VARlABILITY 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 [Mihaljević et al., 1980; Marić et al., 1980; Aćimović and Straser, 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 (Muntaníola-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) northern Yugo-
slavia, Romania, Bulgaria along the Danube river, south-eastern Hungary, and terri-
tories 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 disease 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,

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

The leaf-petiole-stem is the infection pathway for *Diaporthe/Phomopsis helian-
thisi*. The fungus entering through leaves progresses towards the petioles along foliar
veins, and finally gains entrance to the stem. The stem lesions originate from inter-
nal 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. Differentiation 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 peritheciun elongates and ascospores are released.

A colony of isolates described as *Phomopsis helianthi* attained 35-45 mm (aver-
age) in diameter in 4 days and 70 mm in 60 days; the mycelium was mostly sub-
merged 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 correspond-
ing 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 simi-
lar to the pycnidium. The pycnidial wall was multicellular, with heavily pigmented
and sclerotized cells on the outside. Conidiophores were simple, rarely branched,
arisng 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, sig-
moid, 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 length. 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 distinctly 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 occurred sporadically. α- and β-conidia are mutually 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 Bački Petrovac was found on harvest in late fall while the other isolates came from plants examined during vegetation. 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 Bački Petrovac) is a saprobe, not parasite. For example, Mihaljević 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.

Consequently, 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 \( \alpha \)-conidia, not a single Phomopsis species possessing \( \alpha \)-conidia in its pycnidia was reported in the subsequent 17 years following the occurrence 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 occurring in Ohio (USA). In Ohio, pycnidia with predominantly \( \alpha \)-conidia and those with predominantly \( \beta \)-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 occurring 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 \( \beta \)-conidia in the pycnidia.

According to Gulya (1996), Phomopsis helianthi Munt.-Cvet. et al. containing exclusively \( \beta \)-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 occurrence 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 Zakarpaty 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 \( \beta \)-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 \( \alpha \). \( \beta \)- 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 occurred 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. et 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 diseases. 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 occurrence 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 occurrence 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.

REFERENCES


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 investigación de esta enfermedad se diferenciaban según el tipo de conidias (tipos α, β, α y β). Phomopsis helianthi era el único aislado que creaba exclusivamente las conidias β y peritecias en los restos vegetales (Diaporthe helianthi). Otros aislados de Phomopsis eran saprobes 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 péritèches sur les restes de la plante (Diaporthe helianthi). Les autres isolats de Phomopsis étaient saprobes sur les plantes de tournesol.