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A STUDY ON THE SPOROGENESIS AND PATHOGENESIS OF *Sclerotinia sclerotiorum*

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SUMMARY

One of the most important pathogen of our field and culture plant production is *Sclerotinia sclerotiorum* (white mold).

In case of its epidemic spreading it causes significant production losses in sunflower fields.

For this reason we consider it important to know more about the generative reproduction cycle and the interaction of ecological conditions to this pathogen.

To establish the above we have conducted laboratory and field studies during various phenophases of the sunflower. For our experiment we used various genotypes (NK-254, Remil, NS-H-26, GK-70 and SP-1) and have found that the development of *Sclerotinia sclerotiorum* infection very much depends on the phenological state of the host and the microclimatic conditions.

From the infection point of view the dampness of the surroundings and the light conditious (1500-1600 lux is the optimum) were decisive.

The sporulation of the fungus is found at 60-95% relative humidity of the air and the spores germinate in 4-5 hours at 18-25°C.

According to our observation for the mycelial imbedding in the host's tissues the leaves must be wet up to 30-35 hours. On the other hand, regular interruptions of wet period can result in the failure of infection. From the development of epidemic point of view the most critical period is the state of the beginning of the generative phases of budding, blooming and yellow ripening.

Presently, depending on the meteorological factors, the spraying of sunflower plants is justified.

The dampness of suroundings is vital in this regard.

Key words: *Sclerotinia sclerotiorum*, sporogenesis, pathogenesis, sunflower

INTRODUCTION

Sclerotinia sclerotiorum (white rot) belongs to those "multi-host" pathogens which cause significant quantitative and qualitative production losses in many field crops (Molnáré and Vörös, 1963; Békési, 1980; Vörös, 1983.). According to the recent observations, the fungus is capable to parasitise even the young branches of ligneous plants (Ratkos 1986).

The pathogen can infect the plants in two ways. On one hand from the sclerotia in the soil-which is the over-wintering structure of the fungus-micelia develop and infect the vegetative and reproductive surfaces in contact with the soil. On the other hand by the ascospores getting free from the productive bodies, in other words, by the generative reproductive structures which, germinating on the surface of plants, through their enzymes infect the host plants, by embedding into their tissues (Krexner, 1969; Krüger,

1975; Adams and Tate, 1976; Schwartz - Steadman, 1978; Lamarque, 1981; Ratkos et al., 1983). The following ecological factors are highlighted by some authors for the development of apothecia: light and dampness (Honda and Yunoky, 1975; Morell, 1977), the depth of sclerotia in the soil (Williams and Western, 1965; Krüger, 1975), the biological characteristics of the cultivated plants (Williams and Western, 1965), chemical cultivation of soil (Partyka and Mai, 1958, 1962; Jones, 1974), agrotechnical cultivation of soil (Stevens and Hall, 1911).

The ascospores getting free through the explosive movement of apothecia was described by Ratkos (1982).

The use of an integral plant protection technology against the pathogen was indicated by Békési et al., 1980, Vörös, 1983, Ratkos, 1983; 1986; Ratkos et al., 1985.

The importance of genetical protection was highlighted by Vranceanu (1977), Vear and Guillaumin (1977), Lamarque (1981), Tourvieille-De Labrouche (1978), Platonov (1984).

About the importance of agrotechnical protection wrote Petrócz (1982) and Merriman et al. (1979), underlining the hygienic requirements of the plants.

The significance of microclimatic conditions was brought to light by Molnárné and Vörös (1963), Szepessy (1975), Partyka and Mai (1962). Huang (1977), Wörös (1983), and Litkei (1986) wrote about the role and importance of the antagonist and hyperparasitic organisms when studying the possibility of working out biological protection measures. As concerns the chemical protection against the pathogen, diklozolin, inprodion, procymidon, vinklozolin (Enisz, 1985), benlate, karboxin, enovit (Aćimović, 1977), benlate (Marić et al., 1982.), were found to play a prominent role.

In the interest of improving the efficacy of the protection, Enisz (1985), Vörös et al. (1986), Milinkó et al. (1986) wrote about a need to know more about the biology of the pathogen and to work out protection measures. Useful information are at the disposal of specialists, but there are still some blank areas, as for example, the insufficient knowledge of certain parameters of the ecological conditions affecting the host - parasite relationschip, which make it hard to discover those qualitative interrelations which exist between the phenological susceptibility of plants, the reproduction cycle of *Sclerotinia sclerotiorum* and the climatic conditions (Ratkos et al., 1986).

The aim of our research was to find out under what enviromental conditions do apothecia develop and ascospores infect the sunflwer plants, in order to be able to use inoculation tests with similar result as the spontaneous infection and to work out an effective protection technology based on forecasting.

MATERIALS AND METHODS

On the request of the Vegetable Oil and Detergent Producing Enterprise and under the supervision of the Plant Protection Research Centre of the Hungarian Academy of Sciences, tests were carried out by the Research Centre of the Seed Producing and Marketing Company of Nyiregyháza in cooperation with the University of Agricultural Sciences of Debrecen and the Plant Protection and Agrochemical Station of Szabolcs-Szatmár County. The tests vere conducted in laboratory, infection nursery and under field conditions.

The work can be summarized as follows:
- showing up the functional apothecia, i.e., sporogenesis tests;
- inoculation experiments to study the process of pathogenesis;
- determination of infection perod in the field.

The sunflower was used for our inoculation tests and field observations because the pathogen can cause greatest damage to sunflower crops. For the observations of *Sclerotinia sclerotiorum* sporulation we have collected sclerotia from spontaneously infected fields.

In the winter of 1984, to ensure the carpogenesis, we overwintered the collected material in an outside isolator. The planting was done on January 1985. After the infection, the sclerotium was transplanted into perlit with 5-10 cm thickness. After this we put it in a foil-hut. The temperature and light in the foil-hut changed according to the outside daily conditions. We ensured dampness by occasional water spraying depending on temperature change. Of the environmental conditions we observed the temperature, light and relative humidity changes. The development of apothecia was closely followed (Table 1). We recorded their color change and ascospore productivity in function of their lifetime. To collect ascospores, a ventilated spore-trap was used (Ratkos, 1983) The daily rhythm of sporulation was observed and the apothecium and ascospore production measured.

The spores collected in spore traps were blended in 2 ml distilled water, then the concentration was established with the help of Bürker-chamber. The sporulation time was checked in function of the concentration.

We checked the flora of sclerotia and apothecia and identified the flora's elements. The study of the pathogenesis of *Sclerotinia sclerotiorum* was done with the inoculation of sunflower cultivar GK-70 under foil-hut. Sunflower was planted intermittently so at the time of inoculation we had plants with 2-4 and 4-6 leaves. The inoculation was done with $4x10^4$ spores/ml suspension, on 3x50 plants. The time period of leaf dampness-coverage after inoculation was solved by changing the surroundings of plants. In this way the infected plants' dampness coverage was altered every five hours. In one case, between the 15th and the 20th hour we put in a dry period.

The surface of plants was dried by switching light on them (Table 2).

Similarly to the sporogenesis test, we measured the environmental conditions with thermo-hygrograph, lux-meter, soil-thermometer, maximum-minimum thermometers (Table 2).

We studied in an infection nursery the phenological susceptibility responsiveness of the sunflower.

The climatic conditions were ensured by water spraying (Ratkos et al., 1986). Prior to the development of symptoms we measured the surface dampness of the plants by DEFI-DOFA instrument, DEW DINAMICS SYSTEM microprocessor controlled measuring instruments and LUFT instrument. The first instrument was developed by the Plant Protection Department of the University of Agricultural Sciences of Debrecen on the basis of research done by Dr. István Szepessy, professor and head of the above department. The other two instruments were handed to us by the pathology group of NAA Sz. Sz. M. with which they carried out earlier tests.

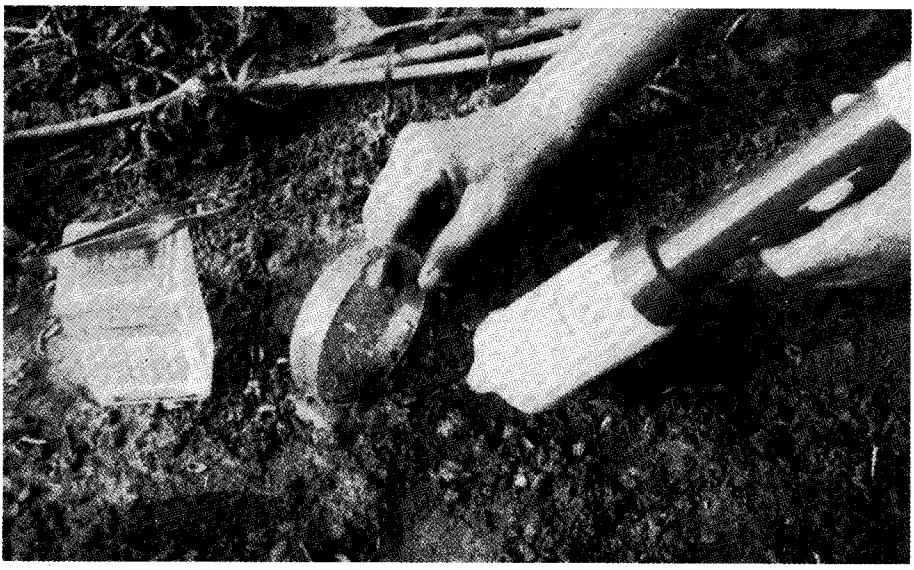

Figure 1. Collection of ascospors of Sc.sc. by spore-trap.

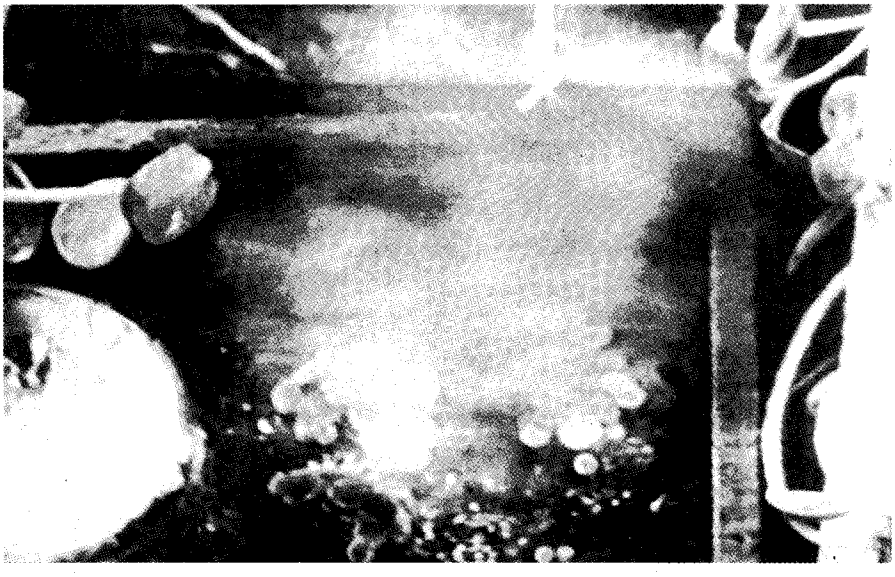

Figure 2. Sporulation of Sclerotinia sclerotiorum (Lib.) de Bary.

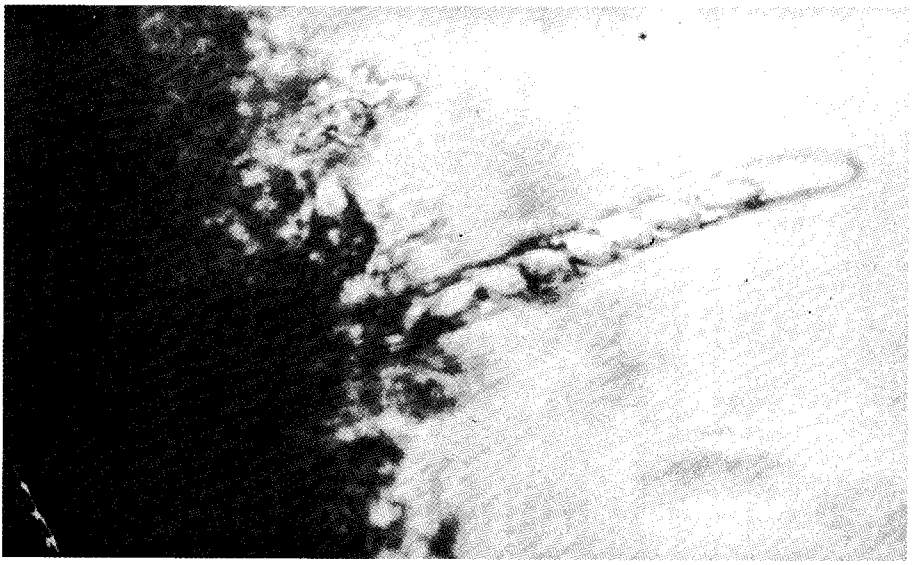

Figure 3. The ascus of Sc.sc. with 8 ascospores inside it.

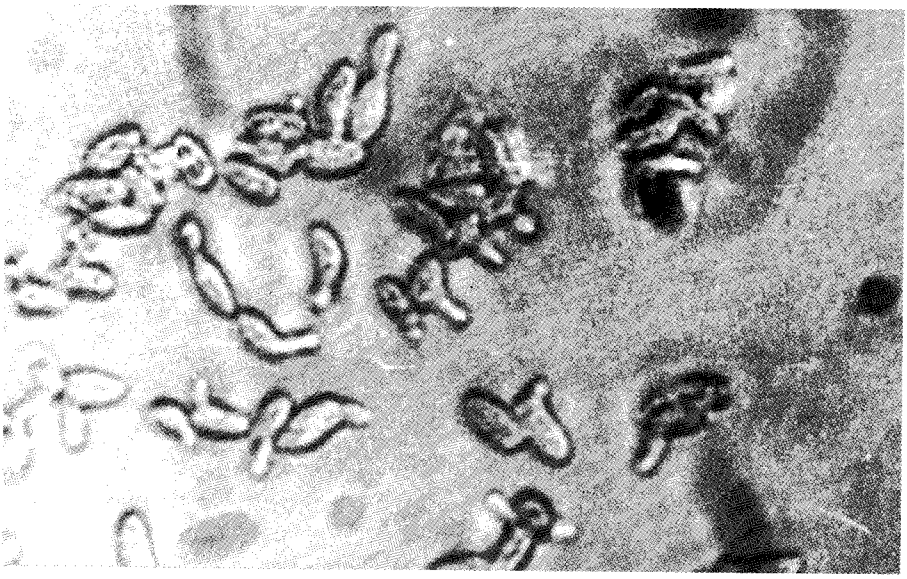

Figure 4. Germinating ascospores of Sclerotinia sclerotiorum (Lib.) de Bary.

The inoculation of sunflower hybrid NK-254 was performed on the 23rd of June (star bud), on the 5th of July (4-5 cm flower bud) and on the 4th of August (half blooming).

We sprayed 3 ml suspension over the leaf, stem, as well as the head. The tests were done on 70x30 cm areas of each of the 32 plants, four times. (Table 3). The same method was used for the simultaneous inoculation of different genotypes. At the opening of the 3rd-4th flower-ring we sprayed 3 ml suspension containing $4x10^4$ spores/ml, over the blossoming heads (Table 4). The genotypes tested were Remil, GK-70, SP-1, NSH-26. We did the inoculation on the 4th of August. The tests were evaluated by variance analyses and the symptoms by inoculation index.

RESULTS AND DICUSSION

The results of the tests carried out under laboratory conditions proved that the over-wintered sclerotium developed apothecia between 10-30°C, the most intensively, between 15°C-25°C and at 85-100% relative humidity. Decisive factors were the soil moisture and the light conditions. Acid and loose type of soils were less favorable, but alkaline soils proved more favorable for producing bodies.

Under ideal conditions the apothecia develop in perlit as well as in soil. The perlit dries out earlier and needs watering more often. Its advantage is that the apothecia are less prone to parasitation then in the soil. The development of apothecia is intensive at 1500-1600 lux (average) light density. In case of less light density, the apothecia are smaller, the stem of apothecia is longer, elongated, similar to the plants produced with less light.

The 3-5 cm soil coverage is ideal. There is no correlation between the shape, size and the number of apothecia except the extreme values. Their life span (the product of a single sclerotium) is 3-4 weeks under balanced conditions. The older apothecia are darker colored and usually infected by saprophytic flora.

The ascospore production from the fully developed apothecia begins 1-3 days after the first appearance. The development is fairly balanced. The sporulation will happen in turgor state with minimum air movement. The microclimate around the apothecia is decisive. According to the measurements, 1% drop in relative humidity will bring about the sporulation. But above 90% relative humidity there is no sporulation. If apothecia are wet, the sporulation again will not take place. In 12 hours the apothecium produces 1-3 ascospores, but with less intensity (Table 1).

A single apothecium produces $2x10^6$ spores on an average. During 4-5 hours the ascospores germinate.

The optimum pH for germination is between 6-7. The development of the mycelium is most intensive between 16-24°C. At high water-column, above 1 cm, the germination will stop. Sticking surface is vital for the germination; at 10^6-10^7 ascospores/ml concentration, self-antagonism will occur and there will be no germination.

Table 1. - Apothecia development of *Sclerotinia sclerotiorum* in foil hut between May 20, and June 24, 1985

Planting: January 10 1985. (ISOLATOR)
Transplanting: April 10 1985. (FOIL-Hut)
Number of apothecia groups: 574 db.
Number of apothecia: 3895 db.
Average number of apothecia: 6785 db.

1.	Light brown:	1104 db	28.34 %		$/1^+/$
2.	Dark brown:	809 db	20.77 %	64.33	$/2^+/$
3.	Grey-brown:	593 db	15.22 %		$/3^+/$
4.	Grey:	159 db	4.82 %		$/1^-/$
5.	Grey-black:	109 db	2.79 %	35.67	$/2^-/$
6.	Black:	1083 db	27.17 %		$/3^-/$
7.	Pink:	38 db	0.97 %		$/4^-/$

$1^+,2^+,3^+$ = healthy
$1^-,2^-,3^-,4^-$ = stem infected
Climatic conditions:

air temperature	16-25°C
soil temperature	10-15°C
relative humidity	75.90 %
light intensity	1500-1600 Lux.

At room temperature the ascospores can keep their vitality up to 3-4 weeks. During this time amino-acids and carbohydrates improve the germination. If the hyphae dry out they lose their vitality. Studying the climatic conditions for the infection, the results showed that the relative humidity during 35-40 hours after infection must be above 85%. During this time the leaf dampness coverage is the basic condition for the mycelium to imbed into the tissue. Until the imbedding, the interruption of the wet period could bring into question the outcome of the infection.

Table 2 - Inoculation of GK-70 sunflower genotype with *Sclerotinia sclerotiorum* ascospore suspension between May 20 and 25, 1985

Leaf dampness coverage (hours)	Infected plants %	Infection index (I.I.)
5-20	4.66	1.21
25	11.33	1.73
30	45.37	2.68
35	76.71	3.12
40	82.00	3.41
45	88.66	3.88
0 - 15 20 - 45 (15 - 20 dry)	12.15	2.11

After the inoculation performed at seedling stage under ideal conditions (90% relative humidity, 1500-1600 lux light density, 15-20°C temperature), the symptoms

Table 3. Inoculation of NK-254 sunflower hybrid by *Sclerotinia sclerotiorum* ascospore suspension in isolator-tunnel apparatus 1985.

Time of Inocul.	Phenophase Time of observation	STAR BUD				GREENBUD				Flower		Lemon ripening		Full ripening	
		VI.30.		VII.10		VIII.25.		VIII.5		VIII.15.		VII.25.		VIII.31.	
		%	Fi	%	Fi	%	Fi	%	Fi	%	Fi	%	Fi	%	Fi
VI.23.	Star bud	14.06	1.21	26.78	2.03	32.03	2.12	35.15	2.24	50.78	2.4	50.78	2.45	53.12	2.65
VII.10.	Green bud	4.65	1.41	6.25	1.34	17.81	1.65	19.36	1.93	54.68	2.18	57.81	2.44	61.71	2.89
VIII.5.	half flowering	3.12	1.20	3.90	1.24	6.25	1.45	14.06	1.42	32.81	1.88	64.84	2.43	66.46	3.44
Ø	–	3.90	1.18	5.46	1.15	7.81	1.32	12.14	1.54	20.31	1.69	28.14	2.00	35.46	2.21
infected plants in %															

After inoculation:

1. week (VI.30, VII.25, VIII.15.) $LSD_{5\%} = 2.79$

2. week (VII.10, VIII.5, VIII.25.) $LSD_{5\%} = 6.81$

3. week (VII.25, VIII.15, VIII.31.) $LSD_{5\%} = 6.97$

Note: Fi. Index of rate of infection.

developed in 4-5 days and the development of fungus was intensive. With aging, at the stage of 2-4-6 leaves, the appearance time of symptoms increased (Table 2).

We have tested the greatest susceptibility of sunflower to *Sclerotinia.*

Following the inoculation on June 23, the infection was 14,06% by June 30 and 26,78% by July 10. As a result of inoculation on July 10,17% of plants showed sings of infection. This value changed to 54,68% by August 5. As a result of the August 5 inoculation the infection by August 15 was 32,81% and by August 25 it was 64,84%.

Table 3 also contains the data of infection index from which the dynamic of infection can be followed. The apothecia appeared on June 11, 1987 in the infection nursery as well as in the field. Their number in the infection nursery was not significant because the sunflower was at the 2-3 leaves stage. The light coverage of soil did not allow the development of apothecia *clusters* in large numbers.

In the field at the beginning of star bud stage there started the *closing* of foliage and the less light coverage of soil is more favorable for the fungus.

Prior to the apothecia appearance 28,5 mm rain fell and there were 5,94 sunny hours, 15,2°C average temperature, 77,8% relative humidity, and the average soil temperature in 5 cm soil was 20,74°C.

The sporulation is most intensive between 8-10 am when the dew dries up.

After rain because of *ascending* air-flow this can happen in any part of the day.

During sporulation the movement of *spores* in the air can be followed by eye within 30-60 cm. (Figure 2)

The drying up of leaf surface roughly coincided with the beginning of sporulation.

The response of the tested genotypes to the inoculation differed in dependence of the genotype and the inoculation method applied.

According to the data in Table 4 the least infected is the French hybrid Remil.

On the basis of the results of the first observation (August 15) following the inoculation, it can be stated that the appearance of *symptoms* was most dynamic and it happened earliest on the blossoming head of the genotype SP-1. The tubular flowers died, the flower stalk became darker, in some places the micelium could be seen.

Tab. 4. - Inoculation of various genotype sunflowers by *Sclerotinia sclerotiorum* ascospores suspension in isolator-tunnel apparatus August. 1985.

Inoculation: 4th of August.

Time of observation	Genotype				
	Remil	GK - 70	SP - 1	NSH - 26	$LSD_{5\%}$
VIII.15.	4.68	4.68	19.53	3.90	3.32
VIII.20.	49.21	64.84	57.03	52.34	4.41
VIII.25.	50.78	66.40	57.81	57.81	4.56
VIII.31.	51.56	65.53	69.53	64.06	5.13
	Remil in %				
VIII.15.	100	100	417.30	84.61	
VIII.20.	100	131.76	115.89	106.36	
VIII.25.	100	130.76	113.84	113.84	
VIII.31.	100	134.85	134.85	124.24	
Average:	100	124.34	195.47	107.26	

It the other genotypes these symptoms were not so expressive except for *brown* coloring on the flower stalk and the loss of turgor of tubular flowers. On August 20 the infection values were close to each other, the difference being attributable to the advancement of pathogenesis.

On the head of SP-1 the micelium infection is excessive, while on the other genotypes it was in traces.

During the 3rd and the 4th survey, wet sunken spots and first sclerotia could be seen on the back of the infected heads.

Regarding the strength of infection there are no spectacular differences. But in the infection percentage mathematically significant differences from the first observation can be found:

1. $LSD_{5\%} = 3{,}32$ 2. $LSD_{5\%} = 4{,}41$
3. $LSD_{5\%} = 4{,}56$ 4. $LSD_{5\%} = 5{,}13$

When studying the sunflower and *Sclerotinia sclerotiorum* host-parasite relationship we must emphasize the sclerotium, ascospore, phenological responsiveness of sunflower and from the ecological conditions the humidity, light and soil temperature conditions.

Between the size of sclerotium and the number of apothecia there can not be found a close relationship. The temperature is not important but the light and dampness are vital.

If the soil is wet but exposed to bright light, the apothecia develop harder.

If the light density drops, then the formation of apothecia starts. Below 1500 lux. the apothecium becomes deformed. Sporulation can be found only at 60-85% relative humidity. The severed apothecia are still functioning up to 24 hours and can be kept for a week. (Figure 3). At room temperature the spores can stay viable for one month, although their germination capacity decreases. For the germination of spores 4-5 hours are needed at 18-25°C.

The phenological susceptibility of sunflower is changeable. It is more responsive at seedling stage then at star-budding, green-budding and at half-blooming.

By the inoculation of the head significant differences can be induced between the genotypes.

Our experiments produced new information profitable for the practical production.

The wider knowledge of the biological and environmental factors of the sporogenesis and patogenesis ensure the possibility of working out the prevention.

To ensure genetical protection we must discover the susceptibility differences by the help of a complex diagnostical method.

The base of this diagnostical method is the application of an inoculation method very close in parameters to the spontaneous infection.

To establish the susceptibility of the stem $4x10^3$ - $4x10^4$ ascospore/ml concentration of inoculum must be put on it at seedling, star bud and green bud stage.

The inoculation of the head at half blooming required the concentration described above.

The theoretical base for the practical use of this method is the assumption that the agronomical responsiveness points of the sunflower are connected to the differentiation of flower stalk, the beginning of budding and pollination.

The infection takes place at star-bud stage on the top leaves, at budding on the upper third of the stem, and at blooming on the flowering side of the head.

The responsiveness of the basal stem can be established by soil infection during the vegetation.

In every phenological stage mentioned the stimulation effect of endogenous materials prevails in the establisment of the host-parasite relationship.

For the chemical protection we got new information about the interaction of ecological conditions of pathogenesis and sporogenesis of *Sclerotinia sclerotiorum*.

The reproduction cycle of the fungus can be expected in the first two weeks of June at 15°C soil temperature, 70% relative humidity, 4-6000 light density, 14°C air temperature (the values are matematical averages.) The sporulation can be expected after 3-5 rainy and humid days. The apothecia will develop in 2-3 days and the sporulation will happen at 60% relative humidity. Pathogen structures are being put in the air for 2-3 weeks by one generation.

According to our measurements, 30-35 - hour leaf dampness coverage is needed for the germination of ascospores and their imbedding into the tissue.

The interruption of wet period will result in the failure of infection.

Our study shows that the generative reproduction cycle of the *Sclerotinia sclerotiorum* can be characterized by 3-4 culminating points.

June 10-20, June 25 - July 15

July 25 - August 10, August 25 - September 10

According to our observation on the bases of phenological susceptibility of sunflower the critical periods coincide with the culminating points of the sporulation.

For this reason we recommend to do the surface plant protection in the following sequence: starbud stage, beginning of bud, half-blooming, third lemon ripening.

The justification of foliage treatment depends on those meteorological factors which determine the moisture content in the enviroment.

Tab. 5. - Meteorological data in the field 5 days prior to the appearance of apotecia

Date	Total rain (mm)	Number of sunny hours	Leaf dampness coverage hours	Air temp. °C	Relative humidity %	Soil temp. °C
June 5-10	28.5	8.32	14.5	21.84	75.4	22.78
July 1-5	17.3	7.46	14.1	16.82	72.8	21.50
July 20-25	8.2	9.16	14.2	19.54	70.2	24.06
August 5-10	6.8	2.32	14.3	18.34	78.86	19.98

CONCLUSIONS

One of the most important pathogen of our field and culture plant production is Sclerotinia sclerotiorum (white mold).

In case of its epidemic spreading it causes significant production losses in sunflower fields.

For this reason we consider it important to know more about the generative reproduction cycle and the interaction of ecological conditions to this pathogen.

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Presently, depending on the meteorological factors, the spraying of sunflower plants is justified.

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ESTUDIO SOBRE LA ESPOROGENESIS Y PATOGENETIS DE *Sclerotina sclerotiorum*

RESUMEN:

Uno de los patógenos mas importantes de campo es *Sclerotium sclerotiorum* (moho blanco). En caso de su expansión epidémica causa pérdidas significativas de producción en las plantaciones de girasol por esta razón es importante conocer mas a cerca del ciclo genrativo de reproducción y la interacción de condiciones ecológicas de este patógeno. Para establcer esto se han conducido estudios de laboratorio y campo durante varias fases en girasol. Para nuestro experimento fueron utilizados varios genotipos (NK-250, REMIL, NSH-26, GK-70 y SP-i) y hemos encontrado que el desarrollo de la infección de *Sclerotinia sclerotiorum* depende mucho mas del estado fenológico del huesped y de las condiciones microclimáticas desde el punto de vista de la infección la humedad de los alrededores y las condiciones de luz (1500-600 Lux is optimo) fueron decisivos. La espurulación del hongo fue encotrada a 60-95% de humedad relativa del aire y las esporas germinaron en 4-5 horas a 18-25°C. De acuerdo con nuestras observaciones para el empotramiento del tejido micelial del patógeno las hojas deben estar mojadas hasta 30-35 horas. Por otra parte la interrupción regular de periodos húmedos puede resultar en el fallo de la infección. Desde el punto de vista del desarrollo epidémico el periodo más crítico es el estado del comienzo de la fase generativa, boton, floración y amarilleamiento en madurez. En este momento dependiendo de factores metereológicos la pulverización de las plantas es justificada. La humedad ambiental es vital desde este punto de vista.

ETUDE DE LA SPOROGÉNÉSE ET DE LA PATHOGÉNÉSE DE *Sclerotinia sclerotiorum*

RÉSUMÉ:

En raison de la menace que constituent les épidémies de *Sclerotinia sclerotiorum* pour les rendements du tournesol, il est important de mieux connaitre le cycle biologique et les interactions des conditions écologiques sur ce parasite.

A partir de différents génotypes (NK 254, Remil, NSH 26, GK 70 et SP-1) nous avons montré en champs et en laboratoire que le développement de l'infection de *Sclerotinia sclerotiorum* est fortement dépendant du stade phénologique des plantes et des conditions microclimatiques.

L'humidité et les conditions lumineuses (optimum de 1500-1600 lux) jouent un rôle prépondérant dans l'établissement de l'infection.

La sporulation du champignon s'accomplie sous une humidité relative variant de 60 à 95% et la germination s'effectue en 4 - 5 heures à 18-25 degrés.

Pour la pénétration des tissus par le mycelium, les feuilles doivent être humidifiées, une interruption de l'humectation foliaire pouvant empêcher l'infection.

D'un point de vue épidémiologique, la phase la plus critique se situe entre la boutonisation et la floraison. Pendant cette période, et en fonction des conditions météorologiques (humidité du milieu environant) un traitement des plantes peut être justifié.