Phot. 4. Morphological structure of *Diaporthe eres* on PDA medium visible under scanning microscope (a), conidiomata (b), alpha conidia (c), conidiophores and alpha conidia (d), phot. M. Wróbel

Table 2. Average dimensions of spores from the own research results compared to those of other authors

Author	Species of fungus	Host plant	Dimensions of conidia	
			Alpha (μm)	Beta (μm)
Own research	*Diaporthe* eres	apple, pear, cherry, plum	(5-)6.2–8.6(-9) × 2.2–3.2	23–34 × 1–2.3
Król [2005b]	*Phomopsis* sp.	apple, pear, cherry	6.8–8.8 × 1.9–3.2	not measured
Baumgartner et al. [2013]	*Diaporthe* eres	grapevine	6.9–7.7 × 2.0–2.4	not measured
Du et al. [2016]	*Diaporthe eres*	betula	6.5–8.5 × 3.0–4.0	not measured
Michalecka et al. [2017]	*Diaporthe eres*	cranberry	6.0–9.0 × 3.0–4.0	18.0–19.0 × 1.0–1.5
Udayanga et al. [2014]	*Diaporthe eres*	alfalfa twigs	(6-)6.5–8.5(-9) × 3–4	(18–)22–28(29) × 1–1.5
Kaliterna et al. [2012]	*Diaporthe eres*	grapevine	5.6–7.9 × 2.0–2.3	16.6–27.7 × 0.5–1.5
Cinelli et al. [2016]	*Diaporthe eres*	grapevine	(5.85)7.71–8.21(13.56) × (1.48) 2.12–2.20(2.70)	(26.61)25.70–26.71(32.86) × (1.03)1.34–1.39(1.64)



Phot. 5. Symptoms of *Diaporthe eres* on infected shoots of tested plants (a), conidiomata (b), creamy-yellow liquid containing conidia known as "cirri"(c), phot. B. Abramczyk

All tested isolates formed alpha and beta little morphologically diversified conidia. Alpha conidia were aseptate, hyaline, cylindrical, slightly elongated, sometimes narrowed at one or both ends, often biguttulate. Sometimes conidia contained 3–4 such drops or even did not have them at all (Phot. 3, 4c, d). Beta conidia were hyaline, filamentous, straight or slightly curved, sometimes containing 1–4 drops of fat on one end or they were granular free (Phot. 3). Furthermore, no differences were observed between the size of conidia derived from apple, pear, cherry and plum trees (Tab. 1). Dimensions of majority of alpha-conidia on PDA medium were mostly in the range of (5-)6.2–8.6(-9) µm × 2.2–3.2 µm, and the beta-conidia in the range of 23–34 µm × 1–2.3 µm, and they were similar to those reported by other authors (Tab. 2, Phot. 4c).

In the pathogenicity tests of *D. eres* in relation to the selected host plants, the first symptoms on all inoculated shoots of tested plants appeared after 14–16 days after inoculation. There were small necrosis of 12–17 mm in diameter occurring near the site of inoculation. They were caused by all tested isolates regardless of the type of plant, from which they originated. They increased in size very fast and often within 2–4 days covered the whole fragment of plant shoot. Shortly thereafter, numerous pycnidia of the fungus began to appear on their surfaces (Phot. 5a, b). At the same time on the control shoots, no symptoms of the fungus were observed. After subsequent 2–3 weeks, the pycnidia began to produce alpha and beta conidia of *D. eres* in a typical form of creamy-yellow liquid – "cirri" (Phot. 5c). As a result of re-isolation, *D. eres* colonies were obtained from the diseased tissue.

DISCUSSION

Results of the pathogenicity tests demonstrated the ability to cross-infect the stems of apple, pear, cherry and plum trees by different isolates derived from these species, and re-isolation of *Diaporthe eres* from infected shoots according to the Koch's postulates confirmed the pathogenic abilities towards the shoots of tested plants. Simultaneously, there were no preferences for infection of a particular plant species by tested isolates. The observed pathogenic abilities in relation to several species of the same family, i.e. *Rosaceae,* indicate the possibility of infecting different species of fruit trees grown in the orchard. Similar properties were confirmed by Uddin et al. [1997, 1998] examining the pathogenicity of isolates obtained from diseased shoots of peach in Georgia and Alabama (USA) in relation to the shoots of peach, apple, pear and plum reported by Schilder et al. [2005] in research on *Phomopsis viticola* with grapevines in the Region of the Great Lakes in North America and Król [2005b] investigating the occurrence of *Phomopsis viticola* on grapevines in Poland. Current studies have confirmed that *Diaporthe eres* is associated with rosaceous fruit trees as a pathogen [Gomes et al. 2013, Udayanga et al. 2014].

Due to the fact that *Diaporthe* spp. have a wide range of host plants [Uecker 1988, Mostert et al. 2001, Crous 2005, Schilder et al. 2005, Król and Kowalik 2010, Gomes et al. 2013, Guarnaccia et al. 2018] and that isolates derived from a single plant species may represent more than one taxon [Farr et al. 1999, Rehner and Uecker 1994], knowledge of the pathogenicity of individual isolate to different host plants is of great importance in describing the new species in this genus.

In this study, the formation of alpha and beta conidia having similar size range has been reported. They were comparable to dimensions observed by other authors [Król 2005b, Kaliterna et al. 2012, Baumgartner et al. 2013, Udayanga et al. 2014, Cinelli et al. 2016, Du et al. 2016, Michalecka et al. 2017] as shown in Table 2.

Small variations in the cultures morphology of *D. eres* regardless of the host plant, presence of both types of conidia having similar size range, together with similar thermal requirements of selected isolates (not published) and the pathogenic abilities reported in this study, confirmed that all tested isolates belonged to the same species of fungus i.e. *D. eres* as was previously identified by Abramczyk and Król using molecular techniques [2016]. This indicates that *D. eres* morphology is positively correlated with genetic characterization, although some authors suggest that many *Diaporthe* species that are morphologically similar proved to be genetically distinct [Luongo et al. 2011, Gomes et al. 2013].

CONCLUSIONS

1. The ability to cross-infect shoots of several plant species of the same family reported in this study is of great practical importance, since isolates of *D. eres* from apple, pear, cherry and plum trees might also infect stems of other plants in orchards.

2. Morphological features of studied *D. eres* cultures were very similar, regardless of the isolate and the host plant, from which they were obtained.

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