THE COMPARATIVE INVESTIGATION OF *PHOMOPSIS HELIANTHI* ISOLATES FROM DIFFERENT EUROPEAN LOCALITIES

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Abstract

The morphological and physiological characteristics of *Diaporthe/Phomopsis helianthi* cultures, originating from 11 various localities in Yugoslavia, Russia and France, were investigated. There is a small difference in colony's morphology and growth rates between Yugoslav and Russian isolates. The differences in average length of ß-conidia can be considered as statistically insignificant.

Introduction

Diaphorte/Phomosis helianthi Munrañola-Cvetković, Mihaljčević *et* Petrov is a sunflower (*Helianthus annuus* L.) pathogen which more than 15 years, with different intensity, causes the damages in the areas under this crop (Mihaljčević *et al.*, 1985, Muntañola-Cvetković *et al.*, 1981a, b). In Yugoslavia, an initial investigation of these fungi started in 1980, when an unexpected invasion of this pathogen heavy damaged sunflower fields. One year later, the causative agent of the disease was identified and described as a new taxon. Since then, a significant improvement in the understanding of the morphology, anatomy and cytology of this pathogen was made, as well as in the mode and course of the infections (Muntañola-Cvetković *et al.*, 1988, 1989, 1990). The obtained results were used successfully in breeding of new *Phomopsis helianthi* resistant sunflower cultivars in Yugoslavia (Škorić *et. al.*, 1994). However, there are still periodical epidemics in certain parts of Europe. In connection with this, there is also the question about similarities and differences between the isolates originating from various European localities.

That was the reason for our initiation of comparative investigations of *P./D. helianthi* isolates originating from spatially remote regions (Rumania, Bulgaria, Hungary, France, Russia, Argentina, United States). The results concerning the morphological

and physiological characteristics of the isolates from Yugoslavia, Russia, and France are presented in this paper.

Materials and Methods

The isolates of *D./P. helianthi* from 11 different localities in Yugoslavia, Russia, and France were studied.

Abbreviation or code used:

Helianthus annuus	= H1
Phomopsis	= P
The year of isolation, 1994	= 94
The month of isolation, September	= S
Countru of isolation, e. g. Yugoslavia	= Yu
The locality, e.g. Rimski Šančevi	= RŠ

The cultures were grown on standard malt-a gar (MA) and potato-dextrose-agar (PDA) media (Booth, 1971) under laboratory conditions (16 hours daylight and 8 hours darkness, and at the temperature of 22°C ± 2°C). The cultures were examined after 7, 14, and 21 days. The macroscopic and microscopic characteristics of the colony were observed. The preparations were coloured with lactophenol and fuchsin acid. The statistical analyses were used for estimations of ß-conidia lengths.

Results and Discussion

Colony morphology. The isolates of Yu group have mainly white airy mycelia, which could become olive green in MA media. All Yu isolates have, in various degree, a wet growth, but generally this growth increases with the time. In earlier works there were no signs of the wet growth (Muntañola-Cvetković *et al.*, 1985).

As far as the appearance of the colony is concerned, Ru isolate is very similar to Yu isolates. A dark pigmentation of the media, beneath the very center of the colony, occurs sporadically with Yu and Ru isolates. This pigment is not in a connection with the reproductive structures that have more pail colour, and it is absent in the media which maintain the natural colour on both, front and the back sides of the plates.

All Fr isolates have wet white mycelia, which could be either, substrate or airy. Fr mycelia are very thin, considerably weaker than that of Ru and Yu isolates. All Fr isolates have wet growth, which occurs in sections, without any regularity. Distinctive black pigment is a typical characteristic for all Fr isolates, and it is specially expressed with H1-P.94S.FrM isolate. This pigmentation occurs in reproductive structure,

beneath and around them at media, so that back side of some plates, containing the cultures rich in reproductive structures, sometimes could be completely black. All these characteristics can be much more intensive in MA media than in PDA media. There is a sectional growth in H1-P.94S.FrP isolate, so that one half of the back sides of some cultures is black, and the other in the natural colour of the media (the phenomenon observed on MA, as well as on PDA media). The mycelia are compact, dry and white in the section of the isolate that is black coloured on the back side. In the section with colourless back side the mycelia are delicate, substrate and extremely wet.

In all of the experiments somewhat higher growth rates were recorded for the group of Fr isolates, as compared with Yu and Ru ones, especially for P1-94S.FrV isolate that had ϕ 8x8 mm.

Reproductive structures. Pycnidia were the only reproductive structure observed in all investigated isolates up to 40 days of age. There were insignificant differences in growth rates and density of reproductive structure formations in all three groups of isolates. The initiation of the pycnidia started, for all isolates, in 7 to 10 days old colonies. They matured relatively in the same time, and in 21-day old colonies pycnidia were completely mature (Tab. 1). There were the differences in the colour of pycnidia, Fr isolates had completely black pycnidia, and Yu and Ru isolate dark brown.

There was no rule in pycnidia arrangement. They were single or organized in the stroma-like structures randomly distributed.

Conidia. In pycnidia, of all isolates, only ß-conidia were identified. Conidia discharge from the pycnidia, in the form of exudate, began on 21st day after inoculation, what is in accordance with the results reported by Franić Mihajlović *et al.* (1994). The colour of the exudate varied from milk-white to amber-yellow without some special regularity relating to group of isolates. The length of 300 conidia (150 from MA media and 150 from PDA media) was measured for each of isolates, and minimum and maximum lengths are shown in Table 1.

The high correlation coefficients ($r = 0.880$-0.964) for ß-conidia sizes on both media are obtained by correlation analysis.

The analysis of the average size of ß-conidia for all isolates, grown on both MA and PDA media, shows that the media had the highest effects on H1-P.94S.FrÄ and H1-P.94S.YuB isolates. H1-P.94S.YuB isolate had the smallest average length (Fig. 1). However, the differences between lengths of ß-conidia between Fr and two other isolate groups can be considered as statistically insignificant.

CODE	colony diameter 7 day old (mm)		pycnidia abundance				size β-con. (μm)	
	MA	PDA	MA (day)		PDA(day)		MA	PDA
			14	21	14	21		
H1-P.94S.YuRŠ	6.0 x 5.0	3.0 x 3.0	2	4	2	4	20.7-34.0	20.7-31.0
H1-P.94S.YuB	4.0 x 3.5	3.0 x 2.5	1	3	2	4	20.7-31.0	20.7-32.5
H1-P.94S.YuT	3.5 x 3.0	2.0 x 2.0	2	4	2	4	17.7-32.5	17.7-31.0
H1-P.94S.YuN	4.0 x 4.0	2.0 x 2.0	1	3	1	2	19.2-34.4	20.7-34.4
H1-P.94S.YuS	3.0 x 3.3	2.5 x 2.0	1	2	2	3	19.2-37.0	19.2-37.0
H1-P.94S.RuK	5.0 x 5.0	3.0 x 3.0	2	3	1	3	19.2-32.5	19.2-34.4
4H1-P.94S.FrM	6.0 x 5.0	2.0 x 2.0	2	4	1	4	20.7-34.4	19.2-38.4
H1-P.94S.FrP	6.0 x 5.0	3.0 x 2.0	1	3	1	2	20.7-37.0	19.2-37.0
H1-P.94S.FrB	4.5 x 4.5	3.0 x 3.0	2	3	1	3	20.7-38.4	20.7-29.6
H1-P.94S.FrŠ	4.0 x 2.0	2.0 x 2.0	1	4	2	4	19.6-34.0	17.7-37.0
H1-P.94S.FrV	8.0 x 8.0	6.0 x 5.0	3	4	2	3	20.7-34.0	17.8-34.0

Tab. 1. Caracteristics of colonies (4- abundant; 3- moderately abundant; 2- rarely; 1- less then 5 pycnidia).

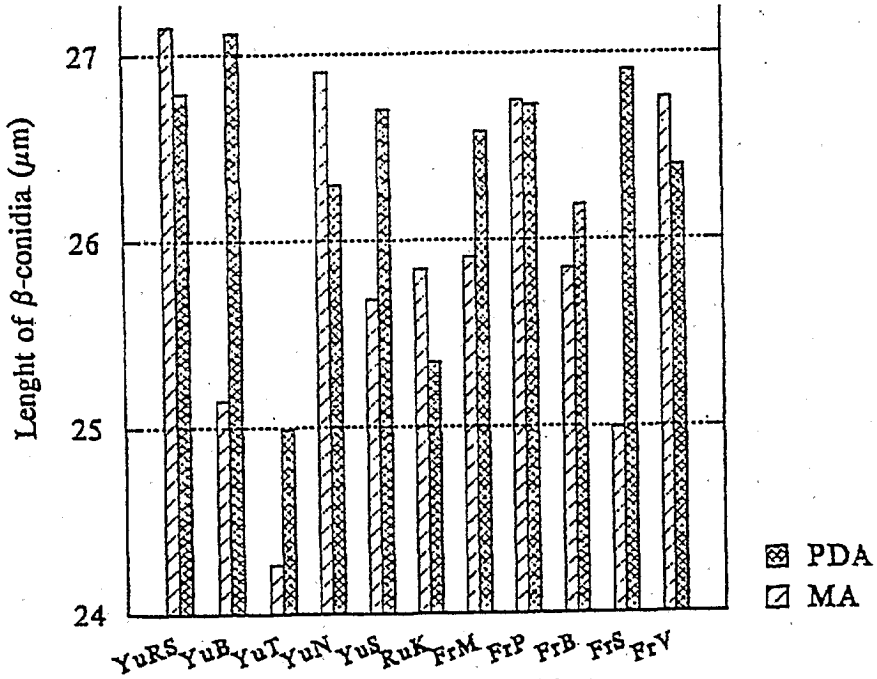

Fig. 1. The average size of β-conidia for all isolates grown on MA and PDA media.

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