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STUDY OF GREY SPOTS ON SUNFLOWER (*PHOMOPSIS HELIANTHI* MUNT.-CVET ET ALL.) IN BULGARIA

A U T O R E F E R A T E

OF A DISSERTATION
FOR AWARDING
THE EDUCATIONAL AND SCIENTIFIC DEGREE
DOCTOR

GENERAL TOSHEVO, 2024

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Proffesional direction:
6.2. Plant protection

Scientific specialty/PhD Programme:
Plant protection (Phytopathology)

Scientific supervisor: Prof. Dr. Valentina Encheva, PhD
Scientific advisor: Prof. Dr. Ivan Kiryakov, PhD

GENERAL TOSHEVO, 2024

The dissertation is written on 149 pages. It contains 26 tables and 31 figures and 3 appendices. The list of used literature includes 147 sources, of which 18 in Cyrillic and 131 in Latin. The dissertation was discussed and directed for defense at an extended meeting of the "Sunflower Breeding" section at the Dobrudzha Agricultural Institute - General Toshevo (Report No. 395/16.10.2024) and by the Scientific Council on Plant Protection at the Agricultural Academy (Report No. 59/08.11.2024 and Order No. RD05-256/14.11.2024). The materials for the defense of the dissertation are available to whom it may concern at the Dobrudzha Agricultural Institute - General Toshevo. The numbering of the tables and figures used in the autoreferate does not correspond to that in the dissertation. The defense of the dissertation will take place on ..... from ..... at Agricultural Academy - Sofia, at a meeting of a specialized scientific jury appointed by order of the President of the Agricultural Academy No. Order No. RD05-256/14.11.2024, composed of:

1. Prof. Dr. Hristo Georgiev Bozukov, PhD - TTPI, Markovo
2. Prof. Dr. Petar Nikolov Chavdarov, PhD - IPGR, Sadovo
3. Prof. Dr. Rositsa Borisova Bachvarova, PhD - ABI, Sofia, retired
4. Assoc. Prof. Dr. Neshka Georgieva Piperkova-Kiryakova, PhD - AU, Plovdiv
5. Assoc. Prof. Dr. Violeta Savova Kondakova, PhD - ABI, Sofia, retired

1. INTRODUCTION

Cultural sunflower is one of the most widespread oilseed crops worldwide. In our country, for 2022, according to FAO data, 9 million decares of sunflower were grown, and production amounted to 21.5 million tons. For the last five business years (2018-2022), both the areas and production of the crop in Bulgaria have increased significantly. Against the background of continuous climate changes and unstable meteorological phenomena (intense, uneven precipitation in the summer, unforeseen droughts), such an increase is also associated with an increase in the risk of the emergence and spread of various phytopathogens.

More than 30 different pathogens attack sunflower and lead to economic losses in production. Gray spots on sunflower are one of the most economically important diseases of sunflower, which can cause serious damage to production, drastically reducing the amount of yield. The disease is caused by the phytopathogenic fungus Phomopsis/Diaporthe helianthi, which was first identified in the 1970s in Europe and in the 1980s in North America. The increase in sunflower production gradually led to the widespread spread of the disease, and at present it is characteristic of all regions where the crop is grown - Europe, Asia, Australia, North America. *Phomopsis helianthi* is a strictly specialized pathogen of sunflower and is difficult to detect outside sunflower crops. However, there are reports that it can be stored in weedy and wild species of the Asteraceae family. At the same time, the development of the disease is determined by high temperatures and a significant amount of precipitation, but under adverse conditions it can persist for a long time in the soil and plant residues. These features are associated with its high adaptive potential to adverse environmental conditions. Nevertheless, at the current stage of development of sunflower production, the genetic and aggressive diversity in the populations of the pathogen is poorly studied, and the search for sources of resistance is relatively difficult, due to the quantitative nature of its manifestation. This necessitates the need for in-depth study of the pathogen's response, and also implies the search for new sources of resistance in order to increase the productivity of cultivated sunflower.

2. AIMS AND SCOPES

The main goal of the study is to study the causative agent of gray spot on sunflower *Diaporthe/Phomopsis helianthi* Munt.-Cvet. et al. under field and laboratory conditions, by establishing the main characteristics of isolates collected on the

territory of the country and their reaction on different sunflower genotypes. To achieve the goal set in this way, the following tasks have been formulated:

1. Study of the distribution of the pathogen on the territory of the country;
2. Establishment of cultural and morphological characteristics of the pathogen in a collection of isolates of different geographical origin;
3. Study of the genetic diversity of the pathogen - mycelial compatibility;
4. Establishing the influence of the host phenophase on the aggressiveness of the pathogen;
5. Study of the aggressive diversity of a collection of isolates with different geographical origins;
6. Study of the inheritance of the reaction to the pathogen in sunflower hybrids with different resistance;

3. MATERIAL AND METHOD

3.1. Collection of plant samples and isolation in pure culture.

For the isolation of Ph. helianthi samples, plant samples were collected from different geographical areas of the country. For this purpose, in 2022 and 2023, sunflower production crops were examined in the phenophase Seed development (BBCH 79; R8), which coincides with the period after mid-July. The samples were collected based on the phenotypic manifestation of the pathogen. Infected parts were cut as segments from the host stem, 30-40 cm long. The samples were labeled by origin and collection date and stored in soda bags until processing, at 4℃ in a refrigerator.

3.1.1. Isolation in pure culture and storage of isolates.

The plant parts were washed in running water for 30 min, followed by two washings with sterile distilled water. After drying in a laminar flow hood, parts up to 5 mm in size were cut from the periphery of the spots. The cut plant parts were immersed in 70% ETOH for 10 sec., then ignited in an alcohol lamp. A part of 1-2 mm in size was cut from the periphery of the spots and placed on Potato Sucrose Agar (PSA) medium, poured into Petri dishes with a diameter of 90 mm, five plant parts per dish. The plates were incubated in a thermostat at a temperature of 22-24^{0}C, in the dark. The development of the colonies was monitored daily. When the colony size reached 15 mm, each colony was transferred to a new nutrient medium, and the isolates were incubated under the same conditions. The obtained pure isolates were maintained on the PSA medium at 4-5^{0}C, and their renewal was carried out every 30 days.

3.2. Cultural characteristics.

3.2.1. Culture media used.

Two culture media were used in the studies – ready-made PDA/PDA (Potate Dextros Agar/Potato-dextrose agar, HiMedia) and PSA/PSA (200 g potatoes, 20g sucrose, 15 g agar).

3.2.2. Study of the cultural characteristics of the isolates.

The study included 50 isolates of different geographical origin. Before conducting the tests, the isolates were cultured on PSA for 7 days. A disk was cut from the periphery of the colonies and placed on new PSA and PDA, poured into 90 mm Petri dishes. For each isolate, three dishes of the respective culture media were used. The incubation of the dishes was carried out in a thermostat at 23 ± 1^0 C in the dark. The following indicators were monitored:

1) Diametrical growth of the isolates in mm; The measurement was carried out from the 1st day of placement to the 7th day of the isolate's development. Since some isolates reach maximum growth (filling the plate) on the 4th day of their development, the results of the first four days were used to determine the values of diametral growth and development rate. The values of diametral growth were determined as the average of the three repetitions, on the 4th day of the measurements.

2) Development rate in mm/h, calculated by the formula:

$$Vd = \frac{k1.D1+k2.(D2-D1)+k3.(D3-D2)+k4.(D4-D3)}{24x24}$$

where k1, k2, k3, k4 – weight coefficients, respectively equal to 4, 3, 2 and 1. D1, D2, D3, D4 – diametral growth of the isolate, respectively on the 1st, 2nd, 3rd and 4th day of its development.

3) Days until the appearance of the first pycnidia – measurements were carried out from the first to the tenth day.

3.3. Establishing the influence of the development phase in sunflower on the reaction to *Phomopsis helianthi.*

The study was conducted under field conditions, during the 2022 and 2023 farming years, on the territory of the Dobrudzha Agricultural Institute - General Toshevo. The reaction of the Favorite variety and the Deveda hybrid to three isolates of

Phomopsis helianthi (*Ph21-614, Ph21-6212, Ph21-423*) was tested. The studied genotypes were sown in rows with a length of 2.40 m, inter-row spacing of 0.7 m and intra-row spacing of 0.30 m. Infection was carried out in three different phenophases of the ontogenetic development of the host BBCH-39, BBCH-51/53 and BBCH-61.

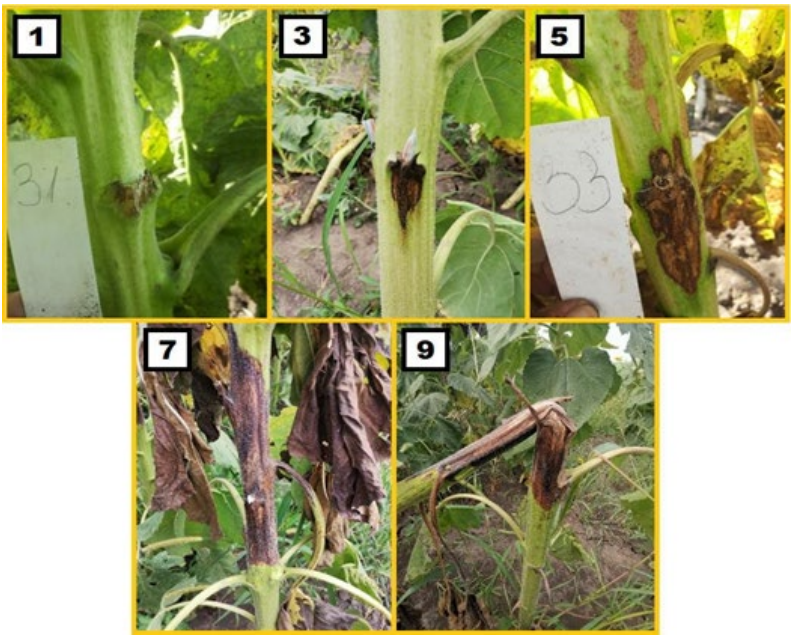

Figure 1. 9-point scale for reporting the reaction to Phomosis helianthi

Inoculation was carried out using the STRAW method (Encheva and Kiryakov, 2002). For this purpose, the petioles of single leaves from the middle tiers of the plant, in the corresponding phenophase, were cut at a distance of 3 cm from the leaf node. A one-sided closed plastic straw (6 x 25 mm) was inserted into the cut, containing an agar disk with mycelium cut from the periphery of a 5-day culture of the isolate on the PSA medium. Five plants of the corresponding genotype were infected with each isolate. The reaction of the samples was recorded 14 days after inoculation on the following 9-point scale: 1-no symptoms, 3-spots on the stem up to 5 cm in size, 5-spots on the stem over 5 cm in size, 7-spot covers the adjacent leaf nodes, 9-stem breakage (Fig. 1). Based on the score, the area under the development

curve (AUDPC) was calculated for each genotype (Simko & Piepho, 2012), as well as the average area under the development curve (AAUDPC) for both genotypes.

3.3. Determination of aggressiveness of *P. helinathi* isolates.

The study included 30 isolates collected from different production areas in Northern and Southern Bulgaria. The aggressiveness of the isolates was determined by infecting the sunflower hybrid Deveda and the variety Favorit under field conditions, on the territory of the DZI - General Toshevo. The genotypes were sown in rows with a length of 2.40 m, inter-row spacing of 0.7 m and intra-row spacing of 0.30 m. The infection was carried out in the BBCH 51 phenophase, using the STRAW method (Encheva and Kiryakov, 2002), with six plants of the respective genotype being infected with each isolate. The reaction of the samples was recorded 14 days after inoculation using the 9-point scale shown in Figure 1. Based on the score, the area under the development curve (AUDPC) for each genotype was calculated (Simko and Piepho, 2012), as well as the average area under the development curve (AAUDPC) for both genotypes.

3.4. Mycelial compatible groups (MCGs).

The study included 31 isolates of *P. helianthi*, collected from four production regions across the country in 2021 (Table 1).

The compatibility of the obtained isolates was established using the method described by Kiryakov and Zhecheva (2019). Four types of culture media were used in the study: PDA, PDA+ 80µl/L with red dye (Christmas red – Sly Commerce Ltd.) for the food industry, PSA and PSA + 80 µl/L. Each of the isolates was combined with the others by placing a 5 mm agar disk, taken from the periphery of a 7-day culture, on pure PDA and PSA and, respectively, PDA and PSA with used dye, at a distance of 3 cm between the isolates in the Petri dish. In parallel, each of the isolates was combined with itself to establish the presence of self-compatibility. The dishes were placed in a thermostat at a temperature of 23 ± 1^0 C. The presence of a compatible/incompatible reaction between the isolates was recorded after 7 and 10 days. Four types of reaction were reported: NC-N – incompatible with necrosis, NC-G-incompatible, the micelles do not approach, with a distance between them, NC-DL-the two lines (black) are spaced apart and C- compatible (Fig. 2).

Table 1. Nomenclature of isolates used by origin

Code of location	Year	Origin	Number of isolates
21-61..	2021	ДЗИ I	4
21-62..	2021	ДЗИ II	8
21-623..	2021	ДЗИ III	2
21-41..	2021	Русе I	5
21-42..	2021	Русе II	1
21-45..	2021	Русе III	2
21-23..	2021	Ямбол I	1
21-24..	2021	Ямбол I	1
21-21..	2021	Ямбол II	2
21-72..	2021	Карнобат	5
Total	-	-	31

The compatibility of the obtained isolates was established using the method described by Kiryakov and Zhecheva (2019). Four types of culture media were used in the study: PDA, PDA+ 80µl/L with red dye (Christmas red – Sly Commerce Ltd.) for the food industry, PSA and PSA + 80 µl/L. Each of the isolates was combined with the others by placing a 5 mm agar disk, taken from the periphery of a 7-day culture, on pure PDA and PSA and, respectively, PDA and PSA with used dye, at a distance of 3 cm between the isolates in the Petri dish. In parallel, each of the isolates was combined with itself to establish the presence of self-compatibility. The dishes were placed in a thermostat at a temperature of 23 ± 1^0 C. The presence of a compatible/incompatible reaction between the isolates was recorded after 7 and 10 days. Four types of reaction were reported: NC-N – incompatible with necrosis, NC-G-incompatible, the micelles do not approach, with a distance between them, NC-DL-the two lines (black) are spaced apart and C- compatible (Fig. 2).

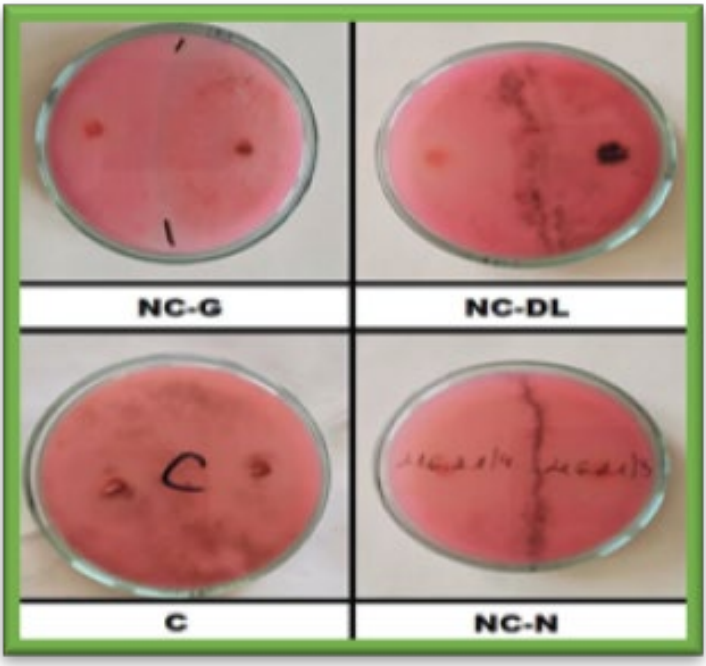

Figure 2. Types of reaction in mycelially compatible groups

1.3. Resistance of sunflower breeding lines and hybrids to *Ph. helianthi*.

To establish the inheritance of resistance to *Ph. helianthi*, 12 sunflower hybrids and their parental lines were studied. The study was conducted in the 2022 and 2023 crop years, under field conditions. The samples were sown in rows with a length of 2.40 m, inter-row spacing of 0.7 m and intra-row spacing of 0.30 m. Two isolates were used for infection – *Ph21-614* and *Ph21-212*. Inoculation was carried out in the BBCH 51 phenophase using the STRAW method (Encheva and Kiryakov, 2002), with each isolate infecting six plants of the respective genotype. The reaction of the samples was recorded 14 days after inoculation using the 9-point scale shown (Fig. 1). Based on the score, the area under the development curve (AUDPC) for each genotype (Simko and Piepho, 2012) was calculated, as well as the average area under the development curve (AAUDPC) for both genotypes. For each genotype, isolate and production year, the heterosis against the better parent (Best Parent Heterosis – BPH), the heterosis against the average parent level (Mid Parent Heterosis – MPH) and the degree of dominance (d/a) were determined, according to the methodology described by Genchev et al. (1975).

1.4. Statistical data processing.

When processing the experimental data, single-factor and multifactor analysis of variance, correlation analysis and cluster analysis were performed. For summarizing, grouping and initial processing of the data, the MS Office Excel LTSC Professional Plus 2021 software product was used. The analysis of variance, the calculation of LSD and the Duncan test were calculated using IBM SPSS Statistics v.19, and the AMMI analysis using the software product AMMISoft v 1.0.

4. RESULTS AND DISCUSSION

4.1. Study of the spread of the pathogen on the territory of the country.

From the plant samples collected during the period 2020-2022 from 10 Districts of Northern and Southern Bulgaria, 190 fungal isolates were isolated (Table 2). The morphological and cultural features of the isolates determined 96 of them as *Ph. helianthi.*

Table 2. Collected and isolated plant samples from the region of Northern and Southern Bulgaria during the period 2020-2022.

Origin	Samples	N. of isolates	Isolates obtained				
			Ph. helianthi	*Ph. macdonaldii*	*Alternaria spp.*	*M. phaseolina*	Други
Silistra	9	18	6	10	3	0	0
Yambol	12	24	16	6	2	0	0
Shumen	13	26	14	4	4	2	0
Ruse	14	28	18	10	0	0	0
St. Zagora	1	2	2	0	0	0	0
Dobrich	25	50	36	10	2	0	2
Plovdiv	4	8	0	4	2	0	2
Burgas	8	16	4	4	4	0	2
Lovech	7	14	0	8	4	0	2
Sliven	2	4	0	4	0	0	0
Total	95	190	96	60	21	2	8

4.2. Establishment of cultural and morphological characteristics of the pathogen in a collection of isolates of different geographical origin.

The cultural features of the studied isolates were established by parallel use of nutrient media - PSA and PDA. The indicators of diametrical growth, colony growth rate and days to pycnidia appearance were monitored.

4.2.1. Diametrical growth and growth rate.

Diametrical growth.

The diametrical growth on the PDA medium, for the 50 isolates studied, varied from 32.8 to 90 mm. The values for the indicator on the PSA medium varied from 40.3 to 90 mm. On average for both media, the colony size ranged from 43 to 98.3 mm, with significant differences being established, both between the isolates and between the values of the indicator for the respective isolate on both media. Table 3 presents the diametral growth of the studied isolates from the respective origins. The isolates from Ruse-2021 had the lowest, average diametral growth on PDA, and the isolates from Silistra-2020 had the highest, and the differences were significant. The average diametral growth on PSA was the lowest in Ruse-2021, and the highest in Silistra-2020. The correlation analysis performed showed a moderate dependence between the two nutrient media for the tested isolates (r=0.425**) and a high dependence between the nutrient media for the individual origins (r=0.837**).

Table 3. Diametral growth of 50 isolates of *Ph. helianthi* on two nutrient media, by origin

Origin	N. of isolates	PDA		PSA		Average	
		D, mm	VC %	D, mm	VC %	D, mm	VC %
Silistra 2020	4	72,4	6,1	83,3	9,3	77,8	10,7
Shumen 2020	6	72,1	4,1	72,7	13,6	72,4	9,9
Radnevo 2020	4	67,1	10,5	73,5	11,2	70,3	11,7
DZI 2020	4	66,1	11	66,8	14,9	66,4	12,9
Yambol 2021	2	55,8	25,5	66,9	37,9	61,3	33,3
Ruse 2021	4	55,3	25,4	53,9	23,1	54,6	23,8
DZI 2021	14	68,8	18,1	71	16,3	69,9	17,1
Karnobat 2021	4	72,1	12,9	76,7	11,3	74,4	12,2
DZI 2022	8	69,2	12,3	74	13,5	71,6	13,3
Average	**50**	**67,8**	**15,9**	**71,4**	**17,8**	**69,6**	**17,1**

LSD0.05: 0.299 for PDA; 1,494 for PSA; 2.113 for average value

Growth rate.

The growth rate of colonies after four days of incubation on the PDA medium varied from 0.148 to 0.98 mm/h, and on the PSA medium from 0.258 to 0.924 mm/h, with significant differences between isolates and mediums. The average growth rate on both mediums varied from 0.427 to 0.951 mm/h.

The growth rate of colonies on both mediums, by origin, is presented in Table 4. The growth rate on the PDA varied from 0.555 (Yambol-2021) to 0.709 (DZI-2021). On the PSA medium, the values of the indicator varied from 0.504 (Ruse-2021) to 0.740 (DZI-2021). A high correlation was found between the rate and diametral growth of isolates in PDA (r=0.782**) and PSA (r=0.785**).

Table 4. Growth rate of 50 isolates of *Ph. helianthi* on two nutrient media, by origin

Origin	Number of isolates	PDA		PSA		Average	
		Vd, mm/h	VC %	Vd, mm/h	VC %	Vd, mm/h	VC %
Silistra 2020	4	0,621	7,2	0,687	15,2	0,654	13
Shumen 2020	6	0,690	6,5	0,727	6,5	0,709	6,9
Radnevo 2020	4	0,571	16,7	0,652	21,8	0,611	20,5
DZI 2020	4	0,566	14,8	0,580	18,5	0,573	16,5
Yambol 2021	2	0,555	25,2	0,641	34,8	0,598	30,6
Ruse 2021	4	0,565	23,5	0,504	9,9	0,535	19,2
DZI 2021	14	0,709	24,6	0,740	24,3	0,724	24,4
Karnobat 2021	4	0,634	4,9	0,738	8,2	0,686	10,3
DZI 2022	8	0,649	16,8	0,707	18,4	0,678	18
Average	50	0,644	20,3	0,686	21,7	0,665	21,2

$LSD_{0.05}$: 0,003 for PDA; 0,014 for PSA; 0,020 for average value

Figure 3 presents the distribution of isolates depending on the average values for diametral growth and growth rate on PDA and PSA media.

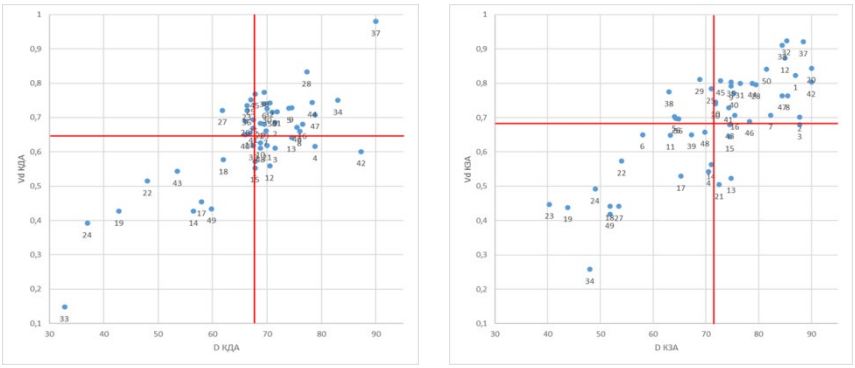

Figure 3. Combination of diametral growth and development rate on PDA (left) and PSA (right)

4.2.2. Pycnidia formation on both media types.

One of the isolates studied did not form pycnidia on PSA medium (Fig. 4). In 14 isolates, pycnidia formation started after 6 days of incubation. The formation of the largest number of pycnidia on this medium was observed during the period 7-8 days. Five isolates did not form pycnidia on PDA medium for the entire incubation period. Pycnidia formation started on the fifth day, with the largest number of isolates forming pycnidia being reported on days 7-8. These results indicate that PSA medium stimulates a higher percentage of pycnidia formation, parallel to a higher rate of colony development.

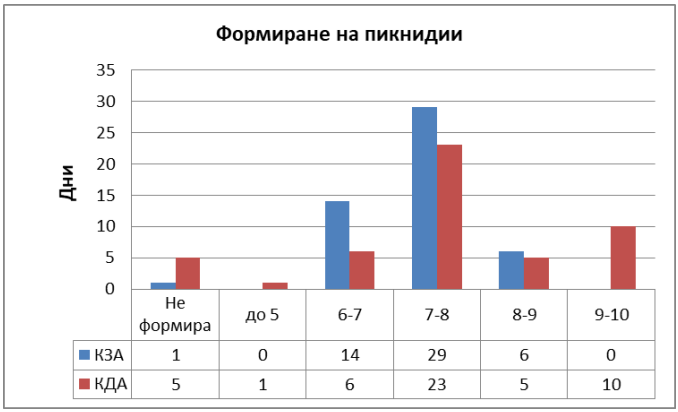

Figure 4. Pycnidia formation on PSA and PDA media (days from the start of incubation)

4.5. Study of genetic diversity in the pathogen – mycelial compatibility.

As a result of the complementary tests, 416 combinations were carried out. The four types of reaction described in the methodology were observed. In 48.1% of the combinations, a fusion of the two colonies without a formed groove (C), which is an indicator of compatibility between the isolates (Fig. 5), was observed. In the NC-DL phenotype, two darkly colored, separated lines were observed, and this phenotype was found in 24.8% of the isolates. A reaction of the NC-N type, in which the phenotype is a narrow, black-colored groove, was found in 19.2% of the samples. The lowest percentage of reactions was of the NC-G type, in which the mycelium did not approach each other - 7.9% of all combinations. The last three phenotypes defined the studied isolates as incompatible.

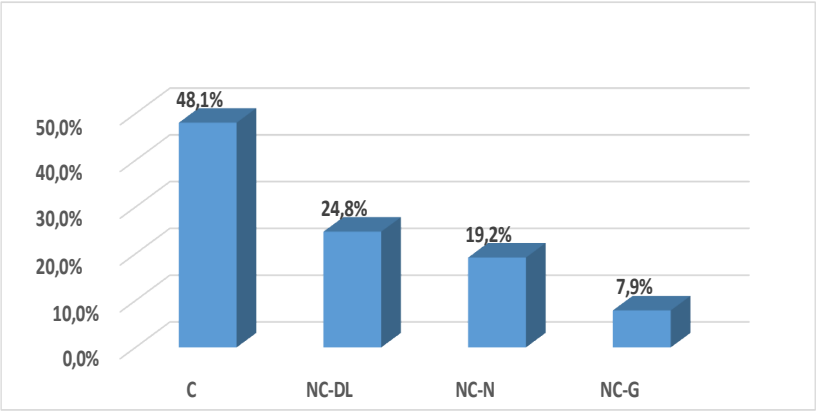

Figure 5. Phenotypic expression of the interaction between 30 isolates in the mycelial compatibility test (%)

Complementary self-compatibility tests showed that two of the isolates (*21-6221* and *21-6222*) were not self-compatible, therefore they were excluded from further tests and assigned to separate MCGs, MCG19 and MCG20, respectively (Table 5). Based on the phenotypic reaction between the remaining isolates, an additional 18 MCGs were formed.

Group MCG1 included 14 isolates and was the largest group out of 20 (Tables 5 and 6). MCG2 included 12 isolates, making it the second largest group. MCG3 included 7 isolates, MCG4 – 5, and MCG5, respectively, 6 isolates. Seven of the groups (MCG7, MCG8, MCG9, MCG10, MCG11, MCG12, MCG13) included 3 isolates each, and one of them (MCG6) had 4 isolates. Five of the groups (MCG 14, MCG15, MCG16, MCG 17, MCG18) were represented by 2 isolates each, and two of them (MCG19 and MCG20) had 1 isolate each and were defined as separate independent groups due to their self-incompatibility.

Table 5. Mycelial compatible groups (MCGs) in the analysis of 30 isolates of *P. helianth*

Group	Isolate	N. of isolates
MCG1	21-412; 21-413; 21-414; 21-415; 21-451; 21-45221-6211; 21-6212;21-6213; 21-6223; 21-721; 21-722; 21-723; 21-724	14
MCG2	21-412; 21-413; 21-414; 21-415; 21-451; 21-452; 21-6214; 21-6224; 21-6223; 21-721; 21-722; 21-723;	12
MCG3	21-414; 21-451; 21-452; 21-611; 21-612; 21-613; 21-614;	7
MCG4	21-412; 21-451; 21-452; 21-6231; 21-6232;	5
MCG5	21-451 21-452 21-241; 21-231; 21-211; 21-212	6
MCG6	21-241; 21-411; 21-423; 21-723;;	4
MCG7	21-411; 21-6212; 21-6214;	3
MCG8	21-411; 21-241; 21-721;	3
MCG9	21-241; 21-411; 21-722;	3
MCG10	21-241; 21-41; 21-724;	3
MCG11	21-411; 21-451; 21-452	3
MCG12	21-212; 21-414; 21-423;	3
MCG13	21-211; 21-423; 21-452;	3
MCG14	21-411; 21-613;	2
MCG15	21-423; 21-612;	2
MCG16	21-414; 21-6232;	2
MCG17	21-241; 21-413;	2
MCG18	21-411; 21-6224;	2
MCG19	21-6221	1
MCG20	21-6222	1

The region of Ruse is characterized by the greatest genetic diversity, in which the eight studied isolates are assigned to 18 out of a total of 20 formed MCGs (Fig. 6). Isolates from the DZI region are part of 9, and from Yambol in 7 of the 20 identified MCGs. The number of established MCGs is the smallest in the Karnobat region. These results show that some groups can be found in more than one region. For example, MCG1 and MCG2 are found both in the DZI region and in the Karnobat and Ruse regions. Group MCG10 is also observed in three of the studied regions - Karnobat, Ruse and Yambol. Some of the MCGs can be found in only one region. Such are groups MCG11, MCG12, which are found only in the Ruse region.

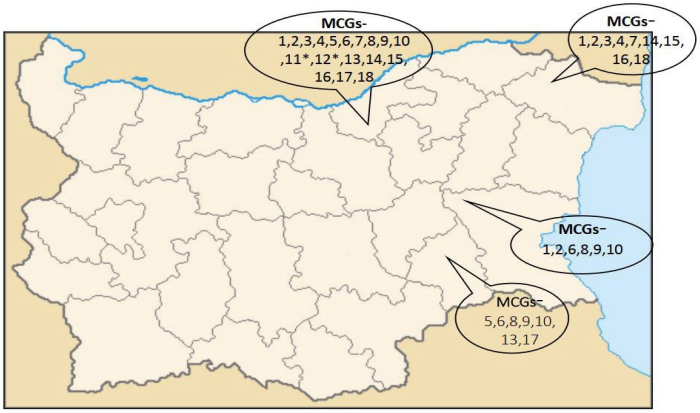

Figure 6. Distribution of MCG by region of origin

Table 6. Distribution of isolates by region of origin

MCGs	Origin				Total number of isolates	MCGs	Origin				Total number of isolates
	DZI	Yambol	Ruse	Karnobat			DZI	Yambol	Ruse	Karnobat	
MCBG1	5	0	6	4	15	MCBG13	0	1	2	0	3
MCBG2	3	0	6	3	12	MCBG14	0	1	2	0	3
MCBG3	4	0	3	0	7	MCBG15	1	0	1	0	2
MCBG4	2	0	3	0	5	MCBG16	1	0	1	0	2
MCBG5	0	4	1	0	5	MCBG17	1	0	1	0	2
MCBG6	0	4	1	0	5	MCBG18	0	1	1	0	2
MCBG7	0	1	2	1	4	MCBG19	1	0	1	0	2
MCBG8	2	0	1	0	3	MCBG20	1	0	0	0	1
MCBG9	0	1	1	1	3	MCBG 21	1	0	0	0	1
MCBG10	0	1	1	1	3	**Shanon's H index**	**2,743**	**2**	**5,434**	**1,6**	**4,958**
MCBG11	0	1	1	1	3	**Simpson Index**	**0,868**	**1**	**0,916**	**0,76**	**0,92**
MCBG12	0	0	3	0	3						

By examining the genetic diversity of 30 isolates of *P. helianthi* based on their grouping into MCGs by origin, the Shannon index and Simpson index were calculated (Table 6). Relatively high diversity was recorded in isolates originating from Ruse (H = 5.434, S = 0.916) and moderate in isolates originating from DZI (H = 2.743, S = 0.868) and Yambol (H = 2.426, S = 0.827). The lowest diversity was found in isolates originating from Karnobat (H = 1.602, S = 0.760). The low values in isolates originating from Karnobat and Yambol are probably due to the small number of isolates that are included in them.

4.6. Determining the influence of the host phenophase on the aggressiveness of the isolates.

The ANOVA for the influence of the phenophase of infection on the response of the hybrid Deveda and the variety Favorit is presented in Table 7. The analysis shows that the most significant influence is exerted by the factors year (29.3% of the total variation) and phenophase (4.95% of the total variation).

Table 7. Analysis of variance on the response of two genotypes to three isolates of *Ph. helianthi* depending on the phanophase of inoculation

Factor	Sum of squares	df	Mean squares	F	Sig.
Isolate (I)	559,144	2	279,572	1,656	0,192
Phenophase (P)	10110,878	2	5055,439	29,939	0,000
Genotype (G)	6414,168	1	6414,168	37,985	0,000
Year (Y)	59750,668	1	59750,668	353,847	0,000
I * P	3073,389	4	768,347	4,550	0,001
I * G	526,478	2	263,239	1,559	0,211
I * Y	108,344	2	54,172	0,321	0,726
P * G	1333,344	2	666,672	3,948	0,020
P * Y	4335,411	2	2167,706	12,837	0,000
G * Y	1314,901	1	1314,901	7,787	0,005
I * P * G	404,522	4	101,131	0,599	0,664
I * P * Y	206,889	4	51,722	0,306	0,874
I * G * Y	43,011	2	21,506	0,127	0,880
P * G * Y	340,278	2	170,139	1,008	0,366
I * P * G * Y	76,222	4	19,056	0,113	0,978
Error	115500,350	684	168,860		
Total	204097,999	719			

The response of the studied genotypes to three isolates of the pathogen, inoculated at different phenophases of their development, is presented in Table 8.

Table 8. Influence of phenophase on the aggressiveness of *Ph. helianthi* isolates in the 2022 - 2023 marketing year.

Isolate	2022											
	Favorit				Deveda				Average			
	P1*	P2	P3	AV	P1	P2	P3	AV	P1	P2	P3	AV
Ph21-614	29,8	28,7	18,2	25,6	42,0	44,1	25,2	37,1	35,9	36,4	21,7	31,3
Ph21- 6212	35,7	26,6	22,4	28,2	50,1	35,0	23,8	36,3	42,9	30,8	23,1	32,3
Ph21-423	37,1	27,0	29,1	31,0	43,8	39,2	29,4	37,5	40,4	33,1	29,2	34,2
Average	34,2	27,4	23,2	28,3	45,3	39,4	26,1	36,9	39,7	33,4	24,7	32,6
Isolate	2023											
	Favorit				Deveda				Average			
	P1	P2	P3	AV	P1	P2	P3	AV	P1	P2	P3	AV
Ph21-614	10,5	13,3	9,1	11,0	15,4	20,3	12,6	16,1	13,0	16,8	10,9	13,5
Ph21- 6212	16,1	13,3	11,2	13,5	20,3	15,4	11,9	15,9	18,2	14,4	11,6	14,7
Ph21-423	14,0	12,6	14,7	13,8	16,1	17,5	14,7	16,1	15,1	15,1	14,7	14,9
Average	13,5	13,1	11,7	12,8	17,3	17,7	13,1	16,0	15,4	15,4	12,4	14,4
Isolate	Средно за 2022-2023											
	Favorit				Deveda				Average			
	P1	P2	P3	AV	P1	P2	P3	AV	P1	P2	P3	AV
Ph21-614	20,1	21,0	13,7	18,3	28,7	32,2	18,9	26,6	24,4	26,6	16,3	22,4
Ph21- 6212	25,9	20,0	16,8	20,9	35,2	25,2	17,9	26,1	30,5	22,6	17,3	23,5
Ph21-423	25,6	19,8	21,9	22,4	29,9	28,4	22,1	26,8	27,7	24,1	22,0	24,6
Average	23,9	20,2	17,4	20,5	31,3	28,6	19,6	26,5	27,6	24,4	18,5	23,5
LSD I	*2,33*	***LSD Y***	*1,90*		***LSD I*Y***	*3,29*		***LSD G*Y***	*2,68*		***LSD I*G*Y***	*4,65*
LSD P	*2,33*	***LSD I*P***	*4,03*		***LSD P*G***	*3,29*		***LSD I*P*G***	*5,70*		***LSD P*G*Y***	*4,65*
LSD G	*1,90*	***LSD I*G***	*3,29*		***LSD P*Y***	*3,29*		***LSD I*P*Y***	*5,70*		***LSD I*P*G*Y***	*8,05*

*P1 -BBCH-39 , P2-BBCH-51/53 и P3 -BBCH-61

The results presented in Table 8 show a significant variation in the response of the Deveda hybrid and the Favorit variety, both in terms of isolates and in terms of the inoculation phenophase. Differences in the response of the genotypes related to the year are also observed. In 2022, the average AUDPC values for the Favorit variety are highest in Phenophase 1 (BBCH-39), with the differences compared to the other phenophases being significant (LSD0.05=4.65). In the second year, no differences were observed in the response to the three isolates between the individual phenophases. The average AUDPC values for the Deveda hybrid in 2022 are highest

in Phenophase 1, with the differences compared to the other phenophases being significant. In 2023 no significant differences were observed between the first two phenophases, but the values of the AAUDPC parameter between Phenophase 2 (P2-BBCH-51/53) and Phenophase 3 were significant.

The results for the individual phenophases by year and genotype as a factor show that the moment of infection has a decisive importance not only for the aggressiveness of specific isolates, but also for the formation of trends between them. The data from the AMMI ANOVA indicate that there is a significant interaction between the phenophases and the isolates used. At the same time, the results for the individual phenophases are not identical, but vary in different ways, depending on the isolate used. This shows that each of the individual phenophases is characterized by different predictability. This is shown by combining the AAUDPC values for each specific phenophase and the IPC1 values for the same phenophase. The results obtained show that the isolates exhibit their maximum aggressiveness when infected in the BBCH-39 phase "before budding" (Fig. 7).

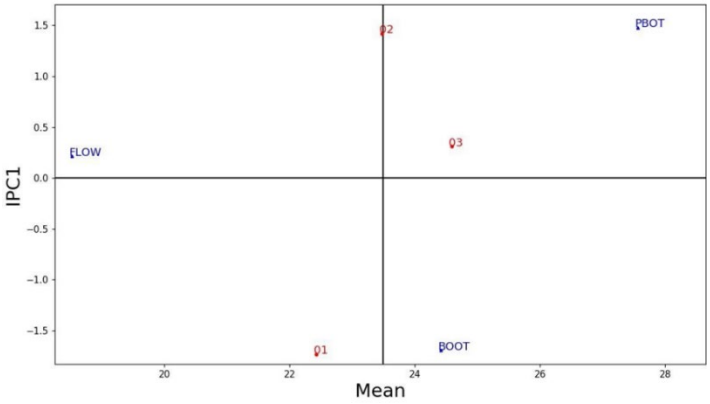

Figure 7. AMMI1 biplot by phenophases for the studied hybrids and years
(PBOT – before budding, BOOT – budding, FLOW – flowering; 01 – isolate Ph21-614, 02 – isolate Ph21-6212, 03 – isolate Ph21-423)

4.7. Study of aggressive diversity in a collection of isolates of different geographical origins.

The ANOVA performed shows a proven influence of the studied factors, both individually and in combination. The data on the aggressiveness of the 32 isolates are presented in Table 9.

Table 9. Aggressiveness of 32 isolates of *Ph. helianthi* expressed by the AAUDCP indicator

№	Isolate	Favorit			Deveda			Average		
		2022	2023	Average	2022	2023	Average	2022	2023	Average
1	21-451	28,0	14,0	21,0	39,7	18,7	29,2	33,8	16,3	25,1
2	21-452	7,0	18,7	12,8	7,0	11,7	9,3	7,0	15,2	11,1
3	21-611	7,0	7,0	7,0	14,0	9,3	11,7	10,5	8,2	9,3
4	21-612	32,7	16,3	24,5	63,0	18,7	40,8	47,8	17,5	32,7
5	21-613	7,0	21,0	14,0	18,7	16,3	17,5	12,8	18,7	15,8
6	21-614	46,7	7,0	26,8	49,0	21,0	35,0	47,8	14,0	30,9
7	21-6211	51,3	23,3	37,3	42,0	11,7	26,8	46,7	17,5	32,1
8	21-6212	39,7	30,3	35,0	7,0	16,3	11,7	23,3	23,3	23,3
9	21-6213	21,0	18,7	19,8	16,3	16,3	16,3	18,7	17,5	18,1
10	21-6214	39,7	16,3	28,0	32,7	18,7	25,7	36,2	17,5	26,8
11	21-6221	21,0	21,0	21,0	46,7	21,0	33,8	33,8	21,0	27,4
12	21-6222	25,7	7,0	16,3	32,7	16,3	24,5	29,2	11,7	20,4
13	21-6223	28,0	16,3	22,2	39,7	7,0	23,3	33,8	11,7	22,8
14	21-6224	7,0	7,0	7,0	23,3	7,0	15,2	15,2	7,0	11,1
15	21-6231	25,7	7,0	16,3	11,7	7,0	9,3	18,7	7,0	12,8
16	21-6232	7,0	25,7	16,3	7,0	7,0	7,0	7,0	16,3	11,7
17	21-721	18,7	7,0	12,8	21,0	9,3	15,2	19,8	8,2	14,0
18	21-722	25,7	21,0	23,3	32,7	32,7	32,7	29,2	26,8	28,0
19	21-723	44,3	16,3	30,3	11,7	11,7	11,7	28,0	14,0	21,0
20	21-724	11,7	9,3	10,5	11,7	11,7	11,7	11,7	10,5	11,1
21	21-241	9,3	7,0	8,2	11,7	21,0	16,3	10,5	14,0	12,3
22	21-231	25,7	11,7	18,7	28,0	21,0	24,5	26,8	16,3	21,6
23	21-411	32,7	7,0	19,8	49,0	11,7	30,3	40,8	9,3	25,1
24	21-412	7,0	9,3	8,2	9,3	21,0	15,2	8,2	15,2	11,7
25	21-413	30,3	7,0	18,7	51,3	21,0	36,2	40,8	14,0	27,4
26	21-414	14,0	9,3	11,7	7,0	14,0	10,5	10,5	11,7	11,1
27	21-415	21,0	9,3	15,2	7,0	16,3	11,7	14,0	12,8	13,4
28	21-421	23,3	7,0	15,2	9,3	23,3	16,3	16,3	15,2	15,8
29	21-422	39,7	7,0	23,3	39,7	18,7	29,2	39,7	12,8	26,3
30	21-423	23,3	7,0	15,2	44,3	28,0	36,2	33,8	17,5	25,7
31	21-211	32,7	7,0	19,8	21,0	21,0	21,0	26,8	14,0	20,4
32	21-212	35,0	25,7	30,3	11,7	28,0	19,8	23,3	26,8	25,1
Average		***24,6***	***13,3***	***19,0***	***25,5***	***16,7***	***21,1***	***25,1***	***15,0***	***20,0***

$LSD_{0,05}$: Isolate (I)- 5,646; Genotype (G)-1,412; Year (Y)-1,412; I x G-7,985; I x G-7,985; G x Y-1,996; IxGxY - 11,292

On average for the two years of study, the AUDPC values for Favorit ranged from 7.0 (*Ph21-6224*) to 37.3 (*Ph21-6211*). AUDPC for Deveda, for the same period,

were in the range of 7.0 (*Ph21-6232*) – 40.8 (P*h21-612*). The lowest aggressiveness of the two genotypes was characterized by isolate *Ph21-611* (AUDPC = 9.3). No significant differences were found between this and 14 of the isolates included in the test (LSD0.05=11.292). The highest aggressiveness, on average for the two years and the studied sunflower genotypes, was characterized by isolate *Ph21-6211* (AUDPC = 32.1), with no differences observed compared to the 15 isolates.

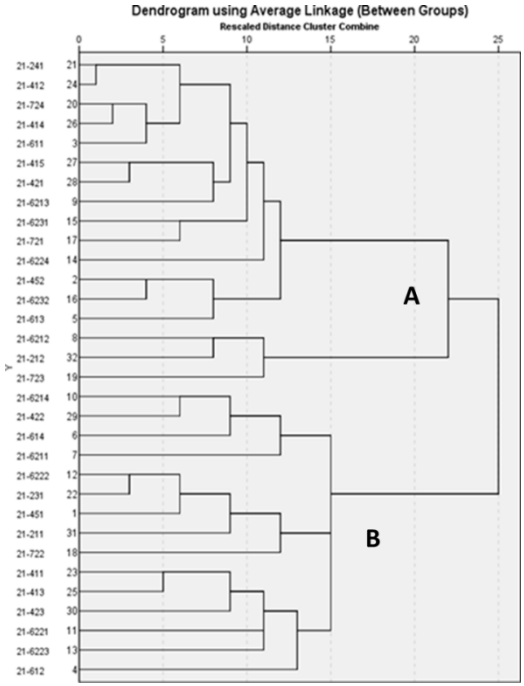

Figure 8. Dendrogram from cluster analysis using the AAUDPC parameter for 30 isolates across two genotypes and two harvest years

Based on the AUDPC values, the studied isolates are grouped into two main clusters. Cluster A includes isolates with low and cluster B with high aggressiveness towards both host genotypes.

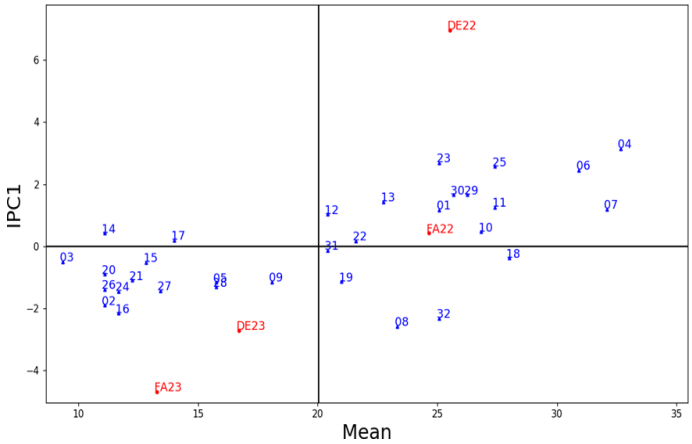

Figure 9. AMMI1-biplot by the AUDPC indicator for the studied isolates. F – Favorit, D – Deveda

The AMMI1 analysis conducted grouped the isolates according to their aggressiveness towards the two genotypes in the respective year of study (Fig. 9). The grouping of the isolates by both methods shows the lack of relationship between the aggressiveness of the isolates and their geographical origin. For example, isolates Ph21-612 and Ph21-611 were isolated from the same location (DZI) in 2021, but are grouped into different groups according to aggressiveness. The analysis of the obtained isolates shows that the conditions during the year of infection have an impact on the aggressiveness of the isolates. This requires the determination of aggressiveness to be carried out in two or more years.

4.8. Inheritance of the response to *Ph. helianthi* in 12 sunflower hybrids and their parental forms.

Results for the studied hybrids and their parental forms in 2022 and 2023 are presented in Table 10.

Table 10. Area under the progressive curve of parental forms and hybrids to two isolates of *Ph. helianthi*

Genotype		2022			2023			Средно		
		Ph21-	Ph21-	AV	Ph21-	Ph21-	AV	Ph21-	Ph21-	AV
217A	M	35,00	30,33	32,67	21,00	25,67	23,33	28,00	28,00	28,00
KM 852 R	F1	35,00	35,00	35,00	49,00	7,00	28,00	42,00	21,00	31,50
217A × KM 825 R	H1	7,00	21,00	14,00	7,00	7,00	7,00	7,00	14,00	10,50
3607 A	M2	7,00	7,00	7,00	7,00	7,00	7,00	7,00	7,00	7,00
KM 172	F2	21,00	16,33	18,67	11,67	7,00	9,33	16,33	11,67	14,00
3607 A × KM172	H2	35,00	11,67	23,33	30,33	7,00	18,67	32,67	9,33	21,00
376A-SU	M3	21,00	21,00	21,00	11,67	11,67	11,67	16,33	16,33	16,33
KZ 23R/8	F3	63,00	44,33	53,67	53,67	25,67	39,67	58,33	35,00	46,67
KZ 23R/8 × 376A-SV	H3	21,00	30,33	25,67	16,33	7,00	11,67	18,67	18,67	18,67
692-1/19A	M4	25,67	39,67	32,67	25,67	7,00	16,33	25,67	23,33	24,50
1065-1/17 R	F4	39,67	16,33	28,00	35,00	11,67	23,33	37,33	14,00	25,67
692-1/19A × 1065-1/17R	H4	21,00	16,33	18,67	16,33	7,00	11,67	18,67	11,67	15,17
1111A (226-2/mA)	M5	58,33	21,00	39,67	7,00	7,00	7,00	32,67	14,00	23,33
KZ 23 R/4	F5	44,33	44,33	44,33	39,67	11,67	25,67	42,00	28,00	35,00
1111A × KZ 23R/4	H5	35,00	16,33	25,67	7,00	7,00	7,00	21,00	11,67	16,33
696-1/19A	M6	58,33	39,67	49,00	58,33	53,67	56,00	58,33	46,67	52,50
958-3/19R/7n	F6	21,00	30,33	25,67	35,00	11,67	23,33	28,00	21,00	24,50
696-1/19A × 958-3/19R/7	H6	39,67	30,33	35,00	11,67	7,00	9,33	25,67	18,67	22,17
664-1/19A	M7	35,00	7,00	21,00	49,00	21,00	35,00	42,00	14,00	28,00
1060-2/19R	F7	39,67	11,67	25,67	49,00	35,00	42,00	44,33	23,33	33,83
664-1/19A × 1060-2/19 R	H7	7,00	16,33	11,67	44,33	16,33	30,33	25,67	16,33	21,00
656-2/19A	M8	30,33	35,00	32,67	49,00	16,33	32,67	39,67	25,67	32,67
1101-2/19R	F8	11,67	25,67	18,67	21,00	11,67	16,33	16,33	18,67	17,50
656-2/19A × 1101-2/19 R	H8	39,67	21,00	30,33	16,33	7,00	11,67	28,00	14,00	21,00
674-2/19A	M9	7,00	30,33	18,67	63,00	63,00	63,00	35,00	46,67	40,83
966-1/19R/2	F9	7,00	16,33	11,67	21,00	11,67	16,33	14,00	14,00	14,00
674-2/19A × 966-1/19	H9	21,00	16,33	18,67	25,67	25,67	25,67	23,33	21,00	22,17
1111A (226-2/21A)	M10	11,67	16,33	14,00	63,00	16,33	39,67	37,33	16,33	26,83
1065-1/17 R	F10	25,67	30,33	28,00	25,67	7,00	16,33	25,67	18,67	22,17
1111 A × 1065- 1/17 R	H10	39,67	16,33	28,00	16,33	7,00	11,67	28,00	11,67	19,83
1252-2/19A	M11	7,00	16,33	11,67	11,67	7,00	9,33	9,33	11,67	10,50
NAS -1R/12	F11	7,00	11,67	9,33	21,00	7,00	14,00	14,00	9,33	11,67
1252-2/19 A × NAS-	H11	16,33	21,00	18,67	11,67	7,00	9,33	14,00	14,00	14,00
1379A	M12	7,00	39,67	23,33	21,00	7,00	14,00	14,00	23,33	18,67
SVD1R	F12	30,33	21,00	25,67	21,00	7,00	14,00	25,67	14,00	19,83
1379A × SVD1R	H12	7,00	21,00	14,00	21,00	7,00	14,00	14,00	14,00	14,00
Average		**26,06**	**23,46**	**24,76**	**27,61**	**14,13**	**20,87**	**26,83**	**18,80**	**22,81**

In 2022, a trend towards a lower reaction compared to the average value for all genotypes was observed in most of the studied hybrids (Table 10). The lowest AUDPC value of all samples was observed in the hybrid combination H7 (664-1/19A ×1060-2/19). Low values were also recorded in the hybrids H1, H12, H4, H9 and H11. The highest aggressiveness, on average for both isolates, was observed in the hybrid H6, H8 and H10. In H1 and H5, differences in reaction compared to the parental forms were observed. Differences compared to the reaction of the maternal forms were recorded in the hybrids H2, H4, H5, H6 and H10. Of these, hybrids H2 and H10 have higher values than the maternal form, and the other three have lower values. In hybrids H3 and H7, values lower than the paternal form are observed.

Examining the reaction of the hybrids and their parental forms in 2023, it was found that the average reaction of all genotypes (AUDPC=20.87) was lower than that of the previous year (AUDPC=24.76) (Table 10). However, this trend is not generally valid for all genotypes, as for some the reaction in 2023 was higher, and for some no difference was observed. The lowest values are hybrids H1, H5, H6 and H11. Also with low AUDPC values are H3, H4, H8 and H10. Only hybrid H7 is characterized by values higher than the average – 30.33.

Regarding the average results of the two years (AAUDPC), results are observed that are more inclined to the results obtained in 2023 (Table 10). With the lowest values are hybrids H1, H11 and H12. Regarding the two tested isolates Ph21-614 and Ph21-211, on average for the tested parental lines and hybrids, Ph21-614 is significantly more aggressive. This trend is maintained for parental lines M2, F2, N3, F3, F4, M6, F6, M7, F7 and hybrids H2, H4, H6 and H8, with the results being similar for both crop years. For all other genotypes, the two isolates show radically different results in the two harvest years.

In the study, the heterosis of the individual accessions was investigated in relation to the genetics of inheritance in *Ph. helianthi* (Table 11). In the 2022 crop year, a high heterosis, both in relation to the better parent and to the average parental level, was observed in hybrids H1, H4, H5, H7 and H12. In hybrids H1 and H5, the tendency for heterosis to be high was observed for both isolates used. Very strong negative heterosis (the hybrid was more susceptible than both parental forms) was observed for hybrids H2, H8, H9, H10 and H11. In all, except H8 and H10, this tendency was observed for both isolates.

Table 11. Heterosis and degree of dominance in the reaction of the parental forms and the hybrid to Ph. helianthi

Hybrid	Parameter	2022			2023			Average		
		Ph21-614	Ph21-211	Average	Ph21-614	Ph21-211	Average	Ph21-614	Ph21-211	Average
217A × KM 825 R	*	80,0	30,8	57,1	66,7	0,0	70,0	75,0	33,3	62,5
	**	80,0	35,7	58,6	80,0	57,1	72,7	80,0	42,9	64,7
	d/a	-	-5,0	-17,0	-2,0	1,0	-8,0	-4,0	3,0	-11,0
3607 A × KM172	*	-400,0	-66,7	-233,3	-333,3	0,0	-166,7	-366,7	-33,3	-200,0
	**	-150,0	0,0	-81,8	-225,0	0,0	-128,6	-180,0	0,0	-100,0
	d/a	3,0	0,0	1,8	9,0	-	9,0	4,5	0,0	3,0
KZ 23R/8 × 376A-SV	*	0,0	-44,4	-22,2	-40,0	40,0	0,0	-14,3	-14,3	-14,3
	**	50,0	7,1	31,3	50,0	62,5	54,5	50,0	27,3	40,7
	d/a	-1,0	-0,2	-0,7	-0,8	-1,7	-1,0	-0,9	-0,8	-0,8
692-1/19A × 1065-1/17R	*	18,2	0,0	33,3	36,4	0,0	28,6	27,3	16,7	38,1
	**	35,7	41,7	38,5	46,2	25,0	41,2	40,7	37,5	39,5
	d/a	-1,7	1,0	5,0	-3,0	-1,0	-2,3	-2,2	1,5	-17,0
1111A × KZ 23R/4	*	21,1	22,2	35,3	0,0	0,0	0,0	35,7	16,7	30,0
	**	31,8	50,0	38,9	70,0	25,0	57,1	43,8	44,4	44,0
	d/a	2,3	-1,4	-7,0	-1,0	-1,0	-1,0	-3,5	-1,3	-2,2
696-1/19A × 958-3/19R/7	*	-88,9	0,0	-36,4	66,7	40,0	60,0	8,3	11,1	9,5
	**	0,0	13,3	6,3	75,0	78,6	76,5	40,5	44,8	42,4
	d/a	0,0	1,0	0,2	3,0	1,2	1,9	1,2	1,2	1,2
664-1/19A × 1060-2/19R	*	80,0	-133,3	44,4	9,5	22,2	13,3	38,9	-16,7	25,0
	**	81,3	-75,0	50,0	9,5	41,7	21,2	40,5	12,5	32,1
	d/a	-13,0	3,0	-5,0	-	-1,7	-2,3	-15,0	-0,5	-3,4
656-2/19A × 1101-2/19R	*	-240,0	18,2	-62,5	22,2	40,0	28,6	-71,4	25,0	-20,0
	*	-88,9	30,8	-18,2	53,3	50,0	52,4	0,0	36,8	16,3
	d/a	-2,0	2,0	-0,7	1,3	3,0	1,6	0,0	2,3	0,5
674-2/19A × 966-1/19R/2	*	-200,0	0,0	-60,0	-22,2	-120,0	-57,1	-66,7	-50,0	-58,3
	**	-200,0	30,0	-23,1	38,9	31,3	35,3	4,8	30,8	19,1
	d/a	-	1,0	-1,0	0,8	0,5	0,6	0,1	0,6	0,4
1111 A × 1065-1/17R	*	-240,0	0,0	-100,0	36,4	0,0	28,6	-9,1	28,6	10,5
	**	-112,5	30,0	-33,3	63,2	40,0	58,3	11,1	33,3	19,0
	d/a	3,0	-1,0	1,0	1,5	1,0	1,4	0,6	-5,0	2,0
1252-2/19A × NAS-1R/12	*	-133,3	-80,0	-100,0	0,0	0,0	0,0	-50,0	-50,0	-33,3
	**	-133,3	-50,0	-77,8	28,6	0,0	20,0	-20,0	-33,3	-26,3
	d/a	-	-3,0	-7,0	-1,0	-	-1,0	1,0	-3,0	5,0
1379A × SVD1R	*	0,0	0,0	40,0	0,0	0,0	0,0	0,0	0,0	25,0
	**	62,5	30,8	42,9	0,0	0,0	0,0	29,4	25,0	27,3
	d/a	-1,0	1,0	-9,0	-	-	-	-1,0	1,0	-9,0

* Heterosis to the better parent better paternal parent,** Heterosis to the average parental level, d/a – degree of dominance.

In the 2023 crop year, a high heterosis, both in relation to the better parent and to the average parental level, was observed in hybrids H1, H4, H6, H7, H8 and H10 (Table 11). The trend was also observed in both isolates used, in hybrids H6, H7 and H8. Very high negative heterosis was found only in one of the hybrids – H2, as in isolate Ph21-614, the heterosis was extremely high, and in isolate Ph21-212 - there was no such effect.

On average for the two crop years, a heterosis was observed in hybrids H1, H4, H5, H6, H7, H10 and H12 (Table 11). In hybrids H1, H4 and H6, the heterosis was high in relation to both isolates, both in relation to the better parent and to the average parental level. This trend shows that these hybrids have increased resistance to the Gray Spot pathogen compared to both parental forms. A negative heterosis effect is observed in hybrids H2 and H11. In hybrids H1, H3, H4, H5, H7 and H12, it is assumed that the high resistance of the hybrid is inherited overdominantly (in H3 it is additive) from the maternal form. This is evidenced both by the AUDPC values for the parental forms and the hybrid, and by the values of the degree of dominance. In hybrid H6, a high heterosis effect is observed compared to the paternal form and a reaction of overdominance to the same parent. This is valid for both tested isolates and in both years of testing. Similar results show that in this hybrid, the resistant reaction is inherited from the paternal form. Since in F6 (958-3/19R/7n) the wild species *Helianthus petiolaris* is involved, the resistance to *Ph. helianthi* is directly related to the genetic material from the wild species. In all other hybrids, the results show that resistance to the pathogen cannot be associated with the participation of the wild species in the parental forms.

5. CONCLUSIONS

1. A total of 95 plant samples were collected during the study period. Of these, 190 isolates were isolated, 50.5% of which were attributed to *Phomopsis helinathi*, and the rest to *Phoma macdonaldii*, the genus *Alternaria* and *Macrophomina phaseolina*.
2. On the PDA medium, the diametral growth (D) varied from 32.8 mm to 90 mm, and the development rate (Vd) ranged from 0.148 mm/h to 0.980 mm/h. Grouping the isolates by origin and years shows that the isolates with the highest D values were those with origins in Silistra 2020, Shumen 2021 and Karnobat 2021, and the isolates with the lowest values in Yambol 2021 and Ruse 2021. Regarding the development rate (Vd), the highest rate was found in origins in DZI 2021 and Shumen 2020, and the lowest in Yambol 2021.
3. On PSA medium, diametric growth ranged from 40.3 mm to 90.0 mm, and the growth rate from 0.258 mm/h to 0.924 mm/h. The highest D values were recorded in the Silistra 2020, Karnobat 2021 and DZI 2022 groups, and the lowest in DZI 2020, Yambol 2021 and Ruse 2021. The isolates originating from DZI 2021 and Karnobat 2021 had a high growth rate (Vd), and the lowest in Ruse 2021.
4. The established positive correlation between the average diametral growth and the average development rate in PSA and PDA (r=0.738) indicates the presence of isolates that react differently in the two nutrient media. The average values for the two media show trends for higher diametral growth and development rate in the PSA media (D=71.4; Vd=0.686), compared to PDA (D=67.8; Vd=0.644). No relationship was established between the studied parameters and the geographical origin of the isolates.
5. The period of pycnidia formation on PDA medium varies from the 5th to the 10th day, with values between the 7th and 9th day prevailing. The period of pycnidia formation on PSA varies between the 6th and 9th day.
6. The 30 isolates studied are grouped into 20 mycelial compatibility groups (MCGs), with two of the groups including only one isolate each, which are defined as incompatible. Isolates from different spatially distant regions fall into the same MCGs. The groups MCG1 and MCG2 (Ruse, DZI and Karnobat) are found with the highest frequency, and the groups MCG11 and MCG12 (Ruse) with the lowest frequency. The analysis conducted

based on mycelial compatibility between the isolates shows low genetic diversity in the populations of the pathogen (Shanon's H index – 4.958; Simpson Index -0.920).

7. The ANOVA conducted for the influence of the phenophase of development on the reaction of sunflower genotypes to two isolates of the pathogen shows a reliable influence of the factor year (29.3% of the total variation) and the factor phenophase (4.95% of the total variation). The obtained results determine the phenophase BBCH 51-53 ("before budding") as the most suitable for assessing the resistance of the breeding materials. Regardless of the phenophase of inoculation, isolate Ph21-423 showed higher aggressiveness than Ph21-614.
8. The average AAUDPC values for all tested isolates and years ranged from 9.3 to 32.7. The highest values for this parameter were recorded for isolates *Ph21-612*, *Ph21-614* and *Ph21-6211*. The analysis of the results shows that the origin of the isolates is not decisive for the aggressive reaction of the pathogen.
9. In hybrid combinations 217A x KM852 R, 692-1/19A x 1065-1/17R and 696-1/19A x 958-3/19R/7n was found a high heterosis in relation to isolates Ph21-614 and Ph21-212, both in relation to the better parent and in relation to the average parental level. A negative heterosis was observed in hybrid combinations 3607A x KM172 and 1252-2/19a x NAS-1R/12. Isolate *Ph21-614* was characterized by the highest aggressiveness towards the 12 studied hybrid combinations and their parents.
10. The high resistance in hybrid combinations 217A x KM852 R, 376A-SU x KZ23R/8, 692-1/19A x 1065-1/17R, 1111A x KZ23R/4, 664-1/19A x 1060-2/19R and 1379A x SUD1R is due to overdominant inheritance (in 376A-SU x KZ23R/8 it is additive) from the maternal form. In the hybrid combination 696-1/19A x 958-3/19R/7n, a high heterosis is observed relative to the paternal form and a reaction of overdominance to the same parent. It has been found that resistance to *Phomopsis helianthi* is directly linked to the genetic material from the wild species *Helianthus petiolaris*, which was involved in the creation of the paternal form of the hybrid.

6. CONTRIBUTIONS

Scientific contributions:

1. This is the first detailed study of *Phomopsis helianthi* in sunflower in Bulgaria, including distribution, cultural, morphological and pathogenic characteristics, in which new research methods were used;
2. For the first time in Bulgaria, the genetic diversity of *Phomopsis helianthi* has been studied, by applying the Mycelial Compatibility Groups (MCGs) method. The analysis of the scientific literature over the past 30 years shows that this method has been used for the first time in the world for *Phomopsis helianthi*;
3. For the first time in Bulgaria, the inheritance of the pathogen response (*Phomopsis helianthi*) in parental forms of sunflower and their hybrids has been studied;

Contributions of a scientific and applied nature:

1. Significant diversity in the aggressiveness of isolates was established, both within a field and between individual fields. The accumulated information allows for the use of the most aggressive isolates in the breeding process.
2. The 5-point scale for assessing damage of Phomopsis helianthi was transformed to 9-point scale system, in order to enable and improve data processing through statistical software;
3. The reaction of hybrids and their parental forms, in which wild annual and perennial sunflower species participate, to artificial infection with isolates of *Phomopsis helianthi* was studied, in order to track the inheritance of resistance to the pathogen;
4. The reaction of different genotypes to infection in certain phenophases of their ontogenetic development was established. This makes it possible to determine the most critical phenophase for infection and, accordingly, to undertake adequate chemical protection;

Publications related to the dissertation:

Petrova, M., Nenova, N., & Encheva, V. (2023). Study on aggressiveness to isolates of *Phomopsis/ Diaporthe helianthi* Munt. – Cvet. et al. on sunflower under field conditions. **Bulgarian Journal of Crop Science, 60(4) 53-58 (Bg).**

Damyanova-Serbezova, R., Petrova, M., Valkova, D., & Drumeva, M. (2024). Testing of spicement of wild sunflower spicies for rezistence to *Phomopsis helianthi*. *Bulgarian Journal of Crop Science, 61(2) 20-28 (Bg).*

Petrova, M., & Kiryakov, I. (2024). Mycelial compatibility of *Phomopsis helianthi* (Munt.-Cvet. et al.) isolates. *Bulgarian Journal of Crop Science, 61(4) 16-24 (Bg).*