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Dedicated to Professor Alina Skirgiełło on the occasion of her ninety-fifth birthday

Characterization of *Phoma negriana* Thüm., a new species from grapevine canes

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The occurrence and elements of morphology of *P. negriana* Thüm. were studied. The fungus cultures were isolated from grapevine canes cultivated in South–East Poland. Grapevine stems from which the cultures of *P. negriana* were obtained showed symptoms of necrosis and bark crashes. The species of the fungus was identified on the basis of pycnidia and conidia morphology, the character of colonies growth and biochemical features of the studied isolates.

Key words: *Phoma negriana*, occurrence, elements of morphology

INTRODUCTION

Fungi from the genus *Phoma* are represented by numerous species grouped into nine sections, selected on the basis of pycnidia structure, especially their cells wall, the presence of chlamydospores including dictiochlamydospores and the appearance of pycnidiospores (Boerema 1997; de Gruyter, Noordeloos, Boerema 1998; de Gruyter, Boerema, Van Der 2002). *Phoma* spp. occur commonly in different geographical regions of the world on the plants from various botanical groups (Sutton 1980; Marcinkowska 1995). Considering manner of life and trophism within *Phoma* spp. saprotrophs, opportunistic pathogenes and specific pathogenes of plants can be distinguished. Among opportunistic pathogenes numerous species occur on above ground parts of fruit-trees and bushes. One of them is *P. pomorum* Thüm., section *Peyronella,* causing necrotic lesions on the leaves and fruit of apple tree (Boerema 1993; Blechtova 1995). *Phoma macrostroma* Mont., sec. *Phyllostictoides*, belongs to the complex of fungi colonizing decayed stems and buds of walnut, ash and beech (Pinter et al. 2000; Griffith, Boddy 1990; Toti et al. 1993). *Phoma exigua* var. *populi* de Gruyter et Scheer, sec. *Phyllostictoides* was recognized as opportunistic pathogen of poplar seedling, *P. exigua* var. *viburni (*Roum. ex Sacc.) Boerema as pathogen of various cultivars of viburnum

and *P. exigua* var. *lilacis* (Sacc.) Boerema of lilac stems (de Gruyter, Scheer 1998). *Phoma herbarum* Westend., sec. *Phoma*, in addition to *Phomopsis viticola* and *Cytospora* sp. was isolated from grapevine stems with symptoms of bark necrosis (Stojanovič 1086). Recently, *Phoma negriana* Thüm., sec. *Phoma* has been recognized as opportunistic pathogen of grapevine in the Netherlands (de Gruyter et al.1998). This species was noted in the vineyards of South Europe earlier, causing disease symptoms on the leaves, fruit and stems (de Gruyter et al. l.c.). In Poland *P. negriana* has not been observed so far.

MATERIAL AND METHODS

Four isolates of *P. negriana* i.e. W 1205, W 1308, W 1075, W 1432 from a collection of this species isolates in our possession, were randomly chosen for the study. These cultures were isolated from grapevine canes cv. Schuyler, Iza and RF-16 with symptoms of necrosis and cultivated in South–East Poland in the years 2000-2003. The occurrence of the fungi was determined on the basis of etiological symptoms present on the surface of diseased canes and on the basis of mycological analysis according to the artificial culture method. The isolation was carried out from superficially disinfected canes using maltose medium (bioMerieux) according to Machowicz–Stefaniak and Kuropatwa (1993). The obtained isolates of *P. negriana* were identificated on the standard media according to a monograph by de Gruyter et al. (l.c.), with regard to the present principles of taxonomy (Boerema 1976; Marcinkowska 1995; Boerema et al. 2004). The inoculum of chosen isolates of *P. negriana* was put in the centre of Petri dishes with solidified media: malt-extract agar (MA), cherry agar (CA) and oatmeal mealagar (OA) (de Gruyter, Noordeloos 1992). The cultures were incubated in thermostat for 7 days at temperature 22°C, without light and during a second week at 13 hours in UV light and 11 hours in the darkness. A description of the colonies morphology, with regards to the principles of Boerema et al. (2004), and the measurment of their diameter were performed after 7 and 14 days of colonies incubation in the thermostat. After 2 weeks of incubation on OA the measurment of 400 conidia (4 isolates x 100 conidia) and 200 pycnidia (4 isolates x 50 pycnidia) were made and the presence of chlamydospores was tested.

RESULTS

The studies indicated that on the surface of grapevine canes in the place of necrotic lesions the pycnidia with conidia with features typical of *Phoma* species were present. A big number of *Phoma* spp. cultures were isolated from these canes. The share of *Phoma negriana* isolates among fungi isolated from grapevine canes in the study years was: 0,4% in 2000; 1,4% in 2001; 0,3% in 2002 and 0,5% in 2003 (0,65% in average).

The studies of selected isolates on the standard media indicated that 7-day-old fungus colonies on the CA medium were rather regular and had the diameter from 28 to 35 mm. In the beginning, they formed soft floccose, white–gray aerial mycelium with cream reverse. After 4-6 days of incubation in the centre of colony the pycnidia appeared and after 7 days conidia were formed. After 14 days of growth, the margin

of colonies was irregular and their diameter ranged from 44 to52 mm (Fig. 1). The aerial mycelium was dark–green or gray. On the whole surface of the colony abundant pycnidia were formed. They were partly immersed in the substrate and secreted the yellow–saffron drops containing conidia. The reverse of 14-day-old colonies was dark–green to almost black.

On OA medium, 7-old-days colonies had a less regular margin and colony diameter from 25 to 28 mm. The aerial mycelium was white–gray and floccose with a rather compact structure. First pycnidia were formed after 4 – 6 days of incubation. After 14 days the colony's diameter was from 35 to 48 mm. The mycelium structure was slightly velvety, green–olivoceous with white margin. Pycnidia were formed on the whole surface of the colony.

On MA medium, the diameter of 7-day-old colonies was from 18 to 22 mm. The colonies were regular with floccose, white–gray surface. The diameter of 14-day-old colonies was from 23 to 35 mm. The colonies were with more or less regular growth, rather compact, of floccose structure, gray–green or gray–black. The reverse was olivaceous–black with a somewhat brighter margin. The formation of pycnidia was poorer than on OA and CA media.

A detailed observation carried out on OA medium allowed to determine that pycnidia of the studied isolates of fungus were formed as singular or with small groups. They were thin–walled, globose or sometimes oval, honey–brown with papillate ostiole (Tab. 1, Figs 2, 3). The majority of pycnidia had 1 ostiole. The surface of the walls was smooth with some hyphal outgrowths. The diameter of the pycnidia was from 72 to 250 μm. Conidia aseptate, oblong with 2 or more guttules (Tab. 1, Figs 4, 5). The conidia had length from 4.2 to 7.88 μm with some reaching 9.88 μm (Tab. 1). The width of conidia ranged from 1.97 to 3.9 μm. Chlamydospores and crystals were not observed in the cultures of the studied isolates of *P. negriana*.

Table 1
Characterization of pycnidia and conidia of *Phoma negriana* (mean for 4 isolates)

Author	Pycnidia		Conidia	
	shape	dimension in μm	shape	dimension in μm
Own data	globose or oval with papillate ostiole	72 - 250	oblong, usually with 2 or more guttules	4.2 - 7.88 (9.85) x 1.97 - 3.9
de Gruyter et al. 1998	globose or irregular, solitary or confluent, glabrous with 1-2 (4) papillate ostiole(s)	70 - 220	ellipsoidal to oblong, with several distinct guttles.	4.5 - 8.5 (10.5) x 2 - 4

In the case of 3 isolates no changes were observed in colony coloration on the media MA and OA after reaction with 1N NaOH. Only in the case of isolate W 1432 on MA medium the change of coloration into red–brown was noted.

DISCUSSION

The obtained results concerning characterization of colonies growth of the studied isolates, the morphology of pycnidia and conidia, compared with a description in a monograph of *Phoma* section (de Gruyter et al. l.c.; Boerema et al. 2004) allowed to identify the studied species as *Phoma negriana.*

Macroscopic features of colonies of the studied isolates of *P. negriana,* especially the growth rate, colouration and mycelium structure showed similarity to a description by de Gruyter et al. (l.c.). The observation indicated that the mentioned feature and character of the colonies growth should have great importance in identification of the species from the genus *Phoma* which corresponds to earlier information of other authors (Boerema 1976; de Gruyter, Noordeloos 1992; de Gruyter et al. l.c.). Considering great morphological similarity of conidia within *Phoma* species, their dimension should be an important but additional diagnostic feature (Boerema 1997; Marcinkowska 1995; Zimowska, Machowicz-Stefaniak 2005). According to the opinion by de Gruyter et al. (l.c.) the reaction of *P. negriana* isolates with 1N NaOH is not specific, which confirms the results of the present study.

The fact of isolation of *P. negriana* isolates from grapevine canes contributs to an increased a number of species described on the stems of this plant, cultivated in South–East Poland.

Considering earlier recognition of this fungus as opportunistic pathogen of the above ground parts of grapevine (de Gruyter et al. 1998), the possible pathogenicity of the fungus towards this plant in climatic conditions of our country require more scrupulous tests.

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Charakterystyka *Phoma negriana* Thüm, nowego gatunku z łozy winorośli

Streszczenie

Przebadano cztery losowo wybrane izolaty *Phoma negriana* z własnej kolekcji kultur tego gatunku. Izolaty wyosobniono w latach 2000-2003 z łozy winorośli odmian Schuyler, Iza i RF-16 z objawami nekrozy i pękania kory. Oznaczano je na pożywkach standardowych: maltozowa (MA), wiśniowa (CA) i owsiana (OA), przy uwzględnieniu aktualnych zasad taksonomii *Phoma* spp. Opis morfologii kolonii oraz pomiar ich średnicy wykonano po 7 i 14 dniach hodowli. Po 2 tygodniach wzrostu na pożywce OA wykonano pomiar 400 konidiów, 200 piknidiów oraz sprawdzono obecność chlamydospor.

Uzyskane wyniki upoważniły do uznania badanego gatunku jako *Phoma negriana* Thüm., sekcja *Phoma.* Makroskopowe cechy kolonii badanych izolatów, zwłaszcza tempo i charakter wzrostu, zabarwienie i struktura grzybni powinny mieć istotne znaczenie przy identyfikacji gatunków z rodzaju *Phoma.* Ze względu na duże podobieństwo morfologiczne zarodników *Phoma* spp., ich wymiary należałoby traktować jako ważną, aczkolwiek pomocniczą cechę diagnostyczną. Uzyskane wyniki potwierdziły niespecyficzny charakter reakcji izolatów *P. negriana* z 1 N NaOH. W Polsce badany gatunek nie był dotychczas notowany.

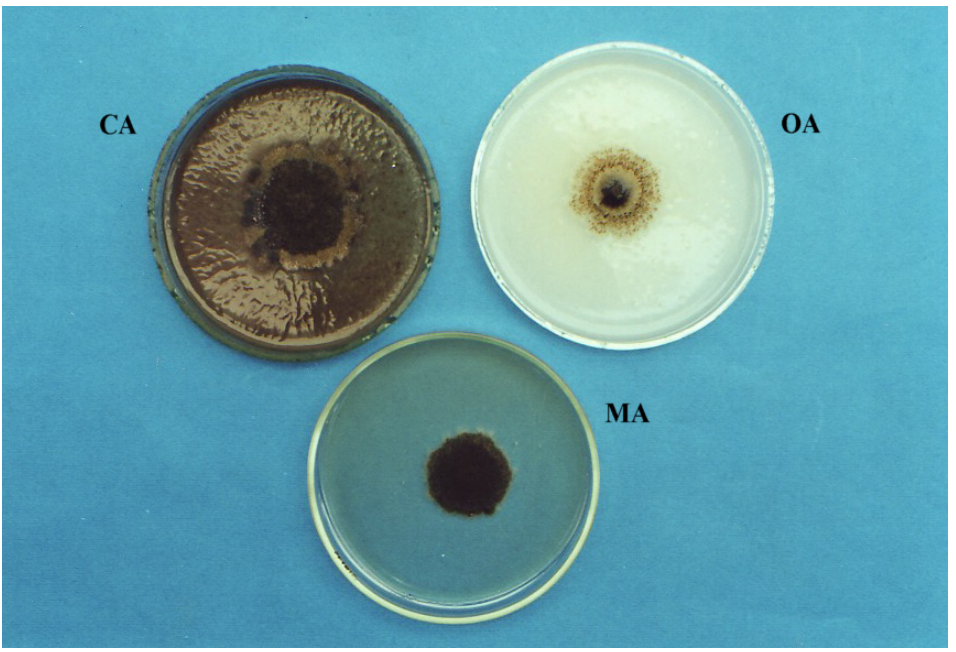

Fig. 1. 14-day-old colonies of *Phoma negriana,* W 1075 on the standard media. Phot. E. Król.

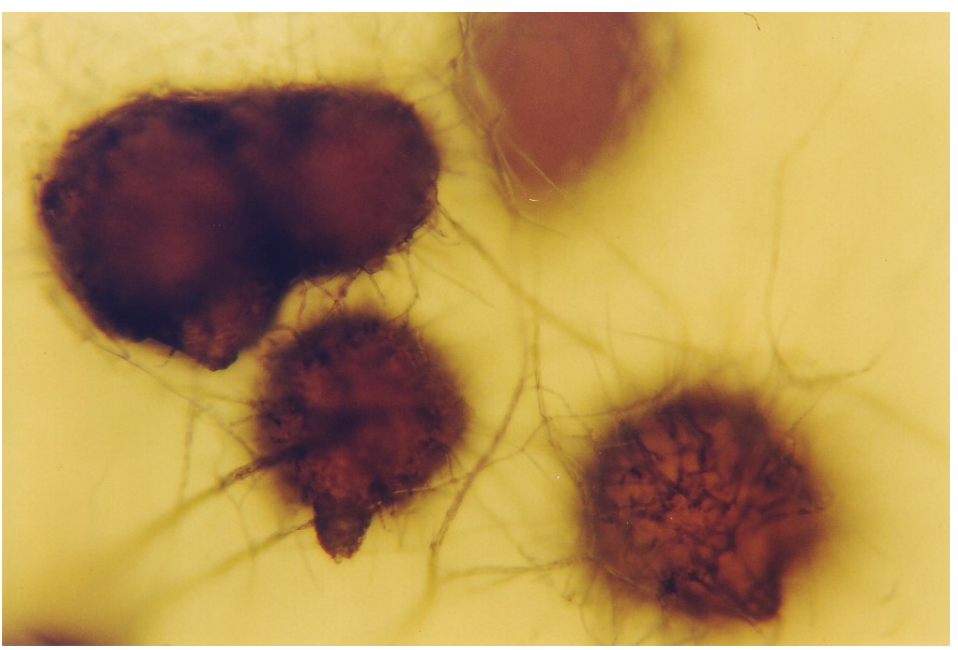

Fig. 2. Pycnidia of *Phoma negriana*, W 1075, 160 x magnification. Phot. E. Król.

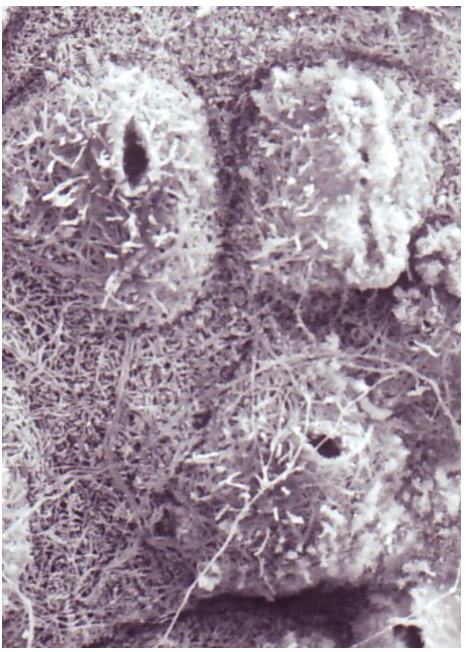

Fig. 3. Pycnidia of *Phoma negriana* (SEM), 200 x magnification. Phot. M. Wróbel.

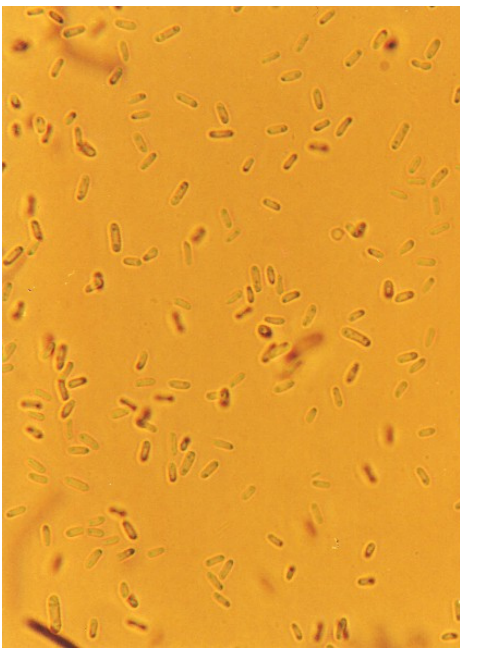

Fig. 4. Conidia of *Phoma negriana*, 640 x magnification. Phot. E. Król.

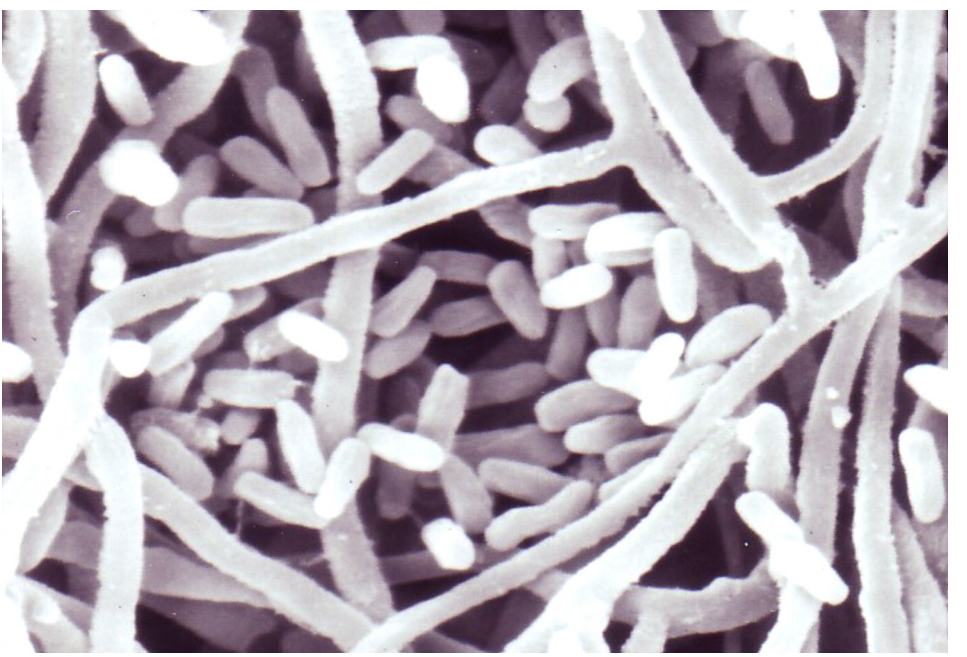

Fig. 5. Conidia of *Phoma negriana* (SEM), 4300 x magnification. Phot. M. Wróbel.