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# VARIABILITY OF *Phomopsis* POPULATIONS IN SUNFLOWER (*Helianthus annuus* L.)

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SUMMARY

Various reports state that the sunflower disease, primarily caused by *Diaporthe/Phomopsis helianthi* Munt.-Cvet. *et al.*, has a wide geographic distribution in Europe, USA, Argentina and Australia. Various *Phomopsis* isolates obtained during this investigation of the sunflower disease differed in conidial type (α, β, α and β). *Phomopsis helianthi* was the only isolate producing exclusively β-conidia and perithecia on debris (*Diaporthe helianthi*). The other *Phomopsis* isolates were saprobes on sunflower plants.

**Key words: sunflower diseases, *Diaporthe/Phomopsis helianthi*, population variability**

## INTRODUCTION

Available data indicate that fungi of the genera *Diaporthe* and *Phomopsis* have been isolated from infected sunflower stems in Yugoslavia (Mihaljčević *et al.*, 1980; Marić *et al.*, 1980; Aćimović and Štraser, 1982), Hungary (Nemeth *et al.*, 1981), Romania (Vranceanu *et al.*, 1983), Bulgaria (Mihailova, 1984), Brazil (Homechin and Franca Neto, 1982), USA (Ohio - Herr *et al.*, 1983; Texas - Yang *et al.*, 1984; Minnesota and North Dakota - Hajdu *et al.*, 1984), France (Regnault, 1985; Lamarque and Pery, 1985), Ukraina and Moldavia (Bogdanova *et al.*, 1986), Russia in 1990 (Skripka *et al.*, 1993) and Australia (Allen *et al.*, 1980).

The alleged wide geographical distribution of the disease is due to the misconception that all *Phomopsis* strains isolated from sunflower are causative agents of the disease caused by *Diaporthe* (*Phomopsis*) *helianthi* (Muntañola-Cvetković *et al.*, 1981). A long-term mycological investigation has shown that the territory of Europe infested by the fungus described as *Diaporthe* (*Phomopsis helianthi* Munt.-

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Cvet. *et al.*) may be divided into two clearly distinguishable areas: 1) northen Yugoslavia, Romania, Bulgaria along the Danube river, south-eastern Hungary, and territories further east, towards the Ukraine and southern Russia and 2) France.

In Yugoslavia, an initial investigation of the fungus started in 1980, when an unexpected invasion by this pathogen caused heavy damages in sunflower yield. One year late, the causative agent of the diseas was identified and described as a new taxa: *Diaporthe* (*Phomopsis*) *helianthi*. Since then, a significant improvement in the understanding of the morphology, anatomy and cytology of this pathogen was made, as well as in its infection pathway (Muntañola-Cvetković *et al.*, 1988, 1989, 1991).

**Morpho-physiological characteristics - *Diaporthe* (*Phomopsis*) *helianthi* Munt.-Cvet. *et al.***

The leaf-petiole-stem is the infection pathway for *Diaporthe/Phomopsis helianthi*. The fungus entering through leaves progresses towards the petioles along foliar veins, and finally gains entrance to the stem. The stem lesions originate from internal pathogen development. Asexual fructifications, pycnidia (the *Phomopsis* state) up to 200 μm in diameter can be found on sunflower stem cankers by midsummer. The pycnidia contain only β-conidia, which fill the conidiomata when these become mature even before the host epidermis is ruptured. Differentitation of the fungal sexual reproductive structures, ascogonia into protoperithecia and perithecia, occurs slowly and unevenly during the autumn and subsequent months. Under wet conditions the rostrum of the perithecium elongates and ascospores are released.

A colony of isolates described as *Phomopsis helianthi* attained 35-45 mm (average) in diameter in 4 days and 70 mm in 60 days; the mycelium was mostly submerged at first, with the aerial part rather scant and arachnoid; later on, it became velutinous or floccose, white, rarely with limited greenish-yellow areas; from the reverse it was dirtywhite at first, or ivory-colored, with brownish dots corresponding to developing or developed fructifications. Anamorphic structures that appeared in 5-10 days, were globose, 170-320 μm in diameter, partially immersed in the pseudostromata, commonly aggregated but also solitary. Among the *Phomopsis* species studied, *P.helianthi* formed the simples type of the stroma, obviously similar to the pycnidium. The pycnidial wall was multicellular, with heavily pigmented and sclerotized cells on the outside. Conidiophores were simple, rarely branched, arising from the innermost layer of cells lining the pycnidia cavity. Conidia were exuded in hyaline or amber-colored droplets; the conidial type was consistent; only β-conidia were found in the pycnidia examined irrespective of the isolation time, age of the culture or the number of transfers to a variety of nutrient media and other substrates (sterilized stem of different host plants). β-conidia were filiform, sigmoid, eventually straight, 22.0-32.0 x 0.5-1.0 μm, unicellular, easily detached from conidiophores.

On nutrient media, the telemorphic state was obtained in very few cases. It consisted of a globose body variable in size but mostly 400 μm in diameter, with the neck up 700 μm (mostly 350-600 μm) in lenght. Asci were clavate-cylindrical 47.5-57.5 x 7.5-12.0 μm. Paraphyses were present at first but disappear with maturity. Ascospores were sub elliptical, with rounded ends, 1-septate, and 12.5-14.5 x 3.25-4.5 μm.

## RESULTS AND DISCUSSION

The identify of the causative agent of the new sunflower disease usually described as *Phomopsis* was a controversial matter since the disease first appeared in Yugoslavia in 1980.

The *Phomopsis* (*Diaporthe*) epiphytotics that broke out in Yugoslavia and Romania in early 1980's led to an intensive search for the fungi from the genera *Phomopsis* and *Diaporthe* around the world. The almost complete absence of literature data on the fungus prior to 1980 was soon replaced by an abundance of data about the parasites from the two genera. Although the descriptions and life cycles of the described parasites differed considerably, they were unanimously labeled as *Phomopsis* sp. or *Phomopsis* ssp. In addition to that, the name *Phomopsis helianthi* Munt.-Cvet. *et al*., started to be used more and more frequently in mid-80's, although it was clear that it covered several distincly different *Phomopsis* species.

The intention of this paper is to give a chronology of appearance of the most frequently cited papers which mention *Phomopsis* sp., *Phomopsis* ssp. or *Phomopsis helianthi* as the agents of the sunflower disease in question because there seems to be a great deal of confusion about the subject matter.

The extensive study of the new disease agent written by Aćimović and Štraser (1982) stated that the parasite produces brownish-black pycnidia and two types of spores (α- and β-conidia) on infected plants as well as in pure culture on different substrates. Studying the relationship between α- and β-conidia, it was found that β-conidia occur regularly in the pycnidia while α-conidia occured sporadically. α- and β-conidia are matually exclusive both in naturally infected samples and on different nutritive substrates. The fungi overwinter in the pycnidium state on infected sunflower debris. Comparing two referent locations, Bački Petrovac and Novi Sad, the authors determined differences in the symptoms caused by isolates, mode and place of development of pycnidia, dimensions of pycnidia and conidia, type of conidia and temperature requirements for conidial development. On this basis of the results obtained, Aćimović and Štraser (1982) concluded that it is very likely that the sunflower is attacked by two *Phomopsis* species. It should be emphasized that the isolate Baèki Petrovac was found on harvest in late fall while the other isolates came from plants examined during vegatation. The tests of the lenght of incubation period conducted by Aćimović and Štraser (1982) never confirmed virulence of the isolate Bački Petrovac. This is clearly stated by the authors *Phomopsis* sp.

(the isolate Baèki Petrovac) is a saprobe, not parasite. For example, Mihaljčević and Muntañola-Cvetković (1985) reported a number of saprobes from sunflower harvest residues.

In their attempt to advocate the name *Phomopsis* sp., Aćimović and Štraser disregarded the fact that they dealt with two distinct organisms of which only one was a parasite, they overlooked the existence of perithecia in the life cycle of the parasite, and, finally, they attributed the capacity to develop α-conidia, established for the saprobe isolated in the location Bački Petrovac, to the pathogenic species which caused the epiphytotics. In their later paper, they omitted the isolate Bački Petrovac and afterwards the established the presence of perithecia on overwintered sunflower residues. Only the fungus labeled as *Phomopsis helianthi* could form these perithecia. The isolate Bački Petrovac may be considered an exception because it had been isolated from a single spot found on sunflower harvest residues, especially when compared against all the other isolates which may be obtained in any years and any location in which *Phomopsis* occurs as a sunflower parasite.

In numerous isolates from infected stem tissues Marić *et al.* (1982) obtained whitish-gray fungal colonies in which β-conidia prevailed. α-conidia could be found in a few pycnidia only. Perithecia were found on infected overwintered harvest residues. The authors labeled this species as *Phomopsis* sp. - isolate 1. When testing fungal colonies on various substrates, these authors found pycnidia with α-conidia in several colonies. However, the isolations from the perithecia which developed in masses on overwintered sunflower stems never produced the same isolate. Marić *et al.* (1982) labeled the fungus that developed perithecia as *Phomopsis* sp. - isolate 2.

Consequantly, Marić *et al.* concluded that the gray spot of sunflower stem is a complex disease caused by two fungi from the genus *Phomopsis*, mentioning also the presence of two more non-identified fungi (isolates 3 and 4) which were capable of developing perithecia and the conidial stage which resembled that of the fungi from the genus *Cephalosporium*.

When proving the virulence of the obtained isolates, Marić *et al.* used as the inoculum a suspension of ascospores from isolate 1 with β-conidia (already mentioned above) - probably *P.helianthi*. However, they did not mention the virulence of either a suspension of α-conidia from pycnidia from isolate 2 or a suspension of ascospores from isolates 3 and 4.

By virtue of the incorrect premise of the complex nature of the disease described as the gray spot of sunflower stem, Marić *et al.* (1982) concluded that the parasite may be transmitted by seed and that it had probably been introduced in Yugoslavia by infected sunflower seed. The fact remains that *Phomopsis helianthi* is capable of developing pycnidia on sunflower seed, but these pycnidia contain only sterile β-conidia. They refer only to isolate 1 whose characteristics are identical to those of *Phomopsis helianthi* Munt.-Cvet. *et al.*

The papers mentioned above are responsible for the false impression that various *Phomopsis* species may be isolated on sunflower in Yugoslavia. With the excep-

tion of the two papers cited previously that reported members of the genus *Phomopsis* capable of developing α-conidia, not a single *Phomopsis* species possesing α-conidia in its pycnidia was reported in the subsequent 17 years following the occurence of the disease on sunflower in Yugoslavia.

Yang *et al*. (1984) published the first verified report of *Diaporthe helianthi* on the cultivated sunflower in Texas and other USA states. The cultures from Texas were similar in many respects (symptoms on plants, morphological-physiological characteristics, responses of sunflower plants to inoculation under controlled conditions, etc.), to the Yugoslav isolates, but they also easily formed perithecia on PDA. Therefore, the isolates from Texas can be considered as a special biotype of *D.helianthi*.

Herr *et al*. (1983) established that *Diaporthe* sp. and its *Phomopsis* anamorph are the primary causal organisms of the stem cancer of sunflower occuring in Ohio (USA). In Ohio, pycnidia with predominantly α-conidia and those with predominantly β-conidia were found at different areas of the same diseases stem of a field-grown sunflower. Preliminary results indicated at the time that the *Diaporthe* sp. and *Phomopsis* anamorph found on sunflower in Ohio might be identical to *D.helianthi* occuring in Yugoslavia.

It should be mentioned that Hajdu *et al*. (1984) isolated *Phomopsis* species in several locations in the USA (Minnesota and North Dakota). Subsequent laboratory tests have shown that the *Phomopsis* isolates from USA and Europe, with the exception of the isolate from Texas, differed considerably. Comparing his results with those of Aæimoviæ and Štraser (1982) and Mariæ *et al*. (1982), Hajdu (1986) found the isolates from Hungary and Yugoslavia to be different in many ways. In reality, however, this is not the case. The Hungarian isolates, same as the Yugoslav ones, develop only β-conidia in the pycnidia.

According to Gulya (1996), *Phomopsis helianthi* Munt.-Cvet. *et al*. containing exclusively β-conidia in the pycnidia started to occur more frequently and more intensively in the USA (Minnesota and North Dakota).

Among the three *Phomopsis* species identified in Argentina, one is definitely *Phomopsis helianthi*. In Australia, the occurence of fungi from the genus *Phomopsis* is associated with the *Phomopsis* complex on soybean, primarily *Phomopsis meridionalis*.

According to Bogdanova *et al*. (1986), the first occurrence of *Phomopsis* in the Ukraine was register in the Uzgorod area of the Zakarpatya region in 1985. The description of the basic characteristics indicates that the fungus in question is *Phomopsis helianthi* Munt.-Cvet. *et al*.

According to the available literature data, there is only one species of *Phomopsis helianthi* Munt.-Cvet. *et al*., the β-conidia - only type, in Europe excluding Ukraine and Russia. Studying the intra-population structure of *Phomopsis* isolated in the Ukraine, Lesovoy *et al*. (1993) concluded that there exists a parasitic population capable of developing α-, β- or both conidial types in the course of vegetation

and on a substrate. The heterogeneity of the *Phomopsis* population led to the authors to label this phytopathogenic fungus as *Phomopsis* sp.

In the period 1988-1989, *Phomopsis* occured on sunflowers in a number of areas in the region of Zakarpatya, Odessa and Kirovgrad (Skripka *et al.*, 1993). The same author reported that the fungus grown on artificial substrates produced cultures with pycnidia containing α-, β- and α- + β-conidia This is an indication of the heterogeneity of the *Phomopsis* populations in these regions. Furthermore, the same paper mentions that *Phomopsis*, when grown on suitable substrates, develops within the temperature range of 7.32°C. This is in disagreement with all data obtained in Yugoslavia. The phytopathogenic fungus *Phomopsis helianthi* Munt.-Cvet. e*t al*. does not develop at the temperatures above 26°C, regardless of the substrate used for its cultivation.

The pathogenic fungus *Phomopsis helianthi* was included in the quarantine list of the USSR in 1986. The 1995 quarantine list of the Russian Federation also includes *Phomopsis helianthi*. Although it is not the purpose of this paper to make comments on any quarantine list including those mentioned above, it is necessary to mention some details that are in collision with the actual/verifiable situation.

*Phomopsis helianthi* Munt.-Cvet. *et al.*, *i.e.*, its teleomorph, *Diaporthe helianthi* Munt.-Cvet. *et al.*, are defined as agents of these disease. All investigations conducted so far have indicated that *P.helianthi* is a parasite of the sunflower as well as that it does not have an alternate host. The occurence of fungi of the genera *Diaporthe* and *Phomopsis* on weed species has been studied over a long period (Mihaljčević and Muntañola-Cvetković, 1985; Mihaljčević and Vukojević, 1994).

The list of countries reported for the occurance of *Phomopsis helianthi* is also open to discussion. The facts mentioned previously indicate that several dissimilar species, including some saprobes, had been described under the common name of *Phomopsis* sp.

A nonspecific characteristics of the species *Phomopsis helianthi* is the exclusive presence of sterile β-conidia (Muntañola-Cvetković *et al.*, 1981; 1985; Mihaljčević *et al.*, 1985). Naturally, it is absolutely acceptable to assume that various *Phomopsis* species may be found on the sunflower, *i.e.*, those possessing various conidial types. In that case, however, we cannot talk about *Phomopsis helianthi* but rather about *Phomopsis* ssp.

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## VARIABILIDAD DE LAS POBLACIONES DE *Phomopsis* EN EL GIRASOL (*Helianthus annuus* L.)

RESUMEN

Diversos informes presentan la enfermedad del girasol causada por *Diaporthe/Phomopsis helianthi* Munt.-Cvet. *et al.* es ampliamente extendida en Europa, EUA, Argentina y Australia. Los aislados de *Phomopsis* obtenidos durante la investigacion de esta enfermedad se diferenciaban según el tipo de conidias (tipos α, β, α y β). *Phomopsis helianthi* era el unico aislado que creaba exelusivamente las conidias β y peritecias en los restos vegetales (*Diaporthe helianthi*). Otros aislados de *Phomopsis* eran saprobies en las plantas de girasol.

## VARIABILITÉ DES POPULATIONS DE *Phomopsis* CHEZ LE TOURNESOL (*Helianthus annuus* L.)

RÉSUMÉ

Différents rapports révèlent que la maladie du tournesol causée surtout par le *Diaporthe/Phomopsis helianthi* Munt.-Cvet. *et al.* est largement répandue en Europe, aux États-Unis, en Argentine et en Australie. Différents isolats de *Phomopsis* obtenus au cours de cette recherche sur la maladie du tournesol se différenciaient par le type conidial (α, β, α et β). Le *Phomopsis helianthi* a été le seul isolat produisant exclusivement des conidies β et des périthèces sur les restes de la plante (*Diaporthe helianthi).* Les autres isolats de *Phomopsis* étaient saprobes sur les plants de tournesol.