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Alternaria helianthinficiens Simmons, Walcz and R. Roberts sp. nov., THE CAUSAL AGENT OF BROWN-RED SPOT, A NEW SUNFLOWER DISEASE

M. Aćimović, N. Lačok

Faculty of Agriculture, Institute of Field and Vegetable Crops
Novi Sad, Yugoslavia

SUMMARY

- *Alternaria helianthinficiens* Simm., Wal. and Rob. was first determined in Yugoslavia in 1987, in the vicinity of Novi Sad and later on other locations.

- Disease symptoms are manifested on sunflower leaf, stem and head, in the form of brown-red angular spots.

- In pure culture on PDA, the fungus produces septated hyphae and multicellular condiophores with conidia.

- The dimensions of conidia are 13.88-114.57 x 1.38-11.08 *m (59.47-3.19 *m).

- Temperatures between 0 and 5 °C are minimum for fungus development, maximum ones are between 35 and 40 °C, while the and optimum is around 15 °C. The temperatures 15, 20 and 30 °C are minimum, optimum and maximum, respectively, for fructification.

- The best media for the development of mycelia were bean and carrot media and carrot and prune media were best for conidia prduction.

- After two or three sowings on nutrient media, the fungus loses its ability of fructification (production of conidia).

- The incubation in greenhouse lasts for two days and after 10 days all plants are dry. In field, *A. helianthinficiens* was more pathogenic than *A. helianthi* but less pathogenic than *Phomopsis helianthi.*

- *A. helianthinficiens* overwinters in the form of mycelia on infected harvest residues and infected seeds, which are the major sources of infection.

INTRODUCTION

Spots of different shape, size and color ranging from gray, light brown to black appear on leaf, stem and head of sunflower. The spots occur on sunflower from the period of emergence to the period of harvest or practically throughout the vegetation period. In the climatic conditions of Yugoslavia, the occurrence of spots is extraordinarily severe in July and August, i.e., at the stage of seed filling, when almost all the leaves wilt (Aćimović, 1966). The early and rapid wilting interrupts seed filling and reduces sunflower yield by 10-20 % (Aćimović, 1969 a,b).

A number of parasitic fungi, systematically classified in several species, are the causal agents of spots on sunflower. However, the spot diseases are most often caused by fungi from *Alternaria* genus, namely, *Alternaria alternata* (*Alternaria tenuis*), *Alternaria zinniae* and *Alternaria helianthi. Alternaria helianthi* is the most pathogenic fungus of the

thre. In fact, it is more pathogenic than any other agent of spot. Investigating *Alternaria helianthi* at the end of the 1960s, we isolated several fungi from the *Alternaria* genus, some of which were pathogenic to sunflower. However, the outbreak of *Phomopsis* on sunflowers in Yugoslavia and other European countries put a stop to the investigation until the problem of *Phomopsis* was solved. We also started to investigate other fungi which cause spot diseases of sunflower and succeeded in determining and describing two new fungi, *Stemphylium helianthi*, an agent of red spot and *Verticillium lateritium*, an agent of sunflower wilt (Aćimović, 1988 a, b, c). In last five years, we have regularly obtained five new, previously unknown *Alternaria* species in isolates taken from the various spots on the above-ground sunflower parts (stem, leaves, head and seeds). Particular attention was paid to two isolates, on taken from light brown spots on the stem which had red-pink surface underneath the spots, another taken from small brown spots on the stem and seeds. Testing their pathogenicity, we found that both isolates were pathogenic on sunflower, the former being more severe than the latter. By using the available literature, we determined the former isolate as *Alternaria helianthinficiens* Simmons, Walcz and R. Roberts and the latter as *Alternaria helianthola* G.N. Rao and Rajangopalan (Simmons, 1986; Rao and Rajangopalan, 1977). These new fungi cause two new diseases in sunflower-producing areas of Yugoslavia and other countries.

We shall describe in this paper the disease symptoms and some characteristics of *Alternaria helianthinficiens*, an economically significant disease in some sunflower-producing areas.

SYMPTOMS AND CONVENTIONAL NAME OF THE DISEASE

We monitored the occurence and the symptoms of the disease on sunflower plants infected spontaneously in field and on the inoculated plants in greenhouse. Under the conditions of spontaneous infection in field, the first symptoms usually occurred after flowering. The disease occured on all above-ground parts, on stem, leaf (lamina, petiole) and head. Although the symptoms occur on all plant parts, they are most characteristic on the stem.

Symptoms on leaves

On the leaves, brown-red spots occur on the lamina, between the nerves. The spots differ in shape and size. At the beginning they are small and polygonal. As the parasite spreads over the leaf tissue, the spots reach the size 0.5-1 cm in diameter. In favourable climatic conditions, the number of the spots increases significantly, the spots merge and the infected leaves wilt. Ten days favorable for fungus development (temperature of 20°C and a light rain) are sufficient for the infected leaves to begin to dry intensively (Figure 1). Except for the lamina, the fungus also attacks the petiole, causing brown-red spots and a premature drying of the lamina (Figure 2).

Fig. 1 *A. helianthinficiens.* Symptoms on sunflower leaf

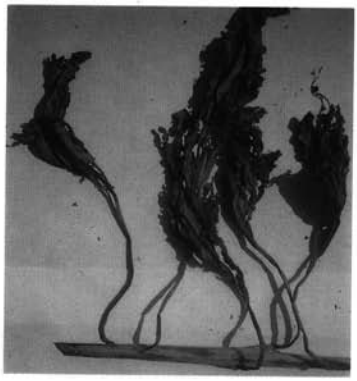

Fig. 2 *A. helianthinficiens.* Sunflower leaves dried by a strong attack of the fungus

Symptoms on the stem

The most characteristic diesease symptoms occur on the stem. The infection court is in the axilla of longer internodes. One internode rarely has only one infection court, usually more. In the beginning, the spots are small, 0.5-1 cm long and 0.5 cm wide (Figure 3). As the fungus progresses into the plant tissue, the spots extend to the lengt of 1-3 cm and the width of 1-2 cm. They usually merge, covering over 50 percent of the stem surface in susceptible varieties. The old spots change their color from light brown to coffee brown.

The spots are spherical, narrowing at the ends (Figure 4). From the stem surface, the fungus progresses into the sclerenchyma (ligneours part) and the core which become pale red (Figure 5). At the end of the vegetation, there are no exterior symptoms of the

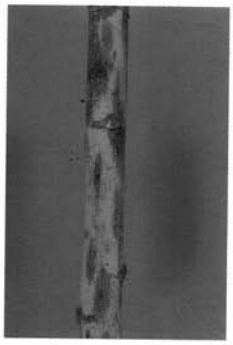

Fig. 3 *A. helianthinficiens.* Symptoms on sunflower stem

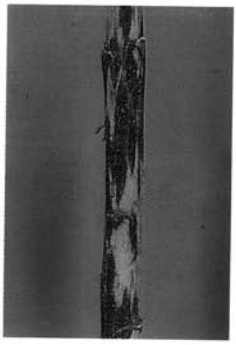

Fig. 4 *A. helianthinficiens.* Characteristic symptoms on the stem - merged spots

Fig. 5 *A. helianthinficiens.* Characteristic symptoms on the stem and on the vertical cross section of stem (J. Walez, Hungary, 1986).

disease on the prematurely dried plants, but red, ovoid areas on the vertical cross section of the stem are reliable signs of the infection by this fungus (Figure 6).

Fig. 6 *A. helianthinficiens*. Symptoms on the vertical cross section of diseased plants at the end of sunflower vegetation

Symptoms on the head

Certain sunflower genotypes display particularly characteristic symptoms on the head. The infection court is on head margin, i.e., on bracts, from which it progresses towards the center of the head in the form of brown-red spots (Figures 7 and 8).

A suspension of conidia in distilled water was used to inoculate 30-day old plants grown in the greenhouse. Already after 48 hours, light brown spots occurred on leaves and stems. After 7-8 days, the plants were completely dry (Figure 9).

In field, the fungus mycelium was used to inoculate the stem core at the beginning of flowering. After 20 days, the inoculated plants displayed first signs of wilt. The change of color was apparent on plant dissects, extending from the inoculation site in the form of brown-pink spots (Figure 10). Since the fungus causes light brown spots on leaf, stem and head and reddish spots inside the stem, our suggestion is to refer to the disease as the brown-red spot of sunflower.

SOME CHARACTERISTICS OF THE FUNGUS

Methodology

During our investigation of *Phomopsis helianthi*, we were collecting samples in infected sunflower plants all over Yugoslavia and especially in Vojvodina Province. The collected material was inspected macroscopically, systematized according to symptoms and finally examined microscopically. Besides *Phomopsis*, a number of previously known, as well as unknown fungi were discovered in the spots of the infected plants.

Our next step was to isolate fungi on the standard medium, PDA. *Phomopsis helianthi* and many other fungi, especially those from *Alternaria* genus, were obtained.

Since 1985 we collected the infected above-ground sunflower parts, whose symptoms clearly differed from the symptoms of *Phomopsis helianthi*. We isolated five various fungi from these spots, which were also isolated before, all belonging to the genus *Alternaria*. In 1987 we isolated a fungus from spots on sunflower stems and heads which had been isolated also in 1980 and 1981. We tested the fungus for pathogenicity on sunflower plants in the field and the greenhouse and achieved positive results.

After that, we extended the investigation to morphology and biology of the fungus.

Fig. 7 *A. helianthinficiens* Characteristic symptoms on the margin of sunflower head

Fig. 8 *A. helianthinficiens*. Symptoms on the head

RESULTS

FUNGUS DEVELOPMENT IN PURE CULTURE

Pure cultures of *Alternaria helianthinficiens* were regularly isolated on the PDA medium from samples of infected plant parts collected since 1987. All isolates from the earlier sunflower genotypes were similar. They developed slowly on the nutrient medium. The mycelium was fairly profuse, moderately consistent, red-brown in color. However, two isolate types could be distinguished according to their color. The type 'A' isolate had a compact reddish brown mycelium, while that of the type 'B' had a light violet central part and reddish brown margins (Figure 11).

The fungus characteristics were studied using the 'A' type isolate, on PDA, at the optimum temperature of 20°C. The fungus development was inspected ocularly and under microscope at certain time intervals.

We shall briefly describe the mycelium ant the conidium with conidiophores which are produced by the fungus in the pure culture on the nutrient medium.

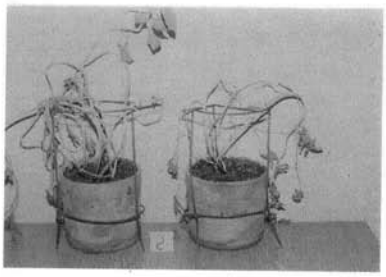

Fig. 9 *A. helianthinficiens*. Symptoms on inoculated young sunflower plants grown in greenhouse

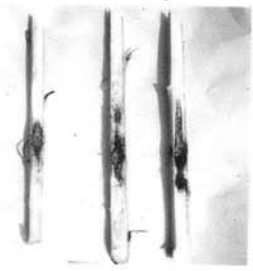

Fig. 10 *A. helianthinficiens*. Symptoms on inoculated stem after 10, 20 and 30 days

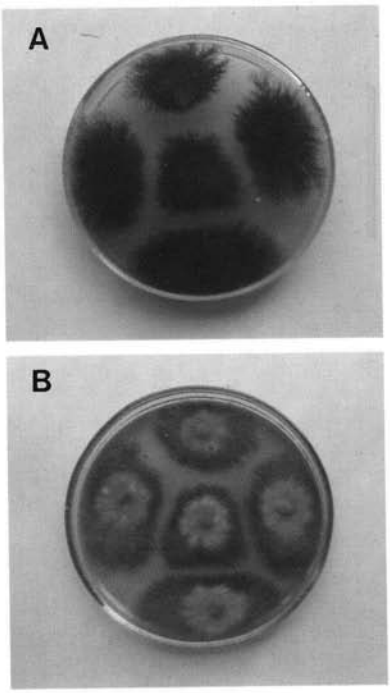

Fig. 11 *A. helianthinficiens.* The pure cultures of 'A' and 'B' isolates

Mycelium

According to Simmons (1986), the mycelium develops rather slowly on the nutrient medium. A fine white film 1 cm in diameter occurs on the medium surface three days after sowing the fungus. At this moment, the mycelium is hyaline, cell walls are thin and tender, rarely divided with almost imperceptible diagonal septa.

A change in color of the mycelium is evident after five days. It becomes yellow-orange and approximately 2 cm in diameter. Under microscope, the mycelium is still hyaline but the cell walls and the diagonal septa are strongly expressed. After seven days, the mycelium turns brown and is approximately 2.5 cm in diameter. The mycelium on the surface is medium profuse. Examined under the microscope, the mycelium is changed, its walls are yellow-brown and the diagonal septa and walls are thicker. After 10 days, the mycelium becomes profuse and medium consistent, 3 to 3.5 cm in diameter. Two types of hyphae could be observed under the microscope the old ones, which finished their growth, whose light brown cells are divided with frequent dark brown septa; the young ones, hyaline or light yellow, are divided with sporadic, almost imperceptible septa (Figure 12).

The fungus develops slowly on the nutrient medium and has two characteristic development phases. The first phase, characterized by rapid growth, lasts for 7 days and the fungus reaches almost 2/3 of its length; in the second phase, which lasts for 21 days, it develops rather slowly covering only 1/4 of its lengts.

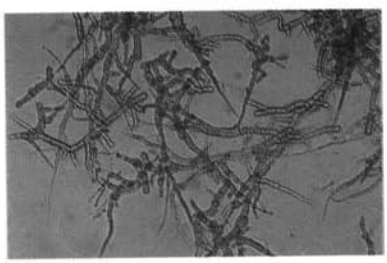

Fig. 12 *A. helianthinficiens.* A view of the mycelium (12.5 x 25 μm)

Conidia

Simmons (1986) gave the only data about the conidium and conidiophores of this fungus. According to these data, the color of the conidium and conidiophores is light brown to dark. Conidia and conidiophores in a 10-day culture are bent and about 200 x 6.5 μm, while the fully developed conidium is 68-18 μm, with 7-8 transversal septa and one longitudinal septum with 3-4 broad cells. In some cases, the dimensions of the conidium itself and conidiophores are 75 x 20 μm and 175-200 x 2 μm, respectively. The conidium, which has

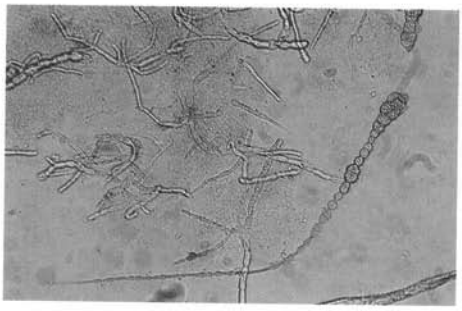

Fig. 13 *A. helianthinficiens*. Production of conidia in a chain (12.5 x 25 μm)

Fig. 14 *A. helianthinficiens*. A view of conidia (12.5 x 25 μm)

7-8 transversal septa, is brown with a green overtone.

According to our investigation, first conidia occurred five days after sowing the fungus on the medium. Eight days after sowing, the number of conidia reached the maximum value.

Bulges appear at the tip of the hyphae prior to the occurrence of conidia. Each bulge usually produces a single conidium, rarely a chain of conidia (Figure 13).

Conidia are different in shape and color, depending on maturity. The are predominantly pear-shaped, either regularly or irregularly. Young conidia are light brown while the old ones are brown. They are divided with transversal (5-7) and longitudinal (16) septa (Figures 14 and 15 a,b,c).

The dimension of conidia is 13.88-113.57 x 11.08-49.86 μm, and 44.54-26.67 μm on average.

Conidiophores

Conidiophores are fibrous, divided with sporadic longitudinal septa. They are unbranched, straight or more or less bent. They are often larger than the conidia, with

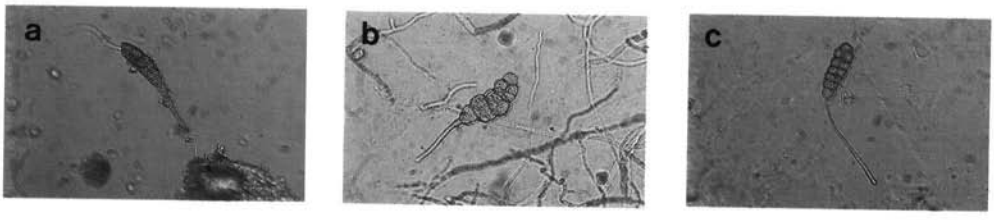

Fig. 15 *A. helianthinficiens*. A view of individual conidia with conidiophores a, b, c (12.5 x 25 μm)

the length and width of 11.08-254.80 μm and 1.38 - 11.09 μm, respectively, or 59.47 - 3.19 μm on average.

In our case, the size of conidia and conidiophores differed more or less from those given by Simmons (1986). Their shape and color are very similar. The differences in size may be due to the isolate itself, the medium and the temperature of cultivation.

EFFECT OF SOME ECOLOGICAL FACTORS ON THE DEVELOPMENT OF THE FUNGUS

There are no literature data on this fungus and this area of study. In our investigation, we tested the effect of temperature and some nutrient media on the development of the fungus. The 'A' type isolate was used.

Effect of temperature

We tested the effect of temperature on the fungus grown on PDA. Identical portions of the pure culture of the fungus were placed in Petri dishes 9 cm in diameter, each with approximately 10 ccm of the nutrient medium. After that, the Petri dishes were put in a polythermostat at 5, 10, 15, 20, 25, 30 and 35°C. The development of the fungus was monitored at certain time intervals for 30 days. During this period, mycelial growth, the presence of mycelial film, its consistency and changes in color, and the occurrence of reproductive organs (conidia) were monitored. The first four parameters were observed ocularly while the occurrence of reproductive organs was examined under microscope. Table 1 presents the results obtained.

The results in Table 1 show that after 3 days the mycelial growth was registered only at 20°C. Scant mycelial growth was detected after 5 days at 15, 20, 25 and 30°C, after 7 days at 5°C and after 15 days at 35°C. The development was uniformly slow at all the temperatures. Not a single isolate succeeded in filling the Petri dish in 30 days. The mycelial growth was most intensive at 20°C, when the diameter of 4.2 cm was achieved in 30 days. Two periods of fungal development were evident at this temperature. In the first period which lasted for 8 days, the fungus developed quickly, while in the second it developed rather slowly. At the other temperatures, the fungal development was slow (Figure 16).

The mycelium developed in and on the medium. The mycelial film developed at all the temperatures, the intensity of development depending on temperature. The mycelium was profuse at 20°C, less profuse at 15 and 25°C and scant at 5, 10, 30 and 35°C.

The consistency of the mycelium was slight at 5, 10, and 35°C, weak at 15°C and medium at 20, 25 and 30°C.

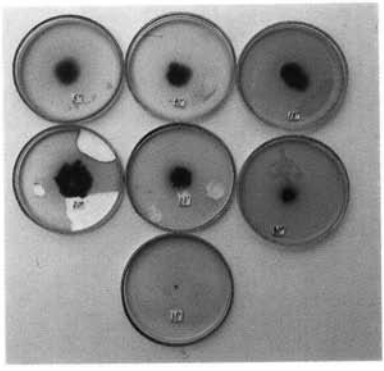

Fig. 16 *A. helianthinficiens*. Effect of various temperatures on fungus development

Tab. 1 Effect of temperature on the development of *Alternaria helianthinficiens*

Phase observed	Period in days	Temperature (in °C)						
		5	10	15	20	25	30	35
Mycelial growth in cm	3	-	-	-	1,10	-	-	-
	5	-	-	0,20	2,20	0,60	0,10	-
	7	0,30	0,20	0,70	2,50	1,10	0,30	-
	8	0,30	0,40	1,10	3,10	1,50	0,40	-
	10	0,40	0,70	2,00	3,30	1,70	0,50	-
	15	0,60	1,40	2,30	3,50	1,80	0,60	0,10
	21	0,90	1,90	2,30	4,00	2,10	0,90	0,10
	30	1,00	2,20	2,50	4,20	2,30	1,00	0,10
Presence of air–borne mycelia	3	-	-	-	+	-	-	-
	5	-	-	+	+	+	+	-
	7	+	+	+	+	+	+	-
	8	+	+	+	+	+	+	-
	10	+	+	+	+++	+	+	-
	15	+	+	+	+++	+	+	+
	21	+	+	+	+++	+	+	+
	30	+	+	+	+++	+	+	+
Consistency of mycelial film	3	-	-	-	+	-	-	-
	5	-	-	+	+	+	+	-
	7	+	+	+	+	+	+	-
	8	+	+	+	+++	+	+	-
	10	+	+	+	+++	+	+	-
	15	+	+	+	+++	+++	+++	+
	21	+	+	+	+++	+++	+++	+
	30	+	+	+	+++	+++	+++	+
Changes in color of mycelial film	3	-	-	-	white	-	-	-
	5	-	-	or.-yell.	or.-yell.	or.-yell.	or.-yell.	-
	7	white	white	or.-yell.	brown	or.-yell.	or.-yell.	-
	8	white	white	or.-yell.	brown	or.-yell.	or.-yell.	-
	10	white	white	brown	brown	or.-yell.	brown-yel.	-
	15	white	white	brown	brown	or.-yell.	brown-yel.	brown
	21	white	white	brown	brown	or.-yell.	brown-yell.	brown
	30	white	white	white	brown	or.-yell.	brown-yell.	brown
Occurrence of repro-ductive organs	3	-	-	-	-	-	-	-
	5	-	-	-	+	-	-	-
	7	-	-	-	+	+	-	-
	8	-	-	-	+++	+	-	-
	10	-	-	-	+++	+	+	-
	15	-	-	+	+++	+++	+	
	21	-	-	+	+++	+++	+	-
	30	-	-	+	+++	+++	+	-

Legend:

-	no occurrence
+	slight occurrence
++	weak occurrence
+++	medium occurrence
++++	strong occurrence

The color of the hyphae and the mycelium was highly dependent on temperature. At the temperatures of 5 and 10°C, the hyphae were hyaline and the mycelium was white. At 15°C, the hyphae and the mycelium were orange-yellow at the beginning, but later turned brown. At 20°C, the hyphae and the mycelium were hyaline and white, respectively, for couple of days and then they turned orange-yellow and brown, respectively. At 25°C, the hyphae and the mycelium were orange-yellow and later brown and black-brown, respectively. At 35°C, the hyphae and the mycelium were brown.

Table 1 shows that the occurrence of the reproductive organs - conidia, depended on temperature. The first conidia occurred after 5 days at the temperature of 20°C, and the maximum fructification took place 8 days later. At 25°C, the fructification was later and medium intensive while at 15 and 30°C the fructification was weak. Therefore, favorable conditions for fructification are between 15 and 30°C.

The above results indicate that the development of the mycelium was temperature-restricted. The minimum temperature was between 0 and 5°C, the maximum between 35 and 40°C, and the optimum about 20°C. The minimum temperature for fructification was about 15°C, the optimum about 20°C and the maximum about 30°C.

Effect of nutrient medium on fungal development

Seven different media were used in this investigation, water agar, PDA, onion medium, bean medium, carrot medium, prune medium and acid-synthetic medium. Identical portions of the pure culture of the fungus were placed in Petri dishes 9 cm in diameter, each with approximately 10 ccm of the nutrient medium. After that, the Petri dishes were put in a polythermostat at 20°C. The development of the fungus was monitored at certain time intervals for 28 days. During this period, mycelial growth, the presence of mycelial film, its consistency and changes in color and the occurrence of reproductive organs (conidia) were monitored. Table 2 presents the results obtained.

It is evident that the tested nutrient media affected the fungus development. The weakest mycelial growth was detected on the water agar, the strongest on the bean medium.

A slight mycelial growth was detected on the water agar, the onion medium and the acid-synthetic medium while on the other four media it was slight to weak.

The hyphae and the mycelium were hyaline only on the water agar, while on the other media the color changed in dependence of the age of the hyphae, ranging from white, orange-yellow, grey-brown to brown.

The occurrence of the reproductive organs - conidia was variable. No fructification occurred on the water agar, it was slight on the bean and onion media, weak on the PDA, slight to weak on the acid-synthetic medium and strong on the carrot and prune media.

Our results show that there existed large differences in fungus development on the different nutrient media with respect to all elements tested: the dynamics of growth, the profusion and consistency of mycelia and the occurrence of conidia. The bean and carrot media were best for mycelial development and the carrot and prune media for the occurence of conidia.

Tab. 2 Effect of nutritive medium on the development of *Alternaria helianthinficiens* (on t = 20°C)

Nutritiv medium	Period in days	Mycelial growth in cm	Presence of air–borne mycelia	Consis-tency of mycelia film	Changes in color of mycelial film	Occurrence ofrepro-ductive organs
Water agar	3	-	-	-	-	-
	5	-	-	-	-	-
	8	-	-	-	-	-
	10	-	-	-	-	-
	17	0,70	+	+	hyaline	-
	19	0,70	+	+	hyaline	-
	28	0,80	+	+	hyaline	-
PDA	3	0,60	+	+	light brown	-
	5	1,20	+	+	light brown	-
	8	1,90	+	+	light brown	-
	10	2,40	+	+	light brown	+
	17	3,30	+	+	light brown	+
	19	3,60	+	+	light brown	+
	28	3,60	+	+	brown	+
Onion medium	3	0,80	+	+	light brown	-
	5	1,50	+	+	light brown	-
	8	1,70	+	+	light brown	-
	10	1,90	+	+	light brown	+
	17	2,10	+	+	light brown	+
	19	2,40	+	+	light brown	+
	28	2,60	+	+	brown	+
Bean medium	3	0,40	+	+	white	-
	5	1,80	+	+	light brown	-
	8	2,80	+	+	light brown	-
	10	3,20	+	+	light brown	-
	17	6,90	+	+	light brown	-
	19	8,60	+	+	light brown	-
	28	8,80	+	+	light brown	-
Carrot medium	3	0,70	+	+	light brown	-
	5	2,00	+	+	light brown	
	8	3,30	+	+	light brown	+
	10	4,20	+	+	light brown	+++
	17	5,50	+	+	light brown	+++
	19	6,20	+	+	light brown	+++
	28	6,30	+	+	brown	+++
Prune medium	3	8,90	+	+	light brown	-
	5	1,40	+	+	light brown	+
	8	1,80	+	+	light brown	+
	10	2,00	+	+	light brown	+++
	17	2,40	+	+	light brown	+++
	19	4,40	+	+	light brown	+++
	28	4,60	+	+	light brown	+++
Acid–synthetic	3	0,70	+	+	light brown	-
	5	1,60	+	+	light brown	-
	8	2,80	+	+	light brown	+
	10	3,20	+	+	light brown	+
	17	4,40	+	+	light brown	+
	19	4,70	+	+	light brown	+
	28	5,00	+	+	brown	+++

Legend:
- \- no occurrence
- \+ slight occurrence
- ++ weak occurrence
- +++ medium occurrence
- ++++ strong occurrence

PATHOGENICITY AND OTHER CHARACTERISTICS OF THE FUNGUS

Pathogenicity of the fungus was tested in the greenhouse on 30-day old sunflower plants and in field at the beginning of flowering. In the greenhouse, the plants were inoculated by spraying on them a suspension of conidia in distilled water; in field, mycelia were injected in the medium part of the stem.

Ten-day old conidia from PDA were used for the suspension. They are capable of germinating in a drop of water, giving one or more infective hyphae (Figure 17). The hyphae penetrate the plant via stomatal apertures or wounded tissues of the leaf, stem or head. Conidia are disseminated by wind, rain drops or insects, specially aphids, during the stages of sunflower budding and flowering. The fungus loses the ability to fructify after two or three sowings on nutrient medium.

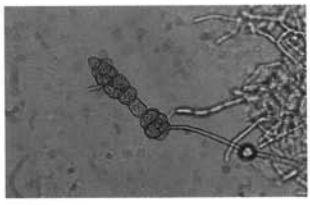

Fig. 17 *A. helianthinficiens.* Germination of the conidium

The inoculation with mycelia proved more suitable for testing the resistance of various sunflower genotypes. It is also a suitable method to revitalize fructification, i.e., for production of conidia.

In the greenhouse, two sunflower genotypes, NS-8 and NS-9, grown in clay pots, were inoculated with the suspension of conidia. We inoculated 25 plants of each genotype which were planted in 5 pots. The plants sprayed with pure distilled water were used as the control. Inoculated plants were put in a moisture chamber at 20°C and after 48 hours transferred to the greenhouse and kept at the relative moisture over 70% and the temperature below 30°C. After 48 hours, a multitude of light brown spots developed on sunflower leaves and stems. Five days later, the severely infected leaves started to dry and ten days later almost all plants of both genotypes were completely dry.

These results indicated that this fungus is pathogenic and hazardous for sunflower. According to the temperature demands of the fungus, its attack may be exected in regions with a temperate climate, i.e., in major sunflower production areas.

The degree of pathogenicity of *Alternaria helianthinficiens* was tested against those of *Alternaria helianthi* and *Phomopsis helianthi*, the two fungi which are pathogenic on most sunflower genotypes, using sunflower genotype NS-9. The inoculi were produced on sterile oat grains. Ten days after insemination, the grains were completely covered by the mycelia of all three fungi. Individual grains were then inserted in incisions made in the medium part of the stem. Ten plants were inoculated with each fungus. External disease symptoms were monitored on all plants and infected stems were dissected after 10, 20 and 30 days to observe the distribution of the fungi in sunflower tissues.

The occurrence of disease symptoms and the distribution of the fungi in the inoculated plants in the course of 30 days are presented in Table 3.

The incubation period of *Alternaria helianthi* was longer (10 days) than the incubation periods of *Alternaria helinathinficiens* and *Phomopsis helianthi* (5 days). The rates of *Phomopsis helianthi*, *Alternaria helianthinficiens* and *Alternaria helinathi* spreading in the

stem in 24 hours were 1 cm, 0.7 cm and only 0.2 cm, respectively. The pathogenicity index of *Alternaria helianthinficiens* was 157% higher than that of *Alternaria helianthi* and 126% lower than that of *Phomopsis helianthi*.

Tab. 3 A comparative test of pathogenicity of the three fungi

Fungus	Incubation	Spot length in cm	Pathogenicity index
Alternaria helianthi	10	7.9	100.00
Alternaria helianthinficiens	5	20.3	256.96
Phomopsis helianthi	5	30.3	383.54

Alternaria helianthi has been considered the most pathogenic fungus of sunflowers in *Alternaria* genus. Our results indicate that the new fungus is more pathogenic and probably more harmful than *Alternaria helianthi*. It appears that sunflower growers are facing a new and serious problem which demands a serious investigation.

OVERWINTERING AND INFECTION SOURCE

A number of spots characteristic for *Alternaria helianthinficiens* were observed on the above-ground parts, particularly on the stem, during inspections carried out in the second part of the vegetation. Mycelia, young and mature conidia were found in the infected tissues. Although the infected harvest residues which overwintered in field were not insepcted next spring, it is quite probable that the fungus is capable of surviving on infected plant parts and producing conidia which may provoke infection in spring.

Infected seeds are another source of the disease. When inspecting the health condition of sunflower seed, conidia and mycelia of the fungus were regularly found. The rate of seed contamination ranged from severe to slight or no infection at all. In our experience, seed-transmitted infections are efficiently controlled with Benlate or Rovral.

It is also necessary to establish whether the fungus attacks only *Helianthus annuus* genotypes or whether other species from *Helianthus* genus and other genea from *Compositae* family too may serve as alternate hosts.

DISCUSSION

According to the literature, several species of *Alternaria* genus are agents of spot diseases in sunflower. Neergaard (1945) and Joly (1963) indicated three species: *Alternaria tenuis*, *Alternaria zinniae* and *Alternaria leucanthemi*.

Nelen and Vasiljeva (1959) described a new fungus isolated from *Leucanthem vulgare* and identified it as *Alternaria leucanthemi*. Later on, they isolated this fungus from infected sunflower.

McDonald and Martens (1963) isolated a fungus on infected sunflowers in Canada and identified it later on as *Alternaria zinniae*.

A few years later, Tubaki and Nishihara (1969) determined *Alternaria helianthi* isolated from infected sunflower. At the same time, Aćimović (1969 a, b) in Yugoslavia isolated and described in detail a new fungus, *Alternaria* sp. He provided the major pathogenic parameters and described the economic importance of the fungus. Later on, Aćimović found that the fungi described by Hansford (1943) in Africa as *Helminthosporium helianthi* Hansf., and Aćimović (1969 a, b) in Yugoslavia as *Alternaria* sp. and *Alternaria helianthi* Tub. and Nish. represent, in fact, the same fungus with the three different names.

A new fungus, *Alternaria helianthicola* was described in India (Rao and Rajagapalan, 1977).

Simmons (1986) described the morphology of two fungi isolated from sunflower, *Alternaria heliathinficiens* and *Alternaria protenta*.

In the course of regular health inspections of sunflowers in Yugoslavia, we collected infected materials from various spots and isolated from them, besides other fungi, a number of fungi from *Alternaria* genus. Five different fungi were isolated in the last six years. Particularly distinguished was a fungus we regularly obtained from large brown spots on sunflower stem. This fungus differed from others in color, mycelial structure, shape and size of conidia and pathogenesis on sunflower. It differed completely from all previously known and described *Alternaria* species. Its mycological features corresponded closely to those of an already described but practically unknown *Alternaria* species, *A. helainthinficiens* Simm., Wal. and Rab. Simmons (1986) described the morphology and determined the fungus but he did not study its phytopathological characteristics. Its symptoms, dispersal mechanism, pathogenesis, possibilities of control and distribution in the major sunflower producing countries are unknown. All we know is that the fungus is present on sunflowers in the USA, Canada, Hungary and Yugoslavia. The real importance of the fungus remains unknown. According to our results, the fungus seems to be economically more important than any other fungus known to be harmful on sunflowers. According to its major temperature and moisture requirements, the fungus is probably present and may cause large economic damage in all countries of the temperate zone, and most sunflower producing countries are located there.

CONCLUSION

- *Alternaria helianthinficiens* Simm., Wal. and Rob. was first determined in Yugoslavia in 1987, in the vicinity of Novi Sad and later on other locations.
- This is a new sunflower parasite, previously known only in few countries.
- Diesease symptoms are manifested on sunflower leaf, stem and head, in the form of brown-red angular spots.
- In pure culture on PDA, the fungus produces septated hyphae and multicellular conidiophores with conidia. Their color varies depending on age. Young conidia (5 days) are hyaline while after 10 days they turn red-brown. Their shape is either regular or irregular pear-shaped. Conidiophores are unbranched, septated, long or short, more or less curved.

- We obtained two isolate types, 'A' and 'B'. The former type, found more frequently, is reddish brown, with medium profuse mycelium. The mycelium of the latter type has light violet central part and reddish brown margins.
- The dimensions of conidia are 13.88-114.57 x 11.08-49.86 μm (44.54-26.67 μm) and of conidiophores 11.08-254.80 x 1.38-11.08 μm (59.47-3.19 μm).
- Testing the effect of temperature on fungus development on PDA, we found that temperature affected the mycelial growth, the presence of mycelial film and its consistency and the production of conidia and their color. Temperatures between 0 and 5°C were minimum for fungus development, maximum ones were between 35 and 40°C, while the and optimum was around 15°C. The temperatures 15, 20 and 30°C were minimum, optimum and maximum, respectively, for fructification.
- The effect of seven different media on the fungus was tested at an optimum temperature. It was found that each medium had specific effects on all the tested parameters. The best media for the development of the mycelium were bean and carrot media and carrot and prune media were best for conidia production.
- After two or three sowings on nutrient media, the fungus loses its ability of fructification (production of conidia).
- The pathogenesis was tested in a greenhouse on 30-day old sunflower plants which were inoculated with water suspension of conidia and in field with mycelia introduced in the stem at the beginning of flowering. Two genotypes, NS-8 and NS-9, were inoculated. The incubation in the greenhouse lasted two days and after 10 days, the plants were dry. In the field, we tested the pathogenesis of *A. helianthinficiens* comparatively with *A. helianthi* and *Phomopsis helianthi*. *A. helianthinficiens* was more pathogenic than *A. helianthi* but less pathogenic than *Phomopsis helianthi*.
- *A. helianthinficiens* overwinters in the form of mycelia on infected harvest residues and infected seeds, which are the major sources of infection.

ALTERNARIA HELIANTHIFACIENS SIMONS, WALCZ ET R. OBERTS SP. NOV., AGENT PATHOGÉNE DE LA TACHETURE ROUGE BRUNE, UNE NOUVELLE MALADIE DU TOURNESOL

RÉSUMÉ

Alternaria helianthifaciens Simm., Wal. et Rob. a été initiallement déterminé en Yougoslavie en 1987, dans les environs de Novi Sad et par la suite dans d'autres localités.

Les symptômes de la maladie se manifestent sur les feuilles de tournesol, les tiges et les capitules sous formes de tâches anguleuses brun rouge.

En culture pure sur PDA, le champignon des hyphes septés et des conidiophores multicellulés avec des conidies.

Les dimensions des conidies sont de 13,88 - 114,57 x 11,08-49,86 m (44,54 x 26,67), celles des conidiophores sont de 11,08-254,80 x 1,38-11,08 m (59,47 x 3,19 m).

Les températures variant de 0 à 5 C constituent les températures minimales de développement et les maximales se situent entre 35 et 40 C, alors que l'optimun est de 15 C. Les températures de 15,20 et 30°C sont respectivement les minima, optima et maxima pour la fructification.

Les meilleurs milieux pour le développement du mycelium sont les milieux à base de carrote et de haricot, tandis que les milieux à base de carrote et de prune se sont révélés les meilleurs pour la production de conidies.

Aprés deux ou trois repiquages sur milieux nutritifs, le champignon perd son pouvoirs fructifères (production de conidies).

L'incubation en serre dure deux jours et aprés dix jours les plantes sont entièrement sèches. En champs, *A. helianthifaciens* est plus pathogéne que *A. helianthi* mais moins que *Phomopsis helianthis*.

A. helianthifaciens se conserve sous forme de mycelium sur des résidus de récoltes infectés et des graines contaminées, qui représentent la principale source d'infection.

ALTERNARIA HELIANTHINFICIENS SIMMONS, WALCZ AND R. ROBERTS sp. NOV. EL AGENTE CAUSAL DE LA MANCHA ROJIZA. UNA NUEVA ENFERMEDAD DEL GIRASOL

RESUMEN

Alternaria Helianthinfeciens SIMM, WAL and ROB fué determinada en Yugoslavia por primera vez en 1987 en la vecindad de Novi Sad y mas tarde en otras localidades. Los sintomas de la enfermedad se manifestaron sobre hojas, tallo y capítulo en forma de machas angulares rojizas. En cultivo sobre PDA, el hongo produce hifas septadas y conidioforos multicelulares con conidias. Las dimensiones de las conidias son 13.88 - 114.57 x 11.08 x 49.86 un (44.54 - 26.67 nm) y de los conidioforos 11.08 - 254.08 x 1.38 - 11.08 nm (59.47 - 3.19 nm). Las temperaturas entre 0 y 5°C. son las minimas para el desarrollo del hongo oscilando las máximas entre 35 y 40°C., mientas la optima está alrrededor de 15°C. Las temperaturas de 15, 20 y 30°C. son respectivamente las minimas, óptimas y máximas para la fructificación. Los mejores medios para el desarrollodel micelio fueron los de judia y zanahoria y para producción de conidias zanahoria y ciruela dos o trés siembras sobre medios nutritivos, el hongo pierde su capacidad de fructificación (Producción de conidias). La incubacion en invernadera dura dos dias y despues de 10 dias todas las plantas se secaron. En el campo *A. helianthinficiens* fué más patogénico que *Alternaria helianthi* pero menos patogénico que *Phomosis helianthi. A. helianthinficiens* inverna en forma de micelio sobre residuos de cosecha y semillas infectadas que son las principales fuentes de infección.