International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 05, 2020
ISSN: 1475-7192

Features of the Propagation of the Ascospores of the Sunflower Phomopsis Blight Pathogen in the Surface Layer of the Atmosphere in the Conditions of the South of Russia

Valentina M. Androsova, Anton O. Didenko,
Aleksandr T. Podvarko and Leonid P. Esipenko

Abstract--- *The present article focuses on the features of the propagation of ascospores of the sunflower phomopsis pathogen in the surface layer of the atmosphere, depending on meteorological conditions, taking into account the development of the fungus in pure culture and on sunflower mulch in southern Russia. The maximum average growth of the mycelium of the fungal isolates transferred to the pure culture from the diseased sunflower stems harvested in different regions of southern Russia was observed at temperatures of 25 and 30°C, between which no statistically significant differences were found. The minimum mycelium growth was detected at a temperature of 32°C; its average value was statistically significantly different from the two previous temperatures. However, both at 30°C and at 32°C, pycnidia did not form, and, therefore, perithecia could not form either. Ascospores, propagating from an artificially created focus of infection, were detected in sunflower crops with the help of special spore traps. It was established that the propagation of ascospores of the sunflower phomopsis pathogen in the surface layer of the atmosphere depended on the degree of infection development on the plant mulch as well as on hydrothermal environmental factors. The propagation started 1-3 days after even minimal precipitation (0.05 mm or less) in a wide range of average daily relative humidity (38-80% or more), at an average daily temperature (13-30°C) and hydrothermal coefficient of more/equal or less than 1. The largest number of days with the presence of ascospores in the surface layer of the atmosphere was at the highest relative humidity (66-80 % and more). Prolonged atmospheric and soil droughts that had a particularly dangerous duration (drought lasted 45 days), accompanied by elevated daily average temperatures (30°C or more) during the growing season of sunflower, followed by severe, snowless winter periods, led to the death and cessation of pycnidia formation. However, these phenomena initiated a significant increase in the number of ascospores in the surface layer of the atmosphere. Abnormally high average daily temperatures (30 °C or more), relative air humidity from 38 to 50% during the growing season of sunflower led to a significant decrease in the propagation of infection.*

Keywords--- *Ascospores, Perithecia, Pycnidia, Propagation, Mulch, Phomopsis.*

I. INTRODUCTION

The accurate taxonomic trivial status of sunflower phomopsis pathogen, which is micromycete *Diaporthe (Phomopsis) helianthi* Munt.- Cvet., Mihaljc. & M. Petrov, was specified by Yugoslavian researchers in 1981 [1]. At

Valentina M. Androsova, All-Russian Research Institute of Biological Plant Protection.
Anton O. Didenko, All-Russian Research Institute of Biological Plant Protection.
Aleksandr T. Podvarko, All-Russian Research Institute of Biological Plant Protection.
Leonid P. Esipenko, All-Russian Research Institute of Biological Plant Protection.

DOI: 10.37200/IJPR/V24I5/PR2020188
Received: 12 Mar 2020 | Revised: 28 Mar 2020 | Accepted: 06 Apr 2020

the anamorphous developmental stage, the fungus forms on the plant mulch diseased by phomopsis the picnidia which contain only β-conodia – single-celled, colorless, elongated or curved cells (in the form of a curved needle) of 21.1-27.2 μm [29, 2]. According to the previous studies, the pycnidia, formed at anamorphous developmental stage of this fungus, usually died in winter [17]. According to other studies, conidal sporulation was found in epidermal and subepidermal tissues on overwinter sunflower stems. [18]. Quick change of dry and rainy weather is favourable to infection development on plant mulch [20]. The role of asexual sporulation remains unclear [19; 28; 46].

The teleomorph stage of sunflower phomopsis pathogen replaces over time the anamorph one on the plant mulch diseased by phomopsis, and plays the key role for survival and growing of fungus not only from year to year, but throughout the life of the host-plant. Ascospores are located in the asci of perithecia formed on plant mulch of diseased sunflower, then they are emitted through rostrum and propagate aerogenically [30]. It was experimentally proved that ascospores are the beginning of infection of sunflower phomopsis [19, 20].

Phomopsis remains one of the most widespread and mostly destructive diseases of sunflower in the world. Seed harvest from diseased sunflower plants may be decreased by 15.9 – 64.7 % [2, 3, 4, 5].

According to the data from European and Mediterranean Plant Protection Organization, the sunflower phomopsis pathogen is presented in European countries: Bulgaria [6, 7], Croatia [8], Hungary [9], Moldova, Romania, Serbia, Slovakia, Spain, Ukraine, Italy, Russia and France. In Asia, it is spread Pakistan, in Africa – inMorocco, in South America – in Argentina and Venezuela, in North America – in Mexico, in the USA - in California [10], Illinois [11], Minnesota, Ohio and Texas [12].

Phomopsis was included in the USSR quarantine list in 1986, and firstly found in Russia in 1990 [13].

In spite of climate conditions changing in the South of Russia in the direction of the drought with abnormal high temperatures and unstable relative air humidity, which are not favourable for pathogen vital activities, the plants diseased by phomopsis are constantly presented in sunflower crops of the Krasnodar Territory creating a potential hazard especially in certain areas [14]. This shows the adaptation of fungus biotypes to unfavourable factors [31].

Currently, other species of sunflower phomopsis pathogen were found by biomolecular analyses first in Australia and then in other countries. There are α as well as β-conidia in pycnidia of these species [15, 16]. In Russia during research works (2005 – 2012 years), only one species of phomopsis pathogen *D. (Ph.) helianthi* was identified on sunflower plant mulch with typical symptoms of phomopsis disease as well as on the species used in our study as infection source [16].

Many researchers have studied the impact of different temperatures and sunlight on the growth and development of fungus in pure culture. It was established that fungus isolates from different geographical locations cultivated on the same medium differ in the growth speed of mycelium. Thus, no pathogen growth was observed at + 5^{o}C and + 37^{o}C. Slow fungus growth was at + 15, + 20 and + 33^{o}C, and optimum temperature range for pathogen development was + 23-27^{o}C. Optimum temperature range for fungus isolates from Moldova and Odessa was + 23-30^{o}C [32]. Isolates from different geographical locations cultivated on the same medium differ not only in the growth speed of mycelium but also in color, type and structure of colonies [33; 34; 35; 32].

DOI: 10.37200/IJPR/V24I5/PR2020188
Received: 12 Mar 2020 | Revised: 28 Mar 2020 | Accepted: 06 Apr 2020

The variability of phomopsis isolates by mycelium growth speed in pure culture and their grade of aggressiveness for vegetative sunflower plants have been studied by many authors. It was proved by French researchers that the mycelium growth speed on artificial medium doesn't correlate with the spot size on the diseased sunflower stems [36]. It was established by American researchers that the impact of weather conditions on the aggressiveness of phomopsis isolates is greater than their geographical origin [37]. Russian researches showed that the aggressiveness of phomopsis isolates from different geographical locations is more due to toxicity of their cultural filtrate than to the growth speed of mycelium *in vitro* [38].

Light has a positive impact on fungus growth speed, its mycelium density and the population of pycnidia. The duration of the photoperiod impacts on pycnidia development, with the increase of which up to 10-12 hours the number of fungus fruits also increases [39].

Formation of fungus teleomorph stage on potato dextrose agar (PDA) and wort agar medium was recorded on the 32-34 days [1; 29; 40; 17]. Perithecia were formed on artificial medium containing a variety of microelements, aminoacids and vitamins, and on sterile fragments of different plants. The obtained results showed that the content of medium is not a key factor in teleomorph formation [41; 42; 43; 44; 45].

The initial development of fungus on sunflower plant mulch begins with mycelium growth. With significant increase of mycelium mass, entwinement and aggregation of hypha occur what leads to the formation of pycnidia initial cells. In future, they transform to pycnidia.

Pycnidia, brown-grey or black in color, spherical in shape, 170-320 μm in diameter, are formed in the end of August – September at high 70 % humidity level and 20-28^{o}C. Pycnidia are formed in epidermal and subepidermal tissue of sunflower stem, individually or in groups, on or under the surface. β – spores developing in pycnidia in moist conditions emerge from stomato of mature pycnidia in the form of creamy white exudate creating a drop above it [49; 50; 46; 51].

Under natural conditions, the formation of organs of the teleomorph stage, perithecia, occurs in phloem and pericycle of sunflower stems during the autumn-spring period. Therefore, perithecia prevailed on plant mulch harvested in spring, while pycnidia were found on stems harvested in early winter [18; 48]. Perithecia have irregular rounded shape, 150-430 × 180-850 μm, mainly black. They are formed on diseased sunflower stems located on the soil surface. The length of rostrum is very variable and usually ranges from 260 to 850 μm. Ascospores are formed in asci, which are club-shaped. The size of asci is 44-67.5 × 4.5-12.0 μm, each of which contains ascospores [2; 53].

Mycelium growth and formation of fungus fruits precede the mature perithecia formation and then its emission through rostrum which is followed by the propagation of ascospores in the surface layer of the atmosphere.

With artificial inoculation of young sunflower plants by β-conidia or ascospores of fungus, the disease is developed only in the latter case [19; 47; 52]. Quick change of dry and rainy weather is favourable to infection development on plant mulch [20]. The role of asexual sporulation remains unclear [19; 28; 47].

For the timely and effective protection of sunflower against phomopsis, it is necessary to determine the flight time of disease pathogen ascospores [17]. Therefore, the features of the propagation of the ascospores of the

DOI: 10.37200/IJPR/V24I5/PR2020188
Received: 12 Mar 2020 | Revised: 28 Mar 2020 | Accepted: 06 Apr 2020

sunflower phomopsis blight pathogen in the surface layer of the atmosphere (flight) have been studied since the 1980ies of the past century to the present [46; 21]. As a result of these studies, disagreements appeared about the role of hydrothermal coefficient (HTC) as a limiting factor of ascospores flight [21, 22].

It was established that propagation of the ascospores of the sunflower phomopsis panthogen under Italian conditions is the function of rains and temperature [23].

To summarize the analyses of the presented information sources, it can be concluded that the biology of the *D.* (*Ph.*) *helianthi* fungus development has been studied in detail. However, the studies of ascospores propagation in the surface layer of the atmosphere were done in the 1980ies of the past century when extreme weather conditions were not so represented as in the beginning of 21st century.

The impact of different temperatures on the growth and development of isolates of sunflower phomopsis pathogen from different geographical locations have been studied by Russian and foreign authors. But despite this, there is a small amount of studies on pathogen isolates from different regions of the South of Russia.

Identification of common factors of ascospores propagation in the surface layer of the atmosphere depending of the meteorological conditions, taking into account the extreme impact of climate change in the surface layer of the atmosphere will allow to evaluate objectively the sunflower phomopsis pathogen development and create more effective protection of crops from disease.

The purpose of this work is to study the features of the propagation of the ascospores of phomopsis pathogen in the surface layer of the atmosphere, taking into account the fungus development in pure culture and on sunflower mulch in the conditions of the South of Russia on the example of Krasnodar city.

II. MATERIALS AND METHODS

Laboratory experiment on the impact of different temperatures and constant illumination (3000 lux) on the growth rate of *D.* (*Ph.*) *helianthi* isolates colony formed in Petri dishes on potato dextrose agar medium (PDA) and located in Sanyo (Japan) climate chambers. Medium preparation and phomopsis pathogen inoculation on the medium were performed according to the methodological procedures developed in All-Russian Research Institute of oil plants, Krasnodar city [31].

Fungus isolates were transferred into pure culture from diseased sunflower stems harvested in different regions of southern Russia Table 1.

Table 1: Phomopsis Pathogen Isolates Transferred from Diseased Sunflower Plants Harvested in Different Regions of Southern Russia

# isolate	Region of harvest	Hybrid/sunflower variety*
6	Krasnodar suburbs	hybrid NSH-630
23	Krasnodar suburbs	variety P-453
48	Gulkevychsky district, Krasnodar Krai	hybrid ПР64А83
49	Mikhailovsky district, Volgograd oblast	hybrid PT31-X
51	Morozovsky district, Rostov oblast	hybrid Signal
52	Ipatovsky district, Stavropol Krai	hybrid Aron
53	Starominskoy district, Krasnodar Krai	variety P-453

* – all hybrids and varieties of sunflower are, to varying degrees, receptive to phomopsis pathogen

DOI: 10.37200/IJPR/V24I5/PR2020188
Received: 12 Mar 2020 | Revised: 28 Mar 2020 | Accepted: 06 Apr 2020

Experiments on study of the development of phomopsis pathogen on sunflower stems (plant mulch) and propagation of ascospores in the surface layer of the atmosphere were performed on crop rotation fields of ARRIBPP (All-Russian Research Institute of Biological protection of plants, Krasnodar city). Sunflower stems with typical symptoms of phomopsis disease were used for studies. Focus of infection was created from these stems and was located on 400-800 m distance from experimental sunflower crops from the prevailing direction of the wind. Part of segments of these stems were placed in Kleban cassettes located on 40 cm supports under environment conditions available after heavy rains.

Analyses of disease symptoms and identification of sunflower phomopsis pathogen on plant mulch were performed by standard methods taking into account the results of last morphological studies [24, 25].

The growth and type of fungus fruits, as well as spores morphology formed in them, were studied on stems both in focus of infection and on stems located in Kleban cassettes daily after each rain during 3-4 days by the method of light microscopy. The part of plant mulch, which were used for creating the focus of infection, were used for the morphological studies of fruits and spores performed under moist conditions of artificial climate chamber. Perithecia ascospores emitted from the plant mulch were picked up on glycerin-gelatin solution-covered glasses above the focus of infection in the same period after rain using DIPCS (device for the identification of plant contamination with spores, Russia). Propagation of the ascospores in the surface layer of the atmosphere above experimental crops of sunflower was recorded using ST-1 (spore trap, Russia) which were placed with a spacing of 0.5 ha at height of 2 m above the soil surface in the center of the experimental sunflower field [26].

Ascospores identified by described methods were studied using light microscopy and compared with the ones on ST-frames. Frames in spore traps were changed daily at the same time.

The number of ascospores on inspection surface of ST-frames was calculated using light microscopy and identified by special formulas [26].

Temperature, relative air humidity and number of precipitations were recorded daily on meteorological station located on the same territory as experimental fields. The hydrothermal coefficient (HTC) was used as complex indicator of moisture conditions which was calculated by Selyaninov formula [27].

The average number of ascospores propagated per day was calculated only on the days of their propagation on average per month of sunflower growing season for each year of study, and also for each month of the growing season (May – August) on average for all years of study (2005-2009; 2011-2012). The obtained data of laboratory study and average number of ascospores on the days of their propagation, and also the corresponding values of HTC were statistically processed and represented by diagram with confidential intervals using Statistica 6 program. The average values of the number of days with ascospores were distributed on different intervals of the daily average relative air humidity. The average number of rains and days with ascospores in the surface layer of the atmosphere during sunflower growth season was identified. The received data was statistically processed using Microsoft Office Exel 2003 program and represented in tabular form with average values and their standard errors.

DOI: 10.37200/IJPR/V24I5/PR2020188
Received: 12 Mar 2020 | Revised: 28 Mar 2020 | Accepted: 06 Apr 2020

III. RESULTS

At an optimum temperature of 25°C for the growth of the fungus, on the second day of cultivation isolates No. 6 (34.5 mm) and No. 53 (31 mm.) had the rapid growth of colonies. The colonies sizes of isolates Nos. 48, 49, 51 and No. 52 were 26.5 – 29.6 mm. On the fourth day of cultivation, the colonies diameter of isolates No. 49 (81.4 mm) and No. 53 (81.2 mm) exceeded the sizes of other isolates. The colonies sizes of isolates Nos. 6, 48, 51 and 52 were 78.3 – 79.9 mm. The slow growth of the mycelium was observed in isolate No. 23. On the second day, its diameter was 22.0 mm and on the fourth day it was 68.8 mm. On the fifth day after plating, the colonies of all isolates completely covered the surface of the medium in Petri dishes and their diameter was 90 mm.

At an unfavourable temperature of 30°C for the growth of the fungus, on the second day of cultivation, isolate No. 49 (27.2 mm) had the rapid growth of colonies. The sizes of its colonies exceeded the colonies sizes of isolates No. 23 (22.0 mm) and No. 51 (26.5 mm) on the second day of their cultivation at an optimum temperature of 25 °C. The colonies size of isolates Nos. 48, 52, 53 varied from 20.6 to 24.6 mm. On the fifth day of cultivation the colonies diameter of isolates No. 49 (79.0 mm) and No. 52 (79.7 mm) exceeded the sizes of other isolates. The colonies sizes of isolates Nos. 48 and 53 were 78.0 and 78.7 mm respectively. The slow growth of the mycelium was observed in isolates Nos. 6, 23 and 51. On the second day, the diameter of their colonies was 19.4, 15.1and 18.9 mm and on the fifth day it was 74.5, 61.0 and 73.2 mm respectively. Six days after the plating, the colonies of isolates No. 6, No. 48, No. 49, No. 51, No. 52, No. 53 completely covered the surface of the medium in Petri dishes. The mycelium of isolates No. 23 had a diameter of 90 mm only on the seventh day of cultivation.

The inhibition of the mycelium growth rate of all isolates was observed at a temperature of 32°C. Isolate No. 48 had a rapid growth of colonies in comparison with other isolates. On the second day of cultivation, its diameter was 11.9 mm and the fifth day was 23.8 mm. On the fifth day of cultivation, isolates Nos. 23, 49, 51, 52, and 53 had the colonies sizes of 20.1 – 21.8 mm. On the twelfth day of cultivation the colonies, the diameter of isolates, No. 48 (72.5 mm) and No. 49 (70.4 mm) exceeded the sizes of other isolates. The colonies size of isolates Nos. 23, 51, 52 and 53 varied from 49.9 to 69.7 mm. The slow growth of the mycelium was observed in isolate No. 6. On the second day of cultivation, its diameter was 9.9 mm; on the fifth day, it was 15.8 mm and on the twelfth day, was 43.7 mm. On the 14th day of cultivation, the isolates colonies were 83.8 and 81.2 mm respectively. The colonies sizes of other isolates (Nos. 6, 23, 49, 51 and 53) varied from 62.3 to 79.6 mm. On the 15th day of cultivation, the mycelium of isolates Nos. 48, 49, 52 and 53 completely covered the surface of the medium in Petri dishes. The colonies of isolates Nos. 6, 23, 51 had the diameter of 90 mm only on the seventh day of cultivation.

The most unfavourable temperature for the growth of the fungus was 35°C. On the third day of cultivation, the growth of the fungal colonies stopped and the next four days it was not observed.

The most favorable temperature for pycnidia formation was 25 °C. The pycnidia formation, as a rule, coincided with the filling of the medium in Petri dishes with the mycelium of the fungus. The isolates were different from each other by quantitative ability of pycnidia formation on the PDA medium. On average, isolate No. 48 formed 15-20 % more pycnidia than other isolates (Nos. 6, 23, 49, 51, 53). All studied isolates did not form pycnidia at high temperatures of cultivation.

DOI: 10.37200/IJPR/V24I5/PR2020188
Received: 12 Mar 2020 | Revised: 28 Mar 2020 | Accepted: 06 Apr 2020

The mycelium colour of the geographical isolates varied from gray-white to white and had a tomentous or procumbent shape.

The fungus growth assessment was done with the help of two-factor cross analysis of variance which is shown in Table 2.

Table 2: Two-factor Cross Analysis of Variance of the Fungus Growth with the Factors "isolate" and "temperature"

Variability	df	mS	F_f	F_{05}	Dispersion	Portion%
Common	139				840.50	100
Between isolates	6	184.69	0.251	2.16	0.00	0
Between temperatures	2	5570.79	7.560	3.06	103.58	12.3
Correlation	12	52.00	0.071	1.82	0.00	0
Residual	119	736.91			736.91	87.7

Note: df - degrees of freedom; mS - mean square; F - Fisher's variance ratio; dispersion - deviation from the mean value; portion - the portion of factor variability in the total variability of the factor

The results of variance analysis show the effect of only the temperature differences ($F_f \geq F05$). At the same time, their portion of factor variability is 12.3%.

The pattern of the differences as comparison of average values is shown in Table 3.

Table 3: Multiple Rank Test Comparing the Effect of Different Temperatures on the Average Growth of the Fungal Colonies

Temperature ^oC	Average growth, mm	Rank test	
32	37.49		****
30	49.46	****	
25	59.76	****	

Note: The position of asterisks on the different vertical lines points to the significance of average differences

According to the Table 3, the maximum average growth of the fungus was observed at temperatures of 25 and 30^{o}C between which no statistically significant differences were found. The minimum fungus growth was detected at a temperature of 32^{o}C; its average value was statistically significantly different from the two previous temperatures.

It was established that in 2005 during the growing season of sunflower, on the plant mulch of the infection focus, first appeared the pycnidia which contained only β – conidia, referring to *D. (Ph.) helianthi.* Pycnidia were localized in the primary infection areas of sunflower stems - leaf axils. The first mass propagation of ascospores occurred on August 13 when there was the replacement of pycnidia in the leaf axils of the plant mulch by perithecia. In the following years (2006, 2007), there was the formation of both pycnidia focus, which propagated on the plant mulch already beyond leaf axils, and perithecia, which replaced them after a while in the same year.

The winters of 2005 and 2007 were short and mild. In 65% of winter ten-day periods, the average temperatures were 1-3^{o}C higher than normal. There was no a sustainable transition through 0^{o}C. The alternation of short frosty and long thawing periods was observed. In 2006, winter was abnormally cold but snowy; the depth of snow cover

DOI: 10.37200/IJPR/V24I5/PR2020188
Received: 12 Mar 2020 | Revised: 28 Mar 2020 | Accepted: 06 Apr 2020

was 30 cm. The negative abnormalities of ten-days temperatures were 10^{o}C and the accumulated negative temperatures (winter severity index) were 256 ^{o}C, with the normal value of 153 ^{o}C. After these overwintering conditions of sunflower mulch, pycnidia continued to appear, and since 2006, the propagation of ascospores has occurred from the first month of the sunflower growing season (May) to its end (August).

It was established that from 2005 to 2007 the average number of ascospores (thousand pieces per day) during the growing season did not differ significantly. Hydrothermal coefficient was 1 only in 2006 and 2005, while in2007 it was less than 1. It was particularly dry in 2007 (hydrothermal coefficient = 0.3). It should be noted that in 2007 there were prolonged atmospheric and soil droughts that had a particularly dangerous duration in August (drought lasted 45 days). This contributed to a noticeable decrease in the formation of pycnidia on the plant mulch (only isolated pycnidia were observed)

The winter of 2008 lasted 52 days and had a small amount of snow with a small and short occurrence of snow cover. In a snowless period (30 days), the temperature of the soil surface during 18 days decreased to 10-16° below zero. With the beginning of the growing season in this year, pycnidia on the plant mulch did not appear more, and the population of propagating ascospores in the surface layer of the atmosphere increased having the maximum value for an entire previous period.

The data on the peculiarities of the propagation of the ascospores of the sunflower phomopsis blight pathogen in the surface layer of the atmosphere and the changes of hydrothermal coefficient average values depending on the year of the research are presented in Figure 1.

The winter periods from 2009 to 2011 were short and mostly warm. In some days, the maximum temperature was 13-18°C above zero. In 2011, the minimum temperature on the soil surface was 7-13°C below zero during 23 days, and in 2012– 10-15 ° C below zero during 6 days. The accumulated negative temperatures were 212°C.

In May, the average monthly temperature during the growing season of sunflower was, as a rule, less than in other months but exceeded a longstanding average monthly temperature from 16.9^{o}C to 4^{o}C. In June, July and especially in August, the average monthly air temperature exceeded the normal value by 1 – 4^{o}C.

As for the growing season, in 2009 precipitation (270 mm) was close to normal. In 2011 and 2012, the amount of precipitation for the growing season did not exceed 70 % of the norm. Hydrothermal coefficient was less than 1. However, there was the propagation of ascospores. Their population was not significantly different from that detected in 2008. However, a downward trend occurred (Figure 2).

In August throughout the research period, there was light precipitation or no precipitation for a long time. Average daily relative humidity decreased to 38-50%, and a temperature was 30°C or more. The minimal hydrothermal coefficient in August was 0.2.

During the growing season of sunflower, the average daily relative humidity varied from 38 % to 80 % or more. The consideration of such average daily relative humidity intervals as 38 – 59, 60 – 65, 66 – 80 and more has shown that the largest number of days with the presence of ascospores in the surface layer of the atmosphere was in the range with the highest relative humidity (Table 4).

DOI: 10.37200/IJPR/V24I5/PR2020188
Received: 12 Mar 2020 | Revised: 28 Mar 2020 | Accepted: 06 Apr 2020

Table 4: The Number of Days with the Presence of the Ascospores of the Sunflower Phomopsis Blight Pathogen in the Surface Layer of the Atmosphere Depending on Relative Air Humidity

Average daily relative humidity, %	The number of		
	days with ascospores in the atmosphere	rains in the growing season	days with ascospores during the season
66 – 80 and more	22.0 ± 1.4	22 ± 2.4	30 ± 1.7
60 – 65	5.0 ± 0.8		
38 – 59	3.0 ± 1.2		

The propagation in the surface layer of the atmosphere started, as a rule, 1-3 days after precipitation (100% of cases). After one rain, there could be more than one propagation of ascospores in the same intervals after the previous one, as the first propagation after rain until the next precipitation. Therefore, in the table the number of days with ascospores in the surface layer of the atmosphere for the season exceeds the number of rains. It was impossible to determine how long it could last because of the following rains. If rains were several days in a row, then the days of the propagation of ascospores followed in a row after the last rain of them or began in one of the last days with small precipitation. In cases of heavy prolonged rains, the propagation of ascospores was delayed for 3-4 days in comparison with the established date after the end of rain.

IV. Discussion Of Results

The study of the fungal isolates growth transferred to the pure culture from the diseased sunflower stems harvested in different regions of southern Russia has shown that the maximum average growth of the mycelium of the fungal was observed at temperatures of 25 and 30^{o}C, between which no statistically significant differences were found. The minimum mycelium growth was detected at a temperature of 32^{o}C; its average value was statistically significantly different from the two previous temperatures. However, both at 30°C and at 32°C, pycnidia did not form, and, therefore, perithecia could not form either.

The research of the ascospores propagation of the sunflower phomopsis blight pathogen in the surface layer of the atmosphere during 7 years showed that it depended on the state of infection on the plant mulch and meteorological conditions.

The propagation of ascospores of phomopsis pathogen started after a replacement of pycnidia by perithecia on the plant mulch of sunflower with typical symptoms of lesion by phomopsis. It occurred in the considered conditions in the same year when pycnidia formed. Pycnidia survived winter if it was warm (2005 and 2007). They could survive a severe winter with the snow cover of 30 cm (2006). In the information sources [17, 18], there were contradictory data due to the fact that during their research there were no described weather factors (the researches were less prolonged) and were carried out in the 1980ies. The conditions have changed a lot for the last period in the direction of frequent prolonged droughts, accompanied by extremely high temperatures (30°C or more). In addition, the fungus, adapting to changing conditions, got new properties. There are the evidences of the researches in vitro.

Pycnidia stopped to form after prolonged atmospheric and soil droughts that had a particularly dangerous duration (drought lasted 45 days) during the growing season of sunflower (2007) followed by severe, snowless

DOI: 10.37200/IJPR/V24I5/PR2020188
Received: 12 Mar 2020 | Revised: 28 Mar 2020 | Accepted: 06 Apr 2020

winter periods (2008). At the same time, these phenomena led to a significant increase in the number of ascospores in the surface layer of the atmosphere.

It was established that ascospores propagate in the surface layer of the atmosphere at hydrothermal coefficient equal and less than 1. It shows a high degree of the fungus adaptation to conditions changing in the direction of drought.

The minimal precipitation (0.05 mm or less) was detected after which the propagation started. The minimal temperature was established at which the propagation of ascospores in the surface layer of the atmosphere started. The interval between the end of precipitation and the beginning of the ascospores propagation was determined. It was established that the largest number of days with the presence of ascospores in the surface layer of the atmosphere was at the highest relative air humidity (66-80% and more). It shows the importance of high relative air humidity for the propagation of ascospores. Moreover, precipitation, as a rule, accompanied by elevated relative air humidity at which, as was shown, the maximum number of days with ascospores in the surface layer of the atmosphere is observed.

These parameters are important for preventive of sunflower with protective agents, which are more effective than those that are used after the propagation especially after the implanting of the infection.

V. CONCLUSION

As a result, the maximum average growth of the mycelium of the fungal isolates transferred to the pure culture from the diseased sunflower stems harvested in different regions of southern Russia was observed at temperatures of 25 and 30^{o}C, between which no statistically significant differences were found.

The minimum mycelium growth was detected at a temperature of 32^{o}C; its average value was statistically significantly different from the two previous temperatures. However, both at 30°C and at 32°C, pycnidia did not form, and, therefore, perithecia could not form either.

The propagation of ascospores of the sunflower phomopsis pathogen in the surface layer of the atmosphere depended on the state of infection on the plant mulch and hydrothermal factors. It was detected for the first time (on August 13, 2005) after the replacement of pycnidia by perithecia of the sunflower phomopsis blight pathogen in the leaf axils of stems (the primary infection area) which formed the focus of infection.

It was established that a frequent change of rainy and dry weather and especially prolonged atmospheric and soil droughts that had a particularly dangerous duration during the growing season of sunflower and following severe, snowless winter periods led to the death and cessation of pycnidia formation. However, these phenomena initiated a significant increase in the number of ascospores in the surface layer of the atmosphere.

The largest number of days with the presence of ascospores in the surface layer of the atmosphere was at the highest relative humidity (66-80% and more).

The propagation of ascospores in the surface layer of the atmosphere started 1-3 days after precipitation (100% of cases)

DOI: 10.37200/IJPR/V24I5/PR2020188
Received: 12 Mar 2020 | Revised: 28 Mar 2020 | Accepted: 06 Apr 2020

The minimal precipitation (0.05 mm or less) led to the propagation of ascospores.

The minimal average daily temperatures of the ascospores propagation was 13^{o}C.

The decrease of the frequency and the amount of precipitation, relative air humidity to 38 - 50 %, the increase of the average daily temperature to 30°C or more, led to a significant decrease in the propagation of infection.

The propagation of ascospores in the surface layer of the atmosphere occurred at hydrothermal coefficient of more/equal or less than 1.

REFERENCES

[1] Muntanola-Cvetkovic M., Mihaljcevic M., Petrov M. On the identity of the causative agent of a serious Phomopsis – Diaporthe Disease in Sunflower Plants / M. Muntanola-Cvetkovic, M. Mihaljcevic, M. Petrov // Nova Hedwigia. – 1981. – № 34. – P. 417– 435.

[2] Maric, A. Prilog proucavanju Phomopsis spp. (Diaporthe sp.) prouzrokovaca sive pegavosti stabla suncokreta / A. Maric, S. Masirevic, S. Li // Zastita Bilja. – 1982. – № 33. – P. 403-419.

[3] Acimovic, M. Phomopsis sp. - An novi parazit suncokreta / M. Acimovic, N. Straser // Zastita Bilja. – 1982. – Vol. 32 (2). – P. 117-158.

[4] Skoric, D. Sunflower breeding for resistance to Diaporthe helianthi / D. Skoric // Helia. – 1985. – № 8. – P. 21-24.

[5] Skripka, O.V. Phomopsis - a dangerous disease of sunflower / O.V. Skripka. - Moscow: Agropromizdat, 1989.-- 4 p.

[6] Bistrichanov, S. Impact of some agrotechnical factors on the diseases' spreading on sunflower in the North Western Bulgaria / S. Bistrichanov, V. Alexandrov, I. Hristov // Pochvoznanie, Agrokhimiya i Ekologiya. – 2000. – № 35(5). – P. 37-40.

[7] Encheva, V 2002. Variation in the aggressiveness of isolates from Phomopsis/Diaporthe helianthi Munt.-Cvet. et al. on sunflower / V. Encheva // Bulgarian Journal of Agricultural Science. – № 8(4). – P.349-352.

[8] Jurkovic, D. Incidence of the most important sunflower diseases in eastern Croatia / D. Jurkovic, M. Culek, // Fragmenta Phytomedica et Herbologica. – 1998. – № 26. – P. 67-75.

[9] Palfi, K. Mire figyeljünk júliusban ? / K. Palfi, A. Pakozdi // Novenyvedelem. – 1999. – Vol. 35. – № 7. – P. 339-341.

[10] Gulya, T.J. Sunflower diseases remain rare in California seed production fields compared to North Dakota. Plant Health Progress [Electronic resource] / T.J. Gulya, S. Rooney-Latham, J.S. Miller, K. Kosta, C. Murphy-Vierra, C. Larson, W. Vaccaro, H. Kandel, J.F. Nowatzki. – 2012. –: http://https://www.plantmanagementnetwork.org/pub/php/research/2012/ sunflower /. – published 14 December 2012 (дата доступа 20.06.19).

[11] Meyer, M.D. First report of Phomopsis stem canker of sunflower in Illinoi s caused by Phomopsis helianthi / M.D. Meyer, G.R. Zhang, D.K. Pedersen, C.A. Bradley // Plant Disease. – 2009. – № 93 (7). – P. 760.

[12] EPPO. PQR database. Paris, France: European and Mediterranean Plant Protection Organization [Electronic resource]. – 2001.: https://gd.eppo.int/taxon/DIAPHE/. – published 21 February 2012 (дата доступа 20.06.19).

[13] Skripka, O.V. Phomopsis of sunflower / O.V. Skripka, V.I. Shelukhin, V.V. Petina, T.P. Serebryakova // Protection and quarantine of plants. - 1993. - No. 8. – P.p. 24-25.

[14] Ismailov V.Ya., Piven V.T., Shulyak I.I., Didenko A.O., Podvarko A.T. Phomopsis on sunflower crops in the Krasnodar Territory / V.Ya. Ismailov, V.T. Piven, I.I. Shulyak, A.O. Didenko, A.T. Podvarko // Oilseeds. - 2014. - issue. 1 (No. 157–158). – P.p. 130-134.

[15] Thompson E.M., Tan Y.P., Young A.J., Neate S.M., Aitken E.A.B., Shivas R.G. Stem cancers on sunflower (Helianthus annuus) in Australia reveal a complex of Pathogenic Diaporthe (Phomopsis) species / E.M. Thompson, Y.P. Tan, A.J. Young, S.M. Neate, E.A.B. Aitken, R.G. Shivas // Persoonia. – 2011. – № 27. – P. 80-89.

[16] Gulya T.J., Hulke B., Seiler G.J., Markell S., Kandel H. Changas in disease incidence over the last decade in the US and around the world, and research to address these new challenges / T.J. Gulya, B. Hulke, G.J. Seiler, S. Markell, H. Kandel // Abstracts 18th Int. Sunflower Conf. – ISA. Mar Del Plata & Balcarce – Argentina. – 2012. – P. 37.

DOI: 10.37200/IJPR/V24I5/PR2020188
Received: 12 Mar 2020 | Revised: 28 Mar 2020 | Accepted: 06 Apr 2020

[17] Fayret J., Assemat P. Evolution du Diaporthe helianthi (Phomopsis helianthi) Munt.-Cvet. et al in differenciation des organe reprodacteurs sur les peants de tournesol aples la period de vegetation / J. Fayret, P. Assemat // Inf. techn. CETIOM. – 1987. – № 98. – P. 2-11.

[18] Mihaljcevic M., Muntanola-Cvetkovic M., Vucojevic J., Petrov M. Sourse of infection of sunflower plants by Diaporthe helianthi in Yugoslavia / M. Mihaljcevic, M. Muntanola-Cvetkovic, J. Vucojevic, M. Petrov // Phytopathology. – 1985. – Vol. 113. – № 4. – P. 334 – 342.

[19] Mihaljcevic M., Muntanola-Cvetkovic M. Responses of sunflower plants to different Phomopsis isolates. 2. From weeds. / M. Mihaljcevic, M. Muntanola-Cvetkovic // Proc. II The International Sunflower Conference. – Argentina, 1985. – P. 419-424.

[20] Acimovic M., Straser, M., Drazik N. Mogucnosti suzbijanja Phomopsis sp. ostalih prouzrokovaca bolesti suncokreta / M. Acimovic, M. Straser,, N. Drazik // Zastita Bilja. – 1982. – Vol. 33. – № 3. – P. 293 – 299.

[21] Piven V.T., Shulyak I.I., Alifirova T.P., Samelik E.G. Phomopsis on sunflower / V.T. Piven, I.I. Shulyak, T.P. Alifirova, E.G. Samelik // Collection of scientific papers “Diseases and pests of oilseeds” / Ed. ed. V.T. Piven. - Krasnodar, 2006.—P.p. 90 - 92.

[22] Androsova V.M. Peculiarities of Ascospore Diaporthe (Phomopsis) helianthi drought summer / V.M. Androsova // Materials of reports of the international scientific-practical conference "Biological plant protection, prospects and role in phytosanitary rehabilitation of agrocenoses and obtaining environmentally friendly agricultural products." - Krasnodar, 2008.—P.p. 565 - 569.

[23] Battilani P., Rossi V., Girometta B., Delos M., Rouzet J., André N., Esposito S. Estimating the potential development of *Diaporthe helianthi* epidemics in Italy / P. Battilani, V. Rossi, B. Girometta, M. Delos, J. Rouzet, N. André, S. Esposito // EPPO Bulletin. – 2003. – Vol. 33. – № 3. – P. 427 – 431.

[24] Valentina Androsova, Irina Balachnina, Thomas Gulya. Structural aspects regarding formation and emission of *Diaporthe (Phomopsis) helianthi* ascospores / V. Androsova, I. Balachnina, T. Gulya // proceedings of the 17th International Sunflower Conference. – Cordoba, Spain, 2008. – Vol. 1, P. 103 – 107.

[25] Androsova V.M., Didenko A.O. Methodical manual on new aspects of diagnostics of the pathogen of sunflower phomopsis / V.M. Androsova, A.O. Didenko - Krasnodar, 2011, 18 p.

[26] Sokolov Yu.G., Evsyukov N.A., Sadkovsky V.T. Recommendations on the use of infection control and environmental parameters in protecting plants from diseases / Yu.G. Sokolov, N.A. Evsyukov, V.T. Sadkovsky. - Moscow: Russian Agricultural Academy, 1994, 33 p.

[27] Selyaninov G.T. World agroclimatic reference book / G.T. Selyaninov. - Leningrad - Moscow: Gidrometeoizdat - 1937, 428 p.

[28] Vukojevic, J. Source of infection of sunflower plants by Diaporthe helianthi in Yugoslavia / J. Vukojevic, M. Petrov // Phytopathol. Z. - 1985. - № 4, P. 334-342.

[29] Muntanola-Cvetkovic, M. Species of Diaporthe and Phomopsisrecorded in Yugoslavia / M. Muntanola – Cvetkovic, M. Mihaljcevic, M. Petrov // Savremena polipriverda. – 1981a. – Vol. 29 (7- 8), P. 293-306.

[30] Assemat, P. Biological culturale ef differeenciation des peritheces in vitro cher Diaporthe (Phomopsis) helianthi Munt.-Cvet. / P. Assemat, J. Fayret // Abstract book «Proc. 12th International Sunflower Conference»/ - Yugoslavia, 1988. – vol. 2, P.84-89.

[31] Araslanova, N.M. The growth rate in the culture of phomopsis isolates at different temperatures and the toxicity of their culture filtrates for sunflower seedlings / N.M. Araslanova, T.A. Chelyustnikova, T.S. Antonova // Oilseeds: Scientific-technical. bull. VNIIMK. - 2013. - Issue. 1 (153-154). – P.p. 60–66.

[32] Skripka, O.V. Biological features of the causative agent of brown-gray spotting of sunflower stems / O.V. Skripka // Mycology and phytopathology. - 1996. –Vol.3. - Vol. 1. – P.p. 56-60.

[33] Muntanola-Cvetkovic, M. Comparisons of Phomopsis isolates obtained from sunflower plant and debris in Yugoslavia / M. Muntanola-Cvetkovic, M. Mihaljcevic, J. Vucojevic, M. Petrov // Trans. Br. Mycol. Soc. – 1985 – № 85. – P.p. 477-483.

[34] Bogdanova, V.N. In time to detect phomopsis / V.N. Bogdanova, L.V. Karadzhova, M.E. Steinberg // Agriculture of Moldova. - 1986. - No. 12. – P.p. 24-25.

[35] Bondarenko, A.I. Phomopsis - a dangerous disease of sunflower / A.I. Bondarenko, M.E. Steinberg, V.N. Bogdanova, L.V. Karadzhova // Mycology and phytopathology. - Vol. 22. - No. 4. - 1988. – P.p. 346-348.

[36] Viguie, A. Interactions between French isolates of *Phomopsis / Diaporthe helianthi* Munt.-Cvet. et al. and sunflower (*Helianthus annuus L.*) genotypes / A.Viguie, F.Vear, D.Tourvieille de Labrouhe // European Journal of Plant Pathology. – 1999. – Vol. 105. – P. 693–702.

[37] Gulya, T.J. Evaluating sunflower germplasm for resistance to *Phomopsis* stem canker / T.J Gulya // Proc. 20th Sunflower Res. Workshop, Fargo. – USA, 1998. – P. 92–94.

DOI: 10.37200/IJPR/V24I5/PR2020188
Received: 12 Mar 2020 | Revised: 28 Mar 2020 | Accepted: 06 Apr 2020

[38] Antonova, T.S. The toxicity of the culture filtrate and the aggressiveness of geographically distant isolates of Phomopsis (Diaporthe) helianthi Munt.-Cvetk., Michal., Petr. for sunflower / T.S. Antonova, N.M. Araslanova, T.A. Chelyustnikova // Oilseeds: Scientific-technical. bull. VNIIMK. - 2006. - Vol. 2 (135). – P.p. 67–72.

[39] Firaz Alam, M. Evaluation of the source and breeding material of sunflower for the defeat of Phomopsis for breeding purposes: author. dis.... cand. S.-kh. Sciences: 06.01.05 // Firaz Alam M.; - Krasnodar, 1995.-- 22 p.

[40] Petrov, M. New findings on the disease of sunflower plants caused by Phomopsis helianthi Munt.-Cvet. et al. (*Diaporthe helianthi* Munt.-Cvet. et al.) / M. Petrov, M. Muntanola-Cvetkovic, M. Mihaljcevic // Archives des Sciences biologiques. – 1981. – № 33 (14). – P. 13-19.

[41] Booth, C. Fungal culture media / C. Booth // Methods in microbiology. – 1971 – № 4. – P. 49-94.

[42] Assemat, P. Differenciation des peretheces de Diaporthe (Phomopsis) helianthi Munt.-Cvet. Et al., parasite du tournesol, en cultures in vitro, incidence des facteurs de Ienvironment / P. Assemat, J. Fayret // Paris: C. R. Acad. Sei. – 1987. –V. 308. – P. 221-224.

[43] Muntanola-Cvetkovic, M. The results obtained until now in investigations of Phomopsis (Diaporthe) helianthi on sunflower. 1. Mycological aspects / M. Muntanola-Cvetkovic, M. Mihaljcevic, J. Vucojevic // Plant protection. – 1988. – № 39 (4). – P. 469-478.

[44] Vukojevic, J. Multidisciplinama studija kompliksa Diaporthe arctii (Lash) Nit u Jugoslaviji (Dissertation). Belgrade, Yugoslavia University of Belgrad faculty of Biology / J. Vukojevic. – Jugoslaviji, 1989. –191 p.

[45] Vukojevic, J. The comparativ investigation of Phomopsis helianthi isolates from different European localities / J. Vukojevic, F. Dubravka, M. Mihajlovic // Plant Pathology. – 1995. – № 10. – P. 173-738.

[46] Siches, B. Phomopsis du tournesol: mise au point d ′une methode de lutte chimicue dans l ′Aude. / B. Siches, G. Fretay, P. Bernard, J. Tamas // Phytoma. – 1989. – № 38. – P. 28-34.

[47] Peres, A. *Diaporthe helianthi* Munt.Cvet. et al.: Elements de biologie et d'épidémiologie appliqués aux essais de lute / A. Peres, Y. Regnault // Proc. 12th Int. Sunflower Conf. – ISA: Novi-Sad (Yugoslavia), 1988. – Vol. 2. – P. 90–95.

[48] Assemat, P. Biological culturale ef differeenciation des peritheces in vitro cher Diaporthe (Phomopsis) helianthi Munt.-Cvet. / P. Assemat, J. Fayret // Abstract book «Proc. 12th International Sunflower Conference». – Yugoslavia, 1988. – Vol. 2. – P. 84-89.

[49] Herr, L.J. Diaporthe stem canker of sunflower / L.J. Herr, P.E. Lipps, B.H. Walters // Plant Disease. – 1983. – № 67. – P. 911-913.

[50] Yang, S. A new sunflower disease in Texas caused by Diaporthe helianthi / S. Yang, R.W. Berry, E.S. Luttrell, T. Vongkaysone // Plant Disease. – 1984. – № 68. – P. 254-255.

[51] Skripka, O.V. The results of the examination for the detection of sunflower phomopsis / O.V. Skripka, L.I. Shcherbakov et al. // Problems of plant quarantine in Russia. - 1991.– No. 1. – P.p. 103-109.

[52] Tourvieille, D. Jugement de la resistance du tournesol au phomopsis saus tunnelen lilet avec humectation controlee / D. Tourvieille, C. Pelletier // Centre Techn. Interprof. Oleagineux Metrop. Paris. – 1988. – № 103. – P. 28-29.

[53] Yakutkin, V.I. Methods for identifying and accounting for sunflower phomopsis / V.I. Yakutkin // Collection of guidelines for plant protection. - St. Petersburg, 1998.—P.p. 191-207.

DOI: 10.37200/IJPR/V24I5/PR2020188
Received: 12 Mar 2020 | Revised: 28 Mar 2020 | Accepted: 06 Apr 2020