PHOMOPSIS SP. – A NEW PARASITE IN SUNFLOWER

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INTRODUCTION

Spots of different shape, size, and colour occur on all above-ground parts of sunflower plants in the course of the vegetative season. The majority of the spots is caused by the wellknown fungi, *Septoria helianthi* Ell. et Kell., *Alternaria helianthi* (Hansf.) Tub. et Nish, *Phoma oleracea helianthi-tuberosi* Sacc.

The symptoms caused by these fungi were described in detail by Josifović and Šutić (1975) and Aćimović (1965 and 1969). In the last two-three years, however, characteristic large spots started occurring on sunflower stems and leaves, the symptoms of which differed from those experienced before. In the course of the vegetative seasons of 1979, 1980, and 1981, we analysed samples of infected plant parts, especially the stem, and regularly obtained *Phomopsis* sp.

In 1979, this fungus launched severe attacks in a few sunflower plots but already in 1980 and 1981 the disease reached epiphytotic dimensions. The pathogen's destructive action brought, considerable yield reductions. We studied the pathogen for the last two years. The aim of this paper is to acquaint the scientific public with some characters of the pathogen.

DISEASE SYMPTOMS

The disease attacks sunflower leaf, leaf petiole, and stem. No symptoms have been registered on the head.

Leaf. The symptoms appear in the form of large brown spots. The infected part of the lamina dries up and the infection spreads down the central nerve to the petiole. The annular infection of the petiole causes a rapid shrivelling of the entire leaf which seems to have been exposed to an open flame.
The symptoms occur at first on the bottom leaves, starting from the second pair of leaves, moving gradually up to the 11 th pair of leaves.

Stem. The symptoms are quite characteristic, starting as large brown spots in the leaf sheath after the leaf itself has dried up (Fig. 1). The spots occur less frequently on the internode. The frequency of the place of infection, and the number and length of spots on the stem are given in Table 1.

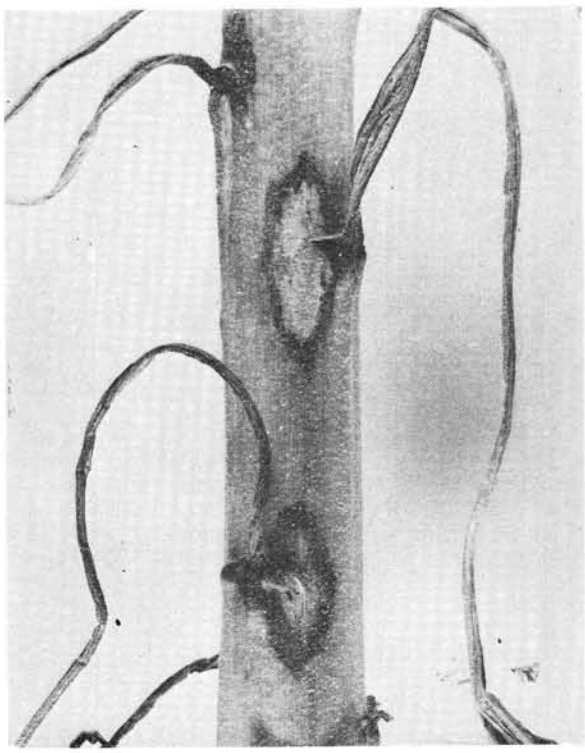

Fig. 1 — Phomopsis sp. Characteristic spots on sunflower stem (Bački Petrovac, 1981).

The place of infection was between the 4 th and 7 th pair of leaves, most frequently between the 5 th and 6 th pair. The number of spots on the stem varied from 1 to 7, or 5 on the average. The length of spots varied from 3.32 to 20 cm, or 7.90 cm on the average.

The pathogen softened up the plant tissue in the spot which frequently caused the breaking of the stems (Fig. 2).

Spots grow to be greyish in colour, brownish-grey or greyish-brown. Brownish-black pycnidia are formed in the central part of the spot (Fig. 3).

Table 1

Place of infection, number and length of spots on sunflower stem

No. of plants	Place of infection (pair of leaves)	No. of spots	Length of spots in cm (average)
1	5	5	8.05
2	7	7	5.72
3	7	6	6.50
4	6	6	11.56
5	6	5	5.78
6	7	7	5.71
7	5	4	6.40
8	5	5	3.32
9	4	1	20.00
10	4	4	6.00
Total	56	50	79.04
Average	5—6	5.0	7.90

Fig. 2 — Phomopsis sp. Sunflower stem broken on the spot of infection.

The occurrence of spots on the stem affects the overall appearance of the plant. If the spots occur before or during flowering, the entire plant becomes pale yellow. The leaves become more and more yellow, starting on the edges of the leaf and later between the nerves. The infected plant loses turgor and wilts. The length of the period between the occurrence of first spots to the wilting depends on plant age and the number of spots. It lasts 10—20 days with young plants, considerably longer with mature plants.

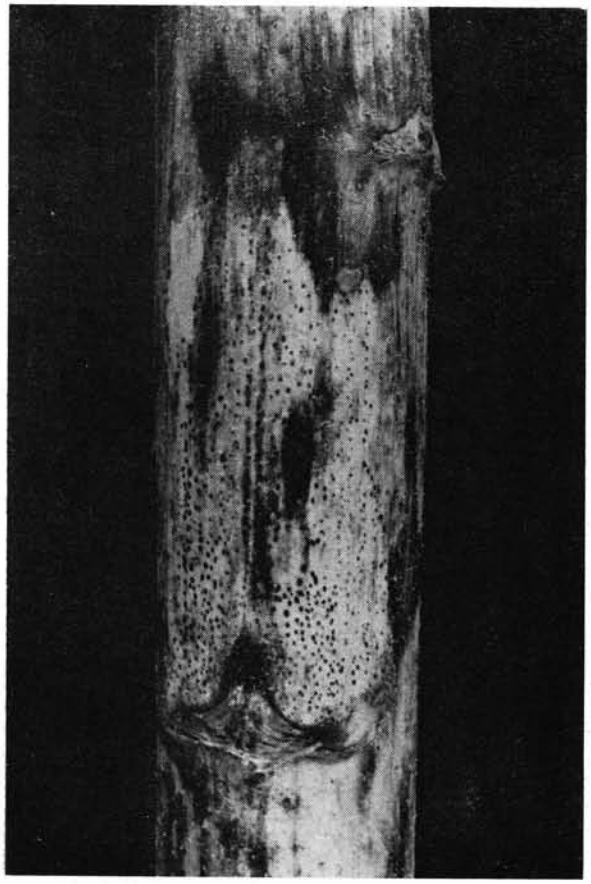

Fig. 3 — Phomopsis sp. Pycnidia on sunflower stem (Bački Petrovac, 1980).

DISEASE OCCURRENCE, NUMBER OF INFECTED PLANTS, AND DISTRIBUTION

Phomopsis has not been registered on sunflower in any European country. It is difficult to say when the pathogen occurred for the first time on sunflower in Yugoslavia. We encountered the pathogen in the fall of 1960 conducting a microscopic survey on a sample of sunflower stem taken in the vicinity of Novi Sad. Much later, in September 1971, it was found again on a sample coming from a plot near Zrenjanin. Finally, in 1979, a 120-ha sunflower plot near Kikinda was found to be severely attacked by the pathogen at the end of the vegetative season.

Macroscopic and microscopic surveys of naturally infected plant did not indicate the presence of reproductive organs of the pathogen. Hyaline mycelia were obtained by isolating a pure culture of the pathogen on potato-dextrose agar. The mycelia produced black pyc-

nidia with stylospores, hinting that we encountered a fungus from the genus *Phomopsis*. After that we examined other sunflower plots which had not been harvested and found that the number of plants infected by the pathogen ranged from 1 to 5%.

It made us suspicious and we conducted careful observations on the occurrence of the disease in 1980 and 1981 in Vojvodina and other parts of Yugoslavia.

In 1980, the disease occured in Vojvodina, northern Serbia, and Croatia. At the end of July and beginning of August, large numbers of infected plants were registered in two parts of Vojvodina, Banat and eastern Srem. The number of infected plants there exceeded 50%. In northern Bačka and western Srem, the infection was less severe and it took place later so that the damages were correspondingly smaller.

In Serbia, high percentages of infected plants were registered in some plots in the northern part of the Republic. Going south, the number of infected plants decreased rapidly. In Bosnia and Croatia, the percentages of infected plants were 7—10 and 2%, respectively. The disease was not observed in southern Serbia and Macedonia. It is probable that the climatic conditions, primarily the insufficient rainfall, prevented the occurrence of the disease.

IDENTIFICATION OF THE PATHOGEN AND NAME OF THE DISEASE

Phomopsis sp. has not been reported to be virulent on sunflower either in Europe or on the other continents. S a c c a r d o (1898) listed 19 fungi which attack sunflower but neither one of those belongs to the genus *Phomopsis*. However, the fungus *Diaporthe ortoceras* (Fr.) Nke., Caul. was registered on the genus *Helianthus* sp. Much later, Wehmeyer (1933) determined the fungus *Diaporthe arctii* (=Diaporthe ortoceras Fr. Nits.) on *Helianthus annuus* L. and *Helianthus giganteus* L.

The pure culture of *Phomopsis* which we isolated was sent for determination to Centraalbureau voor Schimmelcultures, Baarn, the Netherlands and Commonwealth Mycological Institute, London, United Kingdom.

In Baarn, our isolate was determined as *Diaporthe arctii* (Lasch.) Nits. This fungus is polyphagous, subsisting on several plant species. Conversely, in London, the determination was not completed. The report of the Mycological Institute stated that the fungus ought not be determined as *Diaporthe arctii* (Lasch) Nits. because it seemed to be a new specie. The Institute proposed that the fungus retains the name *Phomopsis* sp. until it is studied in more detail. We accepted this proposal and named the disease "brownish-grey spot".

MORPHOLOGICAL, DEVELOPMENTAL, AND ECOLOGICAL CHARACTERS OF THE PATHOGEN

A. MORPHOLOGICAL AND DEVELOPMENTAL CHARACTERS

METHOD

First materials for the study of the pathogen were collected in 1979 in the district of Mokrin, near Kikinda, northern Banat. In 1980 and 1981, we collected a number of samples from all sunflower-growing regions of the country. We took care that the number of samples from a certain region approximates the hectarage under sunflower and the intensity of disease occurrence in this region. The materials collected in Vojvodina came from Pančevo, Vršac, Bela Crkva, Zrenjanin, Kikinda, Subotica, Senta, Sombor, Bačka Palanka, Bečej, Novi Sad, Stara Pazova, Indjija, Ruma, and Erdevik. In Serbia Proper, samples were collected in Negotin, Ćuprija, Veliko Gradište, Kragujevac, and Mladenovac. In Croatia, samples were collected in Osijek and Slavonska Požega. In Bosnia, samples were collected in Brčko and Gradačac.

All samples collected underwent detailed macroscopic and microscopic examinations. The samples interesting for symptoms or reproductive organs were used for the isolation of the fungus. Most interesting isolates were included into further examinations. Some examinations included seven isolates of different origin, five from Vojvodina and two from Serbia Proper.

The isolation of the fungus and a study on the effect of ecological factors were conducted on potato-dextrose agar and malt agar. Developmental and morphological characters of the fungus were studied on the isolates „Bački Petrovac" and "Novi Sad" since these isolates differed macroscopically.

RESULTS

EXAMINATION OF NATURALLY INFECTED PLANT PARTS

In 1980, we collected 14 samples from the following localities : two from Serbia Proper, 11 from Vojvodina, and one from Croatia. Macroscopic examinations of spots indicated the presence of brownish-black pycnidia and slimy exudate which was pale grey and usually dry, in dependence of the origin of the sample. The results of detailed microscopic examinations performed afterwards are given in Table 2.

The results in Table 2 show that only pycnidia were found in the 14 samples, which in turn contained pycnospores and A and B type conidia. The pycnidia of all isolates contained a large number of B conidia (stylospores). Only the isolates "Bački Petrovac" and "Bečej" contained both B conidia and A conidia (spores).

Table 2

Results of microscopic examination of sunflower plants naturally infected by *Phomopsis* sp. in 1980

No.	Locality	Hybrid	Reproductive organs		
			Pycnidia	Type of (conid.) spores	Perithecia
1	Ćuprija	NS-H-27-RM	+	B	—
2	Krnjevo	NS-H-26-RM	+	B	—
3	Pančevo	NS-H-33-RM	+	B	—
4	Sečanj	Proizvodnja	+	B	—
5	Zrenjanin	Proizvodnja	+	B	—
6	Čoka	NS-H-26-RM	+	B	—
7	Djala	NS-H-27-RM	+	B	—
8	Senta	NS-H-26-RM	+	B	—
9	Bečej	NS-H-26-RM	+	A, B	—
10	Novi Sad	NS-H-26-RM	+	B	—
11	B. Petrovac	NS-H-26-RM	+	A, B	—
12	Surduk	NS-H-26-RM	+	B	—
13	Erdevik	NS-H-26-RM	+	B	—
14	Osijek	NS-H-27-RM	+	B	—

Legend :
+ Pycnidia present
— Perithecia absent

Perithecia were not found in either one of the examined samples.

In 1981, we collected 24 samples : six from Serbia Proper and 18 from Vojvodina. Table 3 shows the results of microscopic examinations of the isolates.

Table 3

Results of microscopic examination of sunflower plants naturally infected by *Phomopsis* sp. in 1981

No.	Locality	Hybrid	Reproductive organs		
			Pycnidia	Type of (conid.) spores	Perithecia
1	Negotin	NS-H-27-RM	+	A, B	—
2	V. Gradište	NS-H-26-RM	+	B	—
3	Vlaški Do	NS-H-26-RM	+	B	—
4	Radovanje	NS-H-26-RM	+	B	—
5	Belosavci	NS-H-33-RM	+	A, B	—
6	Mladenovac	NS-H-26-RM	+	B	—
7	Pančevo	NS-H-26-RM	+	B	—
8	Kikinda	NS-H-33-RM	+	A, B	—
9	N. Kneževac	NS-H-33-RM	+	B	—
10	Subotica	NS-H-26-RM	+	B	—
11	Bajmok	NS-H-26-RM	+	B	—
12	B. Palanka	NS-H-62-RM	+	A, B	—
13	B. Petrovac	NS-H-26-RM	+	B	—
14	Bečej	NS-H-26-RM	+	B	—
15	Novi Sad	NS-H-26-RM	+	B	—
16	Kać	NS-H-26-RM	+	B	—
17	Surduk	NS-H-27-RM	+	B	—
18	S. Pazova	NS-H-26-RM	+	B	—
19	Indjija	NS-H-26-RM	+	A, B	—
20	Beška	NS-H-27-RM	+	B	—
21	Ruma	NS-H-27-RM	+	B	—
22	V. Radinci	NS-H-27-RM	+	B	—

Legend :
+ Pycnidia present
— Perithecia absent

Pycnidia were again determined in all samples. Stylospores (B conidia) were found in all of them, spores (A conidia) in seven samples only.

Perithecia were not found.

Comparing the materials coming from the same locality in the two years, we found similarities but also some differences. In both years all isolates produced pycnidia with stylospores (B conidia). Conversely, only two isolates produced A spores in 1980 and seven isolates in 1981. However, the isolates "Bački Petrovac" and "Bečej", which produced A and B conidia in 1980, had only B conidia in 1981 while A and B conidia were produced by other seven isolates.

ISOLATE OF PURE CULTURES

To conduct a detailed study of the pathogen, we isolated pure cultures from infected plant parts collected in different regions in 1980 and 1981. The obtained pure cultures are listed in Table 4. In 1980, we made 13 isolates : two from Serbia Proper, 10 from Vojvodina, and one from Croatia. In 1981, there were eight isolates : one from Serbia Proper and seven from Vojvodina.

Table 4

***Phomopsis* sp isolates from *Helianthus annuus* L. Plants infected in 1980 and 1981**

Republic, province	Isolate	Year of isolate preparation	
		1980	1981
Srbija	„Negotin"	—	+
	„Ćuprija"	+	—
	„Krnjevo"	+	—
Vojvodina			
Banat	„Pančevo"	+	—
	„Sečanj"	+	—
	„Zrenjanin"	+	—
	„Čoka"	+	—
	„Novi Kneževac"	—	+
	„Djala"	+	—
	„Kikinda"	—	+
Bačka	„Novi Sad"	+	+
	„Bački Petrovac"	+	+
	„Bačka Palanka"	—	+
	„Bečej"	—	+
	„Senta"	+	—
Srem	„Surduk"	+	—
	„Indjiia"	+	—
	„Beška"	—	+
	„Grabovici"	—	+
Hrvatska	„Osijek"	+	—

Legend :
+ Fungus isolated
— Fungus not isolated

The pure cultures were isolated and grown on potato-dextrose agar. The isolated had distinct characters (Fig. 4) but nevertheless can be divided into three groups according to the color of mycelial film, fructification, etc., as exemplified by Table 5 which contains data for seven isolates.

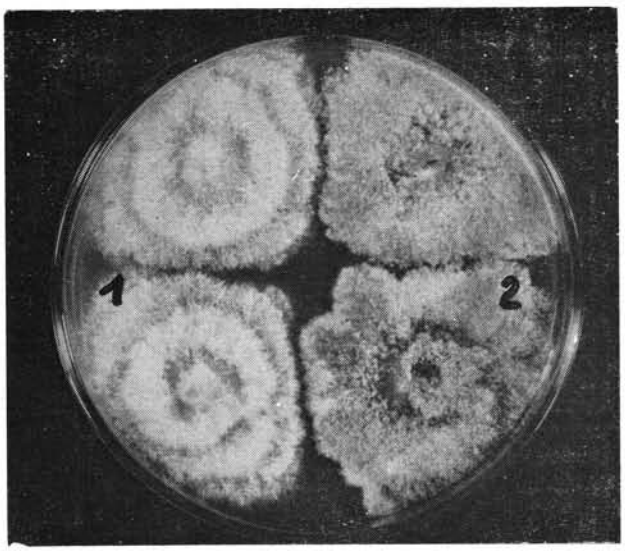

Fig. 4 — Phomopsis sp. Pure cultures of two isolates : 1. "Bački Petrovac", 2. "Bačka Palanka", on potato-dextrose agar.

Table 5

Some characters of *Phomopsis* sp. isolates from *Helianthus annuus* L.

No.	Isolate	Colour of mycelial film	Reproductive organs	
			pycnids	perithecia
1	„Negotin"	White	+	—
2	„Ćuprija"	White	+	—
3	„Kikinda"	White	+	—
4	„Novi Sad"	Brown	+	—
5	„Bečej"	Brown	+	—
6	„B. Petrovac"	White Snow	++++	—
7	„B. Palanka"	White	+++	—

Legend :
— No reproductive organs
+ slight occurrence
++ weak occurrence
+++ medium occurrence
++++ strong occurrence

It is evident that the isolates, grown on potato-dextrose agar at 25°C, differed in the colour of mycelia and in the number of pycnidia produced. The isolate "Bački Petrovac" was the only one to have a snow white mycelial film. This isolate produced also the largest number of pycnidia in pure culture. In case of natural infection, this isolate produced a large number of individual black pycnidia within a whitish-grey spot on the surface of epidermis while the other isolates developed pycnidia underneath epidermis.

Second group is comprised of the isolates forming a white mycelial film. The largest number of isolates from 1980 and 1981 fail into this group, i.e., they were the dominant constituent which caused epiphytotics of brownish-gray spot on sunflowers in Vojvodina and north-eastern Serbia. These isolates differed in the intensity of pycnidium formation : the isolate "Bačka Palanka" produced a large number of medium virulent pycnidia, while the isolates "Kikinda', "Ćuprija", and "Negotin" produced a very small number of pycnidia.

Third group includes a few isolates which formed a brown mycelial film and produced a small number of pycnidia.

Perithecia were not found in either one of the isolates.

It is difficult to decide what caused this differentiation among the isolates, their genetic characters or ecological conditions during their development on a plant in field or on a substrate in laboratory. In fact, we found that some isolates produced a limited number of pycnidia containing an abundance of pycnospores, A and B conidia, only at the optimum temperatures for their development. It is probable that 25°C was not the optimum temperature for all of the examined isolates.

MYCELIUM. Two days after the dissemination of the fungus, a diffuse colony of snow white mycelia, about 2 cm in diameter, appears on the surface of the nutritive substrate. The colony spreads rapidly to cover the entire surface of a 9 cm Petri dish in six days. During that time, the aerial mycelium changes in luxuriance from very low to medium and in compactness from low to medium. The mycelial film hardly changes its colour with age.

A microscopic analysis of a 10-day culture showed that the mycelium was hyaline, divided irregularly by transversal septa. The mycelium branches at the angle of 90°, the most frequent site of branching being the place where a septum joins the cell membrane. The tips of the hypha are rounded. The outer membrane of the mycelium is thicker than the septa, and the cells are granular on the inside. Mycelia and septa thicken with age (Fig. 5). The diameter of the mycelium is 5.54—8.31, or 8.31 micra on the average. The length of cells between septa is 13.85—22.16 or 17.45 micra on the average.

PYCNIDIUM. Pycnidia were found on infected materials gathered in 1980 and 1981. A microscopic analysis showed that they formed individually or in groups. In the former case, their shape was more or less globular, in the latter irregular. Their colour ranged from brown to black, in dependance of the age. The pycnidia were generally formed in the bark with the exception of the isolate "Bački Pe-

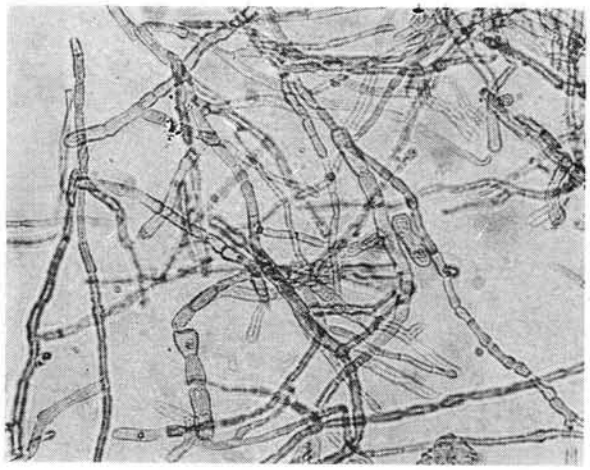

Fig. 5 — Phomopsis sp. Mycelia (12.5×25)

trovac" which formed the pycnidia on it. The pycnidia start appearing on the infected plants in summer and protract their presence to late fall. Their size was variable. With the isolate "Bački Petrovac" their length was 276.80—387.52 or 354.30 micra on the average, and the width was 276.80—366.76 or 337.35 micra on the average.

A detailed observation of the formation of pycnidia was conducted on potato-dextrose agar at 25°C. Fully formed pycnidia were found 10 days after the dissemination of the fungus. They increased in number for the subsequent five days and then stopped. The size of the pycnidia on potato-dextrose agar was 256.04—283.70 or 274.37 micra on the average for length and 193.76—221.44 or 207.44 micra on the average for width.

The above figures show that the pycnidia size was affected by the nutritive substrate. The pycnidia from naturally infected sunflower plants were larger than those from potato-dextrose agar.

PYCNOSPORES. Two types of pycnospores, A and B type (technically called A and B conidia) develop in the pycnidia present in naturally infected sunflower parts or in culture. A conidia are elliptical, hyaline, and monocelled, with a smooth outer membrane and two characteristic vacuoles resembling oily drops positioned at the ends of the inside. Very seldom one vacuole is at an end while another one takes the central position in the pycnospore. The elliptical shape and the two oily drops in A conidia are characteristic for the genus *Phomopsis* (Fig. 6). A conidia also form on potato-dextrose agar, the optimum temperature for their development being between 25 and 30°C.

The size of A conidia from the isolates "Bački Petrovac" and "Novi Sad' is given in Table 6. It is evident that A conidia from naturally infected materials were larger than those from potato-dextrose agar in the case of the isolate "Bački Petrovac". This is an indication that nutritive substrate affects the

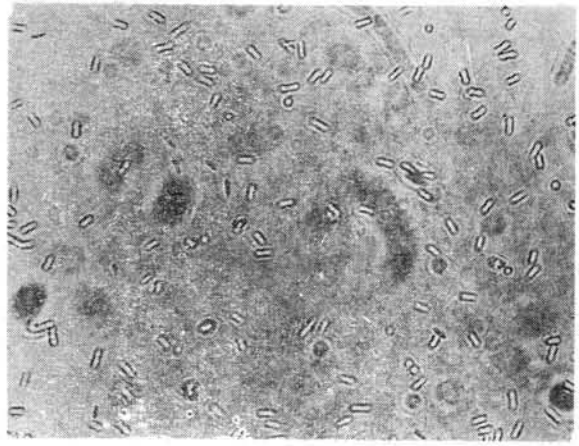

Fig. 6 — Phomopsis sp. A conidia (12.5×25)

Table 6

Size of A conidia in two *Phomopsis* sp. isolates

Isolate	Origin	Length	Average	Width	Average
„Bački Petrovac"	naturally infected material	8.11—13.85	10.84	2.77—5.54	3.75
	potato-dextrose agar	5.19—10.30	8.08	1.73—3.46	2.48
„Novi Sad"	naturally infected material	11.08—16.62	14.40	2.77—5.54	4.29

size of A conidia, same as with pycnidia. In the case of the isolate "Novi Sad", the differences were even larger.

A conidia start germinating at 20—25°C in six hours, reaching the maximum germination after 24 hours when the branching of hyphae begins.

B conidia are monocelled and hyaline. They are sticklike, bent to a varying degree at one or both ends (Fig. 7). They do not germinate in a waterdrop, and their role in the development of the fungus and the effecting of infection is unclear.

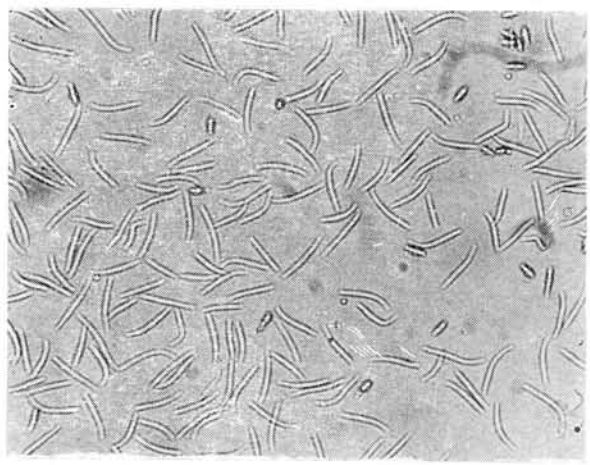

Fig. 7 — Phomopsis sp. B conidia (12.5×25)

The size of B conidia from naturally infected materials and potato-dextrose agar is shown in Table 7. The size was highly variable. With the isolate "Bački Petrovac", B conidia from naturally infected materials were somewhat larger than those from potato-dextrose agar. With the isolate "Novi Sad" the differences were larger, i.e., B conidia of the isolate "Novi Sad" were larger than those of the isolate "Bački Petrovac".

Table 7

Size of B conidia

Isolate	Origin	Length	Average	Width	Average
„Bački Petrovac"	naturally infected material	22.16—27.16	25.78	1.35—4.15	2.45
	potato-dextrose agar	16.62—44.32	24.46	1.38—4.15	2.90
„Novi Sad"	naturally infected material	22.16—38.78	30.96	2.77—6.93	4.42

It should be emphasized at this point that there existed a strict negative correlation between the number of A and B conidia, i.e., a large number of A conidia implied a small number of B conidia, and vice versa. B conidia were prevalent with both naturally infected materials and pure culture although the ratios varied from isolate to isolate. The ratios also differed in dependance of the nutritive substrate and temperature.

PERITHECIUM. It has been observed earlier that some *Phomopsis* species, for example *Diaporthe phaseolorum* Sacc. on soybean, have not only the pycnidium stage in their development cycle but also form sexual organs, perithecia.

Examining the infected sunflower parts in 1979, 1980 and 1981, we could find exclusively pycnidia.

Furthermore, no organs of perithecium type were detected when growing the fungus in pure culture on different substrates and the optimum temperature or on potato-dextrose agar at different temperatures.

The overwintered infected plant parts and pure cultures contained an abundance of pycnidia but no perithecia.

B. EFFECT OF SOME ECOLOGICAL FACTORS ON THE DEVELOPMENT OF THE FUNGUS

This part of the study included the effects of temperature, moisture, light, and different nutritive substances on the development of the fungus, mostly the isolates "Bački Petrovac" and "Novi Sad". The latter isolate came from the experimental field Rimski Šančevi of the Institute of Field and Vegetable Crops in Novi Sad.

EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF THE FUNGUS

Experiments were conducted on potato-dextrose agar. About 10 ccm of the substrate were placed into 9-cm Petri dishes and equal parts of pure cultures of the isolates "Bački Petrovac" and "Novi Sad' were disseminated.

After the dissemination, Petri dishes were kept at the temperatures of 0, 10, 15, 20, 25, 30, and 35°C. Fungal development was followed at the intervals of two, three, and four days.

With both isolates we followed the growth of mycelia, occurrence of aerial mycelia, compactness of mycelial film, changes in mycelium colour, and the occurrence of reproductive organs.

The results obtained for the isolate "Bački Petrovac" are given in Table 8. It may be

Table 8

Effect of temperature on "the development of *Phomopsis* sp., isolate Bački Petrovac"

Phase observed	Period in days	Temperature in °C						
		0	10	15	20	25	30	35
Mycelial growth in cm	2	—	—	—	1.06	2.12	2.35	—
	4	—	—	1.30	4.30	7.20	5.92	—
	6	—	—	2.70	6.92	9.00	9.00	—
	10	—	—	3.47	9.00	9.00	9.00	—
	12	—	0.37	3.75	9.00	9.00	9.00	—
	14	—	0.37	4.75	9.00	9.00	9.00	—
	17	—	0.95	6.00	9.00	9.00	9.00	—
Presence of airborne mycelia	2	—	—	—	+	+	++	—
	4	—	—	+	++	++	++	—
	6	—	—	+	++	+++	+++	—
	10	—	—	+	++	+++	+++	—
	12	—	+	+	++	+++	+++	—
	14	—	+	+	++	+++	+++	—
	17	—	+	++	+++	+++	+++	—
Consistency of mycelial film	2	—	—	—	++	++	+++	—
	4	—	—	+	++	+++	++++	—
	6	—	—	+	++	+++	++++	—
	10	—	—	++	+++	+++	++++	—
	12	—	+	++	+++	+++	++++	—
	14	—	+	++	+++	+++	++++	—
	17	—	+	++	+++	+++	++++	—
Changes in colour of mycelial film	2	—	—	—	+	+	+	—
	4	—	—	+	+	+	+	—
	6	—	—	+	+	+	+	—
	10	—	—	+	+	+	+	—
	12	—	+	+	+	+	+	—
	14	—	+	+	+	+	+	—
	17	—	+	+	+	+	+	—
Occurrence of reproductive organs (pycnids)	2	—	—	—	—	—	—	—
	4	—	—	—	—	—	—	—
	6	—	—	—	—	—	—	—
	10	—	—	—	—	+	++	—
	12	—	—	—	+	++	+++	—
	14	—	—	—	+	++	++++	—
	17	—	—	++	++	++	++++	—

Legend :
— no occurrence
+ slight occurrence
++ weak occurrence
+++ medium occurrence
++++ strong occurrence

seen that in only two days the mycelia started growing at the temperatures of 20, 25, and 30°C. In the course of further mycelial development, the growth was rapid at all three temperatures but still the fastest at 25°C. The growth was slow at 10 and 15°C and the mycelia did not develop at 0 and 35°C. Only the temperatures of 20, 25, and 30°C ensured the maximum development to 9 cm.

At 10°C, the aerial mycelia formed a very thin white film, at 15°C the film was very slight to slight, at 20 and 25°C the film was very slight to medium luxuriant, and at 30°C the film was weak to strong.

The compactness of the mycelial film was very weak at 10°C, very weak to weak at 15°C, weak to medium at 20 and 25°C, and medium to strong at 30°C.

The mycelial film remained snow white or white from the beginning to the end of the experiment, with all temperatures.

Pycnidia were found within the temperature limits of 15 and 30°C. The occurrence was very weak at 15°C, very weak to weak at 20 and 25°C, and weak to strong at 30°C.

The above data show that the minimum temperature for the examined isolate was between 5 and 10°C, the maximum between 30 and 35°C and the optimum between 25 and 30°C, i.e., 27°C.

Table 9 contains data on the effect of temperature on the development of the fungus from the isolate "Novi Sad". After two days, the fungus started to develop only at 20°C, after six days at 25°C, after 10 days at 15°C, and after 12 days at 10°C. In all the cases the fungus reached its maximum development in 12—17 days. The occurrence of the aerial mycelial film could be rated similarly. The colour of the mycelial film remained unchanged (white) at all temperatures througout the period of observations.

The occurrence of pycnidia was very low to low at all temperatures. The most rapid development was registered at 25°C.

It may be concluded that the minimum temperature for this isolate was between 0 and 10°C, the maximum around 25°C, and the optimum around 20°C.

The examined isolates of *Phomopsis* sp. differed considerably in the marginal temperatures necessary for their development.

EFFECT OF LIGHT ON THE DEVELOPMENT OF THE FUNGUS

The experiment was conducted on malt agar ; 10 ccm of the substrate were placed into 9-cm Petri dishes and equal parts of the pure culture of the isolate "Bački Petrovac" were disseminated. The dishes were then placed into two thermostats at 25°C. The outer metal door of one of the thermostats was left open to allow diffuse light to reach the dishes. The other culture developed in dark.

Table 9

Effect of temperature on the development of *Phomopsis* sp. isolate „Novi Sad"

Phase observed	Period in days	Temperature in °C						
		0	10	15	20	25	30	35
Mycelial growth in cm	2	—	—	—	1.80	—	—	—
	4	—	—	—	2.00	—	—	—
	6	—	—	—	2.20	1.50	—	—
	10	—	—	1.33	9.00	5.50	—	—
	12	—	1.16	9.00	9.00	9.00	—	—
	14	—	8.00	9.00	9.00	9.00	—	—
	17	—	9.00	9.00	9.00	9.00	—	—
Presence of air-borne mycelia	2	—	—	—	+	—	—	—
	4	—	—	—	+	—	—	—
	6	—	—	—	+	+	—	—
	10	—	—	+	++	+	—	—
	12	—	—	+	++	++	—	—
	14	—	++	++	++	++	—	—
	17	—	++	++	++	++	—	—
Consistency of mycelial film	2	—	—	—	+	—	—	—
	4	—	—	—	+	—	—	—
	6	—	—	—	+	+	—	—
	10	—	—	+	++	+	—	—
	12	—	+	+	++	++	—	—
	14	—	++	++	++	++	—	—
	17	—	++	++	++	++	—	—
Changes in colour of mycelial film	2	—	—	—	+	—	—	—
	4	—	—	—	+	—	—	—
	6	—	—	—	+	+	—	—
	10	—	—	+	+	+	—	—
	12	—	+	+	+	++	—	—
	14	—	+	+	+	++	—	—
	17	—	+	+	+	++	—	—
Occurrence of reproductive organs (picnids)	2	—	—	—	—	—	—	—
	4	—	—	—	—	—	—	—
	6	—	—	—	+	+	—	—
	10	—	—	+	+	+	—	—
	12	—	+	+	+	++	—	—
	14	—	++	++	++	++	—	—
	17	—	++	++	++	++		—

Legend :

— no occurrence
\+ slight occurrence
++ weak occurrence
+++ medium occurrence
++++ strong occurrence

In this experiment, we measured the diameter of the fungal colony, estimated the compactness and colour of the mycelial film, and observed the occurrence of pycnidia and A and B conidia. The results obtained are given in Table 10. It may be seen that light affected positively the fungal development. The mycelial growth was faster, the compactness of the mycelial film was increased, and pycnidia and spores were more numerous (Table 10). Both types of spores developed in light, only B conidia in dark. There were no differences in the colour of the mycelial film.

EFFECT OF NUTRITIVE SUBSTANCE ON THE DEVELOPMENT OF THE FUNGUS

Six nutritive substrates were examined : carrot agar, pea grain agar, bean grain agar, soybean grain agar, potato-dextrose agar, and acid-synthetic agar. The isolates "Bački Pe-

trovac" and "Novi Sad" were examined at 25°C. Petri dishes contained 10 ccm of the substrates.

Equal parts of the isolates were then disseminated and the fungal development was followed at certain intervals. We observed the development of mycelia, the occurrence of the aerial mycelia, the compactness and colour of the mycelial film, and the development of reproductive organs — pycnidia and perithecia.

Table 10

Effect of light on the development of *Phomopsis* sp. on malt agar, isolate „Bački Petrovac"

Combination	Days	Phase observed: Mycelial growth	Consistency of myc. film	Change in colour of mycel. film	Occurrence of reproductive organs: Pycnids	Conidia: A	Conidia: B
Light	3	4.5	++	White	—	—	—
	4	5.8	++		—	—	—
	6	7.0	++		++	—	—
	10	9.0	+++		++++	++	++++
	15	9.0	+++		++++	++++	++++
Dark	3	3.5	+	White	—	—	—
	4	5.4	+		—	—	—
	6	5.5	++		—	—	—
	10	6.0	+++		+	—	—
	15	9.0	+++		++	—	+++

Legend :
— no occurrence
+ slight occurrence
++ weak occurrence
+++ medium occurrence
++++ strong occurrence

The obtained results are shown in Tables 11 and 12. The former table contains data for the isolate "Bački Petrovac". It is evident that the examined substrates did not secure the uniform development of the fungus. In the period of 11 days during which we followed the fungal development, the mycelial growth reached its maximum with only three substrates : pea grain agar, bean grain agar, and potato-dextrose agar. The mycelial growth was considerably less intensive on the other substrates, especially on soybean grain agar.

The occurrence of the aerial mycelia was very low on soybean grain agar and it ranged from very low to low on the other substrates.

The compactness of the mycelial film on carrot agar, pea grain agar, potato dextrose agar, and acid-synthetic agar was very low to low, on bean grain agar and soybean grain agar very low.

The mycelial film underwent very slight changes in colour on all substrates.

Only pycnidia could be found. Their presence was established on pea grain agar and potato-dextrose agar, weak occurrence ; bean grain agar and acid-synthetic agar, very weak occurrence. Pycnidia were not registered on carrot agar and soybean grain agar.

Table 11

Effect of nutritive medium on the development of *Phomopsis* sp., isolate „Bački Petrovac"

Nutritive medium	Period in days	Mycelial growth in cm	Presence of airborne mycel.	Consis. of mycelial film	Changes in colour of myc. film	Occurrence of pycnids
Carrot agar	3	—	—	—	—	—
	7	2.66	+	+	+	—
	11	4.00	++	++	+	—
Pea seed agar	3	2.63	+	+	+	—
	7	6.50	+	+	+	++
	11	9.00	++	++	+	++
Bean seed agar	3	2.90	+	+	+	—
	7	6.25	++	+	+	—
	11	9.00	++	+	+	—
Soybean seed agar	3	0.20	+	+	+	—
	7	1.80	+	+	+	—
	11	2.20	+	+	+	—
Potato-dextrose agar	3	3.25	+	+	+	—
	7	7.86	++	+	+	+
	11	9.00	++	++	+	++
Acid-synthetic agar	3	1.97	+	+	+	—
	7	3.16	+	++	+	—
	11	5.33	++	++	+	+

Legend for columns 4, 5 and 7 : column 6 : + white color

— no occurrence
+ slight occurrence
++ weak occurrence
+++ medium occurrence
++++ strong occurrence

Table 12

Effect of nutritive medium on the development of *Phomopsis* sp., isolate „Novi Sad"

Nutritive medium	Period in days	Mycelial growth in cm	Presence of airborne mycel.	Consis. of mycelial film	Changes in colour of myc. film	Occurrence of pycnids
Carrot agar	3	2.16	+	+	+	—
	7	6.66	+	+	+	—
	11	9.00	++	++	+	—
Pea seed agar	3	3.15	++	+	+	—
	7	7.25	+++	+++	+	+
	11	9.00	+++	+++	+	+
Bean seed agar	3	2.70	+	+	+	—
	7	7.00	+++	+++	+	—
	11	7.50	+++	+++	+	—
Soybean seed agar	3	2.00	+	+	+	—
	7	4.00	++	++	+	—
	11	4.50	++	++	+	—
Potato-dextrose agar	3	3.20	++	++	+	—
	7	8.00	++	++	+	—
	11	9.00	++	++	+	++
Acid-synthetic agar	3	2.60	+	+	+	—
	7	6.50	++	++	+	—
	11	9.00	++	++	+	++

Legend for columns 4, 5 and 7 : column 6 : + white color

— no occurrence
+ slight occurrence
++ weak occurrence
+++ medium occurrence
++++ strong occurrence

Table 12 contains data for the isolate "Novi Sad". The substrates affected non-uniformly the mycelial growth. The maximum growth (9 cm) was found with carrot agar, pea grain agar, potato-dextrose agar, and acid-synthetic agar. The growth was less intensive with bean grain agar and soybean grain agar (Figure 8).

After the sterilization, the nutritive substrates and sterilized plant parts were used to disseminate on them the pure culture grown on potato dextrose agar. After the dissemination, Petri dishes were placed in a thermostat at 27°C. Observations were made at 3-day intervals for 12 days.

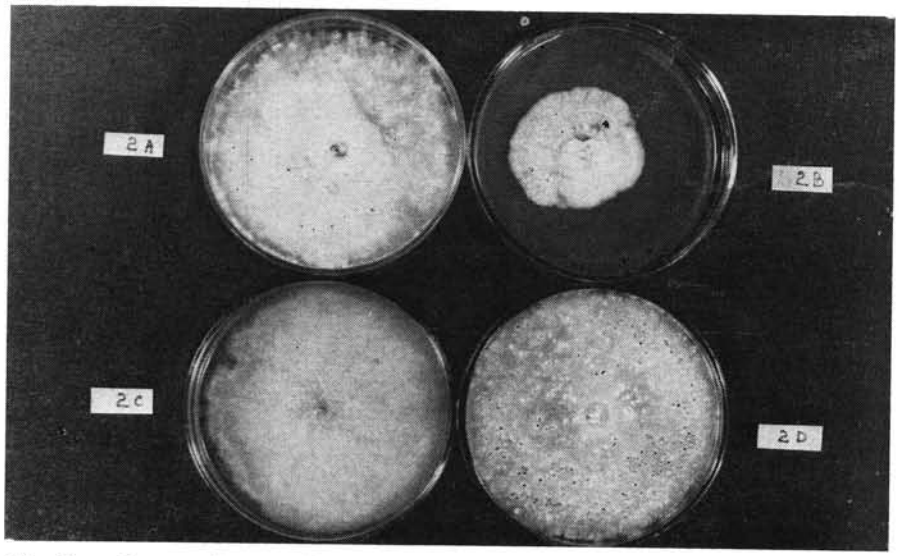

Fig. 8 — *Phomopsis* sp. Effect of nutritive substrates on fungal development ; 10-day old culture ; isolate "Novi Sad".
2 A — potato-dextrose agar, 2 B — soybean seed agar, 2 C — bean seed agar ; 2 D — pea seed agar.

Pea grain agar and bean grain agar stimulated the development of the aerial mycelia and the compactness of mycelial film. The other four substrates had a less stimulating effect.

The colour of mycelial film remained the same with all substrates throughout the period of observation.

Pycnidia were found on pea grain agar, bean grain agar, very weak occurrence ; and potato-dextrose agar and acid-synthetic agar, weak occurrence. Pycnidia were found on carrot agar and soybean grain agar.

The above data show that the two isolates, grown at the optimum temperature and the same nutritive substrates, differed considerably in the micelial growth, i.e., the rate of development, compactness, luxuriance, and especially in the development of reproductive organs, pycnidia.

EFFECT OF NATURAL NUTRITIVE SUBSTRATES ON THE FRUCTIFICATION OF *PHOMOPSIS* SP.

In this experiment we used the following natural nutritive substrates : fresh sunflower agar, fresh soybean agar, sterilized sunflower stem, sterilized sunflower leaf, sterilized soybean stem, sterilized soybean leaf, and sterilized soybean pod. For the combinations 1—6, sunflower and soybean plant parts used for the making of nutritive substrates were taken at the stage of flowering, for combination 7 after grain filling.

During the first observation, thin white mycelia were found on the surface of all substrates. A weak occurrence of pycnidia was registered during the second observation. The number of pycnidia varied from substrate to substrate, as shown in Table 13.

Table 13

Effect of nutritive medium on the fructification of *Phomopsis* sp., isolate „Bački Petrovac"

No.	Nutritive medium	Occurrence of reproductive organs	
		Pycnids	Perithecia
1	Agar of fresh sunflower plants	++	—
2	Agar of fresh soybean plants	++	—
3	Sterile sunflower stem	++++	—
4	Sterile sunflower leaf	++++	—
5	Sterile soybean stem	++++	—
6	Sterile soybean leaf	++++	—
7	Sterile soybean pod	++++	—

Legend :
— no reproductive organs
+ slight occurrence
++ weak occurrence
+++ medium occurrence
++++ strong occurrence

The data in Table 13 show that pycnidia were formed on all substrates. Pycnidia were the least frequent on fresh sunflower agar and fresh soybean agar, the most frequent on sterilized sunflower stems and leaves and sterilized soybean stems, leaves, and pods.

EFFECT OF MOISTURE ON THE GERMINATION OF CONIDIA

This part of the study included :

— the germination of conidia at 100% moisture : pycnidia with A and B conidia were placed in wet Petri dishes ;

— the germination of conidia in a drop of distilled water.

For these experiments we used pycnospores of the isolates "Bački Petrovac" and "Novi Sad,, from pure cultures grown on potato-dextrose agar for 15 days. The germination of pycnospores was followed for 24 hours at 23°C.

A and B conidia were not found to germinate at 100% moisture. However, the conidia did germinate in a drop of distilled water, as shown in Table 14.

Table 14

Germination of A and B conidia distilled water drops, isolate "Bački Petrovac"

Time in hours	Conidia	
	A spores	B spores
1	—	—
2	—	—
4	—	—
6	+	—
12	++	—
17	+++	—
21	++++	—
24	++++	—

Legend :
— no germination
+ weak germination
++ medium germination
+++ strong germination
++++ very strong germination

A conidia started to germinate in six hours and kept germinating throughout the experiment. The intensity of germination was medium in 12 hours, high in 17 hours, and very high, i.e., maximum, in 21 hours.

A conidia germinated at one end, both ends, or in the middle, producing a threadlike hypha which in turn forms a bulge at its tip — an appressorium, and then starts branching and septating (Fig. 9). B conidia failed to germinate in the 24 hours of the experiment.

INCUBATION

Incubation period was studied in a greenhouse, on young sunflower plants, and in a polythermostat, on sunflower leaves.

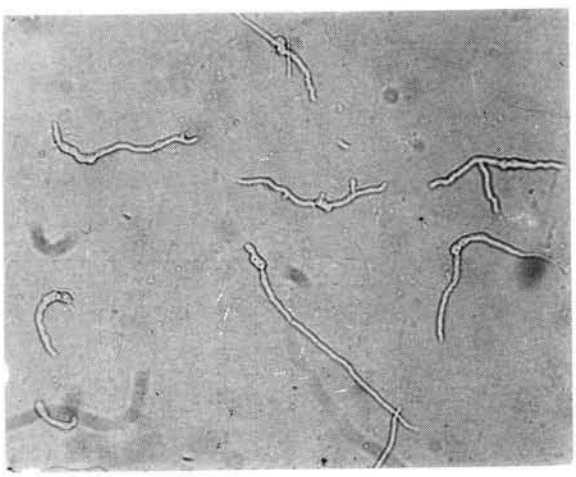

Fig. 9 — Phomopsis sp. Germination of A conidia in waterdrop after 24 hours (12.5×25).

The materials for both experiments were obtained by planting the hybrid NS-H-33-RM in a greenhouse. When the experimental plants reached the age of 45 days, they were prepared for inoculation with a suspension of pure cultures of the isolates "Bački Petrovac" and "Novi Sad", grown on potato-dextrose agar. Twenty pots with five plants in each were selected. The plants in 10 pots were sprayed with the suspension of the isolate "Bački Petrovac", the other 10 pots with the suspension of the isolate "Novi Sad". After the inoculation, the plants were kept for 48 hours in a chamber at 100% moisture. The vitality of pycnospores (A conidia) was checked before the inoculation. Their germinability exceeded 50% after 24 hours. The efficiency of inoculation was followed for 20 days. During that period, the temperature in the greenhouse was kept within the limits of 14 and 29°C.

The inoculated plants showed no trace of infection in 20 days.

The same procedure was repeated in a polythermostat using sunflower leaves. Healthy leaves of 60-day old plants of the hybrid NS-H-33-RM were removed and placed in large Petri dishes with wet filter paper. We made sure that the ends of the petioles of the examined leaves were in contact with the wet filter paper in order to keep the leaves fresh as long as possible. We were successful in it since the leaves stayed fresh and green for more than 10 days, i.e., to the end of the experiment. A suspension of A conidia from the isolates "Bački Petrovac" and "Novi Sad" was then sprayed on the front side and the reverse side of the examined leaves and the inoculated leaves were placed in the polythermostat at the temperatures of 0,10, 15, 20, 25, 30, and 35°C. The results of this experiment are shown in Table 15.

We can see that there existed large differences in the limit temperatures for infection as well as in the period of incubation. The

Table 15

Effect of temperature on the incubation length in two *Phomopsis* sp. isolates

Temperature °C	Incubation in days		
	„Bački Petrovac"	„Novi Sad"	Control
0	—	—	—
10	—	—	—
15	—	10	—
20	—	7	—
25	4	7	—
30	3	—	—
35	2	—	—

isolate "Novi Sad" required a 10°C lower temperature for infection than the isolate "Bački Petrovac" — the former effected infection between 15 and 25°C, the latter between 25 and 35°C.

The period of incubation was 7—10 days with the isolate "Novi Sad", 2—4 days with the isolate "Bački Petrovac". Pidopličko (1978) stated that the incubation period of *Phomopsis dauci* Arx. was 4—6 days.

The short incubation period mostly at higher temperatures (above 25°C) of the isolate "Bački Petrovac" and the long incubation period at lower temperatures (below 25°C) of the isolate "Novi Sad", during long rainy spells, enabled the occurrence of epiphytotics of *Phomopsis* sp. in sunflower.

PATHOGENIC CHARACTERS OF THE PARASITE

Numerous species from the genus *Phomopsis* have been found and described on a number of cultured plants. The best known are *Phomopsis viticola* Sacc. on grapevines, *Phomopsis dauci* Arx. on carrots, Diaporthe *(Phomopsis) phaseolorum* Sacc. on soybeans. These as well as the other *Phomopsis* species have been studied in detail on different plant species. In Yugoslavia, only *Phomopsis viticola* has been studied (Radman, 1973).

There exist very scanty data on the occurrence of *Phomopsis* or *Diaporthe* on the genus *Helianthus* or on *Helianthus annuus* L. First reports on the occurrence of a fungus from the genus *Diaporthe (Phomopsis)* on the genus *Helianthus* sp. made Saccardo (1898) and Wehmeyer (1933) in the USA who found *Diaporthe arctii* (Fr.) Nits. on *Helianthus annuus* L. and some other species of the genus *Helianthus.*

We concentrated our attention on the pathogenicity of *Phomopsis* sp. on *Helianthus annuus* L., *Helianthus* sp., and *Helianthus tuberosus* L. in conditions of field infection. When evaluating the pathogenicity of *Phomopsis* sp. on these species, hybrids and varieties, we considered the number of infected plants and the number and size of spots on them. The last two parameters were rated on the scale 0—4, 0 being healthy plants, 4 heavily infected ones.

The results of the evaluation of the three sunflower species for the resistance to *Phomopsis* sp. are given in Table 16.

Table 16

Pathogenicity of *Phomopsis* sp. on three species of the genus *Helianthus*

Plant species	No. of infected plants in %	Disease manifestation
Helianthus annuus L. (NS-H-26-RM)	100	3
Helianthus sp. *common*	100	4
Helianthus tuberosus L.	—	0

It can be seen than *H. annuus* and *Helianthus* sp. had the maximum number of infected plants — 100%, but the severity of the disease was different. *H. annuus* had a smaller number of spots which were also smaller in size than those on *Helianthus* sp. On the other hand, no symptoms of the disease could be found on *H. tuberosus* although we examined more than 50 varieties of different origin. Therefore, it may be concluded that the genus *Helianthus* includes some resistant species which most probably possess genes of resistance to the pathogen.

The majority of the examined hybrids and varieties displayed a high degree of susceptibility in field trials and in commercial production. However, it was noticed with interest that the damages were much lower if the disease occurred in the post-flowering period, as illustrated by the data on four domestic hybrids and one Soviet variety in Table 17.

The degree of pathogenicity of *Phomopsis* sp. depended largely on the time of the occurrence of the disease, regardless of the num-

Table 17

Pathogenicity of *Phomopsis* sp. on some hybrids and varieties depending on the time of disease occurrence

Hybrid-Variety	No. of infected plants in %	Disease manifestation at the stage of development		
		before flowering	flowering	after flowering
NS-H-26-RM	100	4	3	2
NS-H-27-RM	100	4	3	2
NS-H-33-RM	100	4	3	2
NS-H-62-RM	100	4	3	2
VNIIMK 8931	100	4	3	2

ber of infected plants. If the infection took place before flowering, the manifestation of the disease was drastic. The consequence was a large-scale drying of the plants, no matter if there were one, two, or more spots on the stem. The spots on the leaves did not cause the drying of the whole plant.

In all cases of the occurrence of the disease before flowering the yields were reduced by 50% or even more. Conversely, the large-scale attacks at the stage of milk maturity reduced the yields by 10—20%, while there were no direct damages if the disease occurred at the stage of wax maturity, unless the stems break. An explanation for this phenomenon is that young sunflower tissue is tender allowing the pathogen to penetrate easily to the conductive vessels into which it secretes toxin which are then distributed to all plant parts, causing the disorganization of the cells of the host plant and its rapid drying. With older plants which completed the development of all tissues, cell membranes are thicker and the penetration of the pathogen slower, mitigating the consequences of the disease.

Besides the plant species mentioned above, we also tested the soybean variety Wells for the resistance to *Phomopsis* sp. The inoculation, by the toothpick method, with the pure culture of the isolate "Bački Petrovac", was made in summer 1981. Young bean plants grown in greenhouse, were inoculated by spraying them with a suspension of A conidia. The inoculated soybean plants showed the signs of infection and pycnidia appeared on the site of infection 30 days later. The infection of bean plants in greenhouse was equally successful. The inoculated plants dried in 20 days and pycnidia were found on their stems.

A microscopic analysis of pycnidia taken from infected bean and soybean plants showed that the pycnidia belonged to *Phomopsis* sp., with characteristic pycnospores, i.e., A and B conidia.

OVERWINTERING AND SOURCES OF INFECTION BY *PHOMOPSIS* SP.

After the sunflower harvest in fall 1980, samples of infected plants were collected in order to study the hibernation habits of the pathogen. The samples were divided into three equal parts :

1. samples strewn over the soil surface,
2. samples buried at the depth of 10 cm,
3. samples buried at the depth of 10 cm also.

Vials containing pure cultures of the isolate "Bački Petrovac" were placed by sunflower stems. The vials were wrapped in plastic bags to prevent the access of water to the pure cultures.

At the end of April 1981, the materials of the second variant were dug out and left on soil surface.

Detailed macroscopic and microscopic analyses of the materials under examination were performed at 30-day intervals during winter and at 15-day intervals during spring and summer. The last set of analyses was conducted on July 31, 1981.

Sunflower stem an root residues were regularly collected from April 1 to October 30, 1981 in the plots in which sunflowers were grown the previous year.

During the analyses of variants 1, 2, and 3, pycnidia with pycnospores (A and B conidia) were regularly encountered both on sunflower stems and in pure cultures.

Likewise, mature pycnidia could be found throughout the vegetative season of 1981 on randomly collected harvest residues from 1980 (Fig. 10).

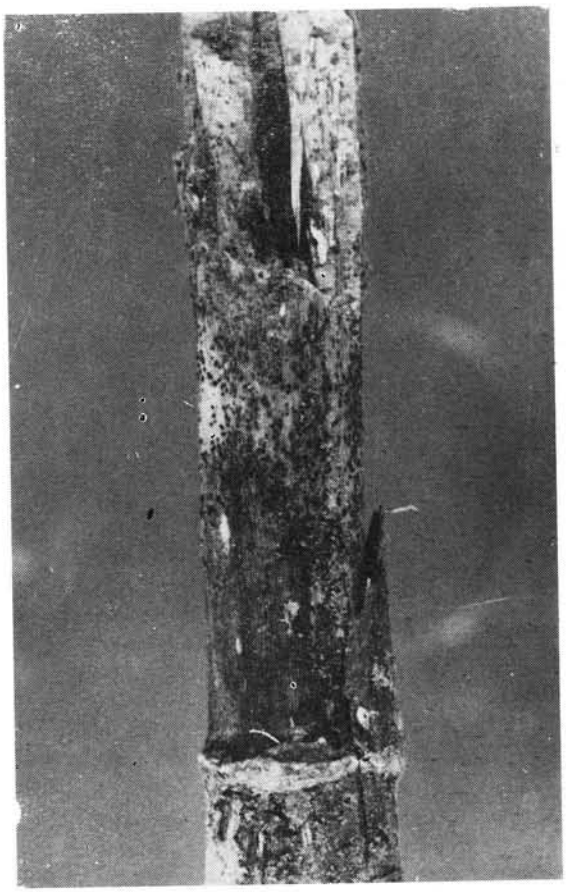

Fig. 10 — Phomopsis sp. Pycnidia on overwintered sunflower stem.

In this experiment, perithecium type black corpuscules were found on sunflower stems and harvest residues. One type of these perithecia was immature, without asci with ascospores. Another type, with more mature perithecia, belonged to the genus *Leptosphaeria*. It is certain that *Phomopsis* sp. overwinters in the form of pycnidia in infected sunflower residues. Furthermore, they can be found there throughout the vegetative season. Pycnidia are

formed from June to September with normal planting, and even in October with late planting or double cropped sunflower.

It is also certain that a large number of pycnospores (A and B conidia) is formed in pycnidia during the vegetative season. A conidia germinate in water drops effecting infection during the vegetation.

It is difficult to say whether the fungus produces perithecia or not. Perhaps they are produced very seldom and we could not find them on account of their limited number. We invest hopes in our further studies to find a reliable answer to this question.

The infection may also be transmitted by seed. In the course of 1980 and 1981 we examined a number of seed samples collected in plots which had suffered an intensive attack of the disease and which had a large number of infected plants. Although we did not find the seed to be infected by *Phomopsis* sp., it does not mean that the fungus is not transmitted by seed.

This paper does not contain a complete list of the pathogen's hosts. Besides the plant species mentioned before, we found *Phomopsis* sp. on several plant species from the spontaneous flora, most frequently from the genus *Sonchus* spp., which grow in sunflower plots and their vicinity, in ditches, roadsides, and pastures. This genus is close to *Helianthus* because both genera belong to the same family. It is essential to examine all plant species from the spontaneous flora for the reaction to *Phomopsis* sp. because they are all latent sources of infection.

DISCUSSION

The fungus *Diaporthe ortoceras* (Fr.) Nits. Caul. was described to inhabit the genus *Helianthus* sp. by Saccardo (1898). Much later, Wehmeyer (1933) reported *H. annuus* and *H. giganteus* as alternate hosts of *Diaporthe arctii* (Lasch.) Nits (=*Diaporthe ortoceras* (Fr.) Nits.) in the United States. However, the occurrence of the pathogen on these two plant species was classified as a sporadic phenomenon. These scanty data comprise the entire literature on the occurrence of fungi from the genus *Diaporthe* and *Phomopsis* on sunflower.

In Yugoslavia, *Phomopsis* sp. was registered for the first time in 1979 but already in 1980 and 1981 the fungus launched epiphytotics with serious consequences. It seems appropriate to put a question how such intensive attacks took place in the last two years when the pathogen has not been observed earlier. It should not be affirmed that the pathogen had not been present in sunflowers before. It had been there but we failed to discover it, most likely because sunflower stems and leaves are covered by conspicuous spots, caused by *Alternaria helianthi* (Hansf.) Tub. et Nish, and *Phoma oleracea* var. *helianthi tuberosi* Sacc. in the second half of summer. The brownish-gray spots caused by *Phomopsis* are then camouflaged by brownish-black and black spots caused by *Alternaria, Phoma,* and other fungi.

If our memory can be trusted, the *Phomopsis* type spots could be seen in Vojvodina even 20 years ago.

In 1980 and 1981, *Phomopsis* attacked sunflowers earlier, in the first part of the vegetative season, when the plants were most liable to infection. Numerous brownish-grey spots, especially on the stem, were clear symptoms which helped us identify the fungus. Why did *Phomopsis* occur so early in the last two years ? Reason should be seeked for in favourable climatic conditions, primarily the amount of rainfall in the first half of the vegetation. A large-scale and intensive infection took place in all regions which had frequent downpours in the first half of the vegetation (May and June) i.e., in Vojvodina and northern Serbia. In other regions, the infection was low or did not occur.

Our results indicate that temperature is not a limiting factor for the effecting of infection in a larger part of Yugoslavia because the required temperature range is 15—30°C, the optimum being between 20 and 30°C, and these temperatures are regularly encountered during the sunflower vegetation.

In 1981, *Phomopsis* sp. was also registered in Hungary and Bulgaria, and it is reasonable to assume that it occurred in Romania because low yields, on the level of those we obtained in Banat, were reported for some regions of that country (Aćimović, 1981).

There is also the question of the origin of the pathogen. The hypotheses are many, the reliable data are few. The first one is that the pathogen has been brought into the country from abroad some time ago, with infected seed. However, this hypothesis has a serious shortcoming : the pathogen has not been reported to inhabit sunflower anywhrere in the world. There have been several instances of a minor pathogen becoming a serious problem for a country or region following its transmission from the native country. If this hypothesis is valid, the pathogen must have come from a country from which we used to import sunflower seed, for commercial production or experiments. Those are primarily the USSR and the USA although those might have been other countries, too.

The second hypothesis is that *Phomopsis* was transferred to sunflower from another crop. Soybean is the only field crop grown in Yugoslavia that is susceptible to *Phomopsis*. Several Yugoslav phytopathologists support this hypothesis.

It is also possible that *Phomopsis* was transferred from a related plant species from the

spontaneous flora which grows in sunflower plot as a weed. We did find *Phomopsis* on some members of the family Compositae.

The above hypotheses are interesting, but there are no reliable data to support any of them. Additional research is required to clarify this question.

Our study showed that *Phomopsis* sp. has spread to the major sunflower-growing regions of Yugoslavia where it causes considerable damages to the crop.

Studying the morphological and biological characters of the pathogen, we found the isolates examined to differ by large in the dynamics of mycelial development, colour of mycelial film, and the formation and size of pycnidia and pycnospores (A and B conidia).

Comparative examinations of some biological characters of the isolates "Bački Petrovac" and "Novi Sad" indicated the presence of certain differences in relation to the symptoms caused on infected plants. The isolate also differed in temperature requirements for development. The isolate "Bački Petrovac" required the minimum temperature of 5—10°C, the optimum temperature ranging between 25 and 30°C. For the isolate "Novi Sad", these values were 5°C, 25°C, and 20°C, respectively.

CONCLUSIONS

The authors studied *Phomopsis* sp. on sunflower in Yugoslavia in the period 1979—1981 and drew the following conclusions :

1. *Phomopsis* sp. in sunflower was observed for the first time in 1979 in the vicinity of Kikinda. Already in 1980 and 1981 it spread to the major sunflower-growing areas : Vojvodina, northern Serbia, Slavonia, and northeastern Bosnia.

2. In Vojvodina, the number of infected plants was 94.00% in Banat, 50% in Srem, and 20% in Bačka. In Serbia, Croatia (Slavonia), and Bosnia, the percentages of infection were 10%, 1.50%, and 5—10%, respectively.

3. The largest damages were registered in Vojvodina (Banat, southern and central Bačka, eastern Srem) and northern Serbia, resulting from the early occurrence of the disease and the number of infected plants.

4. Disease symptoms were manifested on sunflower leaves and stems in the form of large brownish-gray spots which caused a partial or complete destruction of infected plants. The symptoms were most clearly pronounced on the stem.

5. The parasite produced brownish-black pycnidia and two types of spores (A and B conidia) in the spots on infected plants as well as on different substrates in pure culture.

6. Studying the relationship between A and B conidia, it was found that B conidia occurred regularly in pycnids while A conidia occurred irregularly. A and B conidia were mutually exclusive both in naturally infected samples and on different nutritive substrates.

7. In the course of the study, we made 20 *Phomopsis* isolates : 16 from Vojvodina, three from Serbia, and one from Croatia. They had some similarities but also certain differences in the morphological, biological, and ecological requirements for development.

8. With the isolate "Bački Petrovac", the size of pycnids was (275.80—387.52×276.80—366.76) (354.30—337.35) microns in the case of naturally infected material and (256.04—283.70×193.76—221.44) (274.37—207.44) microns on potato-dextrose agar.

9. With the same isolate, the size of A conidia was (8.11—13.85×2.77—5.54) (10.84—3.75) microns and the size of B conidia was (22.16—27.16×1.35—4.15) (25.78—2.45) microns. With the isolate "Novi Sad", the size of A conidia was (11.08—16.62×2.77—5.54) (14.40—4.29) microns and the size of B conidia was (22.16—38.78×2.77—6.93) (30.96—4.42) microns.

10. The isolates "Bački Petrovac" and "Novi Sad" were examined for their reaction to different temperatures. For the former isolate, the minimum temperature for development was between 5 and 10°C, the maximum between 30 and 35°C, and the optimum between 25 and 30°C ; for the latter isolate, the minimum temperature was 5°C, the maximum 25°C, and the optimum 20°C.

11. Light affected positively the rate of mycelial development and the number of pycnids, A and B conidia, but it did not induce changes in the colour of mycelial film.

12. The colonies of both isolates developed well on potato-dextrose agar and pea grain agar ; the colonies of the isolate "Novi Sad" developed well on carrot agar.

13. Nutritive substrate affected positively the development of pycnids. The maximum number of pycnids was obtained on sterile sunflower leaves and stems and soybean leaves, stems and pods.

14. The germination of A conidia in a waterdrop started after 6 hours to reach the maximum after 21 hours.

15. The isolate "Bački Petrovac" could perform infection between 25 and 35°C, the isolate "Novi Sad" between 15 and 25°C, with the incubation period of 2—4 and 7—10 days, respectively.

16. The isolates "Bački Petrovac" and "Novi Sad" displayed considerable differences in the biological and ecological requirements for development giving us ample room to conclude that here we deal with two *Phomopsis* species which attack sunflower or maybe with two ecotypes of the same species.

17. *Phomopsis* displayed a high degree of pathogenicity on varieties and hybrids of *H. annuus* and on undetermined species of *Helianthus* sp. It was not pathogenic on *Helianthus tuberosus.*

18. The pathogen overwinters at pycnidium stage in infected harvest residues.

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PHOMOPSIS SP. — UN NOUVEAU PATHOGÈNE DU TOURNESOL

Résumé

Phomopsis sp. a été observé pour la première fois sur le tournesol en Yougoslavie en 1979. Au cours des deux années suivantes, la maladie a pris un caractère explosif, spécialement dans les régions du Banat (une moyenne de 94% plantes atteintes), de Srem (50%) et de Bačka (20%), ce qui a déterminé une forte baisse du rendement. Le parasite vit sur les feuilles et sur la tige, où il forme de larges taches brunes-grises. Le champignon peut être identifié par les picnidies de couleur brun foncé et deux types de spores (conidies A et B) qui paraissent sur les zones infectées ou sur des substrats de culture pure. Les conidies B, contrairement aux conidies A, se retrouvent régulièrement dans les picnidies. Les deux types de conidies s'excluent mutuellement, autant dans les cas d'infection naturelle que sur les différents substrats nutritifs. Les 20 types isolés de plusieurs zones de culture du tournesol, présentent de nombreuses ressemblances mais aussi des différences morphologiques et biologiques. Les conidies A, les conidies B et les picnidies peuvent varier comme dimension. Les exemplaires isolés à Bački Petrovac se développent à une température minimale de 5 à 10°C, une température maximale de 30 à 35°C et une température optimale de 25 à 30°C ; pour les champignons isolés à Novi Sad, les températures étaient respectivement de 5°C, 25°C et 20°C. La lumière a une influence positive sur le développement du mycélium, le nombre de picnidies et de conidies A et B, mais ne provoque pas de changements dans la couleur de la pellicule mycélienne. Les colonies des deux types se sont bien développées sur milieu nutritif avec agar et dextrose de pomme de terre et sur agar avec farine de pois ; les colonies provenant de Novi Sad ont bien évolué aussi sur agar avec carotte. Le plus grand nombre de picnidies a été obtenu sur les feuilles et les tiges de tournesol et de soja ainsi que sur les cosses de soja. Dans une goutte d'eau les conidies A commencent à germer après six heures et atteignent le maximum après 21 heures. Pour les formes provenant de Bački-Petrovac, l'infection a lieu à 25—35°C, et pour celles provenant de Novi Sad, à 15—25°C, la période d'incubation étant de 2—4 et respectivement de 7—10 jours. Les différences morphologiques et biologiques mentionnées permettent de croire que les deux formes sont des écotypes différents de la même espèces ou bien des espèces différentes du genre *Phomopsis.* Ce champignon parasite un grand nombre de génotypes cultivés de tournesol et une espèce sauvage non déterminée du genre *Helianthus,* mais il n'est pas pathogène pour *H. tuberosus.*

PHOMOPSIS SP. — NUEVO POTÓGENO DEL GIRASOL

Resúmen

Phomopsis sp. fue notado por primera vez al girasol en Yugoeslavia en 1979. En los siguientes dos años esta enfermedad ha estado difundiéndose explosivamente, sobre todo en las regiones Banat (una media de 94 por ciento plantas infectados), Srem (50 por ciento) y Bačka (20 por ciento) reduciendo sustancialmente la producción. El parásito se instala sobre las hojas y especialmente sobre el tallo produciendo manchas amplias, de color castaño-gris. Se reconce por picnidios castaño-oscuros y dos tipos de esporos (conidios A y B) surgidos en las zonas infectadas, así como en diferentes substratos de cultura pura. Los conidios B, a diferencia de los A, han aparecido con regularidad en picnidios. Los dos tipos de conidios han mostrado ser mutualmente exclusivas, tanto en pruebas infectadas naturalmente, como también en diversos substratos nutritivos. Los 20 aislados procedentes de diferentes zonas de cultura de girasol han mostrado diferencias con respecto a los aspectos morfológicos y biológicos. De este modo, se han registrado tamaños diferentes en los picnidios, los conidios A y en los conidios B. Para la aislada de Bački Petrovac, la temperatura mínima para el desarrollo fue entre 5 y 10°C, la temperatura máxima entre 30 y 35°C y la óptima entre 25 y 30°C, mientras que para la aislada de Novi Sad la temperatura mínima fue de 5°C, la máxima de 25°C y la óptima de 20°C. La luz ha influído positivamente la cuota del desarrollo micélico, el número de picnidios y de conidios A y B, pero no ha traído cambios al color del film micélico.

Las colonias de las dos aisladas arriba mencionadas se han desarrollado convenientemente en medios de agar con dextrosis de patatas y en agar con harina de semillas de guisante ; las colonias de las aisladas de Novi Sad han evoluado también en agar con zanahoria.

El número máximo de picnidios se ha obtenido en hojas y tallos estériles de girasol y soja, así como sobre vainas de soja. La germinación de los conidios A en gotas de agua empezó tras seis horas y llegó al máximum tras 21 horas. Las infecciones se han producido a las temperaturas de 25—35°C en el caso de la aislada de Bački Petrovac y de 15—25°C en la de Novi Sad, con un período de incubacioń de 2—4 y respectivamente 7—10 dias. Las importantes diferencias morfológicas y biológicas arriba especificadas sugieren que las dos aisladas pueden ser ecotipos diferentes de las mismas especies, o hasta especies diferentes del gene Phomopsis. El patógeno ha atacado un gran número de genotipos de girasol cultivado y una especie salvaje nodeterminada del gene *Helianthus,* pero no se ha mostrado patogénico para *H. tuberosus.*