INVESTIGATIONS RELATED TO THE PROGNOSIS OF SUNFLOWER STEM CANKER *(DIAPORTHE-PHOMOPSIS)*

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Among the main pathogens which have raised problems in Europe, in the last five years one could include the parasite *Diaporthe helianthi* Munt.-Cvet. et al. with its conidial form *Phomopsis helianthi* which causes stem canker.

The pathogen was observed for the first time in a sunflower crop in Vojvodina, Yugoslavia (Mihalčević et al., 1980) and later on was also detected in Hungary (Mémeth et al., 1981), Bulgaria (Ignatov, 1984) and France (Lamarque, 1985). In Romania, the pathogen was identified in Bihor County in 1981 (Iliescu and Csép, 1982). Research works performed in Yugoslavia (Muntanola-Cvetković, 1981; Acimović and Straser, 1981) and in Romania, (Ciurea et al., 1983; Iliescu et al., 1983 a; Ioniţă et al., 1985) clarified some aspects related to the fungus biology and pathology, as well as to the prevention and control methods, Herr et al. (1983), described this disease in Ohio—U.S.A. considering the possible similitude between the pathogen observed in Europe and that one described in North America. The works undergone in Yugoslavia (Marić, 1984) and in Romania (Iliescu et al., 1983 b; Baicu et al., 1985), tried to establish the range of fungicides efficient in controlling *Diaporthe-Phomopsis* as well as the necessary equipment, according to the vegetation phases of treatment application.

In the framework of that integrated control, warning of chemical treatments represents an important link which leads directly to reduced pesticide and energy consumption and at the same time avoids environmental pollution.

Literature does not mention any results obtained in the field of disease prognosis and warning of treatment application.

This paper based upon information about parasite biology and ecology tries to establish the main elements required for the prognosis of the fungus development in a certain period of time, with a view to determine the adequate moments when chemical treatments should be applied.

MATERIALS AND METHODS

The biological material used in laboratory works originated from the north-western part of the country where the attack level caused by *Diaporthe-Phomopsis* has reached its maximum intensity.

The limiting temperatures as well as the optimum ones for *in vitro* growth and development of the pathogen were established on PDA medium at a pH=6.5—7.0, the determinations being done at an interval of 72 hours. In order to get information about the influence of the environmental factors upon the perithecia development, asci maturation and ascospores release, as well as upon pycnospores on natural substratum, segments of sunflower stems which showed disease symptoms were kept and stored for overwintering under the following variants:

— vertical position at soil surface (V_1);

— horizontal position at soil surface (V_2);

— buried horizontally in the soil, at a depth of 5 cm (V_3), 10 cm (V_4), 15 cm (V_5), 20 cm (V_6), 25 cm (V_7), 30 cm (V_8).

The experiments were carried out during 1982—1983 and 1983—1984, in four replications, in different sunflower fields in Bihor County and Ilfov Agricultural District where a heavy attack of stem canker was registered in the previous year. Periodically, beginning with January, samples were taken from each variant, examining both the dynamics of perithecia growth and maturation under natural conditions and the parasite viability and capacity to form mature perithecia, after keeping them for 30 days in moist chambers, at 20°C.

The test of viability was carried out monthly, using the samples taken out during the second decade of each month (January—July). The moment of perithecia maturation and ascospores and pycnospores release was estimated by installing blades covered with vaseline as traps at the wintering places. The reading of the blades was carried out each five days establishing the time when the charge of *Diaporthe-Phomopsis* propagules in the air was maximum. The data related to the biology of the fungus obtained in laboratory were correlated with those obtained under natural conditions in 1982—1984 and with the meteorological data specific to each year. Blunck's equation was used in order to establish the development duration after estimating the thermic constants of the parasite growth and development *in vivo* and *in vitro* (Săvescu and Rafailă, 1978):

$$K = X_n (t_n - t_o)$$

where the development constant (K) represents the product between the development duration (X_n) and the effective temperature ($t_n - t_o$).

RESULTS AND DISCUSSION

The laboratory investigations related to the influence of temperature upon the growth and development of *Phomopsis helianthi* (Table 1) revealed that the microorganism began its biological activity at 14°C, a temperature that could be considered as the thermic threshold of growth or minimum biological threshold (t_o); it developed adequately at 26°C and its growth was stopped at 32°C (the maximum biological threshold=T). Sporulation began at 18°C (the low thermic threshold for sporulation t_n=18°C), temperature assimilated with the threshold of prolificity (O) with its optimum at 26°C (O_1).

Observing in the laboratory the sporulation process at 18°C (O) and 26°C (O_1) one could note that, in the first case, the ascospores were formed after 63 days while in the second case the sporulation period was of only 21 days. Therefore, the development duration depended on temperature at a relative humidity of over 90%, being of 21 days at the optimum temperature of 26°C (O_1) and of 63 days at the temperature of 18°C (O). In both cases, the sum of the optimum temperatures which exceed 14°C remained stable and it cumulated 252°C, which represents the development constant (K).

According to Blunck's formula,

at t_n=18°C, K=63 (18—14) =252°C.
at 0_1=26°C, K=21 (26—14) =252°C.

The equation can be represented by a hyperbola (Figure 1). Thus the length of development duration as well as the time of maturation beginning and ascospore release can be determined on the basis of temperature, taking into account the formula:

$$X_n = \frac{K}{t_n - t_o}$$

The results obtained *in vitro* were confirmed by those obtained under field conditions. From data presented in Table 2 and Table 3 referring to the dynamics of perithecia maturation after the parasite overwintering under different field conditions it came out that in 1983, the first perithecia began to appear on the buried stems, even from the end of April, but their maturation took place in the third decade of May only on stems buried at a depth of 5 cm or on those which were horizontally placed at the soil surface. The stems which were deeper buried began to decay, the parasite being removed at the same time with the decomposition of the substratum.

Table 1

Influence of temperature on *in vitro* growth and development of *Phomopsis helianthi*

T°C	Colony diameter (mm) at: 3 days	6 days	9 days	12 days	15 days	18 days	21 days	Sporulation (21 days)
10	0	0	0	0	0	0	0	—
12	0	0	0	0	0	0	0	—
14	0	0	3	5	7	8	9	—
16	0	8	20	30	35	45	50	—
18	0	15	25	33	45	50	70	*
20	9	29	40	50	70	70	70	***
22	15	32	70	70	70	70	70	***
24	16	50	70	70	70	70	70	***
26	15	34	70	70	70	70	70	***
28	13	33	70	70	70	70	70	**
30	6	25	30	43	43	43	43	*
32	0	0	5	10	10	10	10	—
34	0	0	0	0	0	0	0	—

— = lack of sporulation.
* = low sporulation.
** = medium sporulation.
*** = heavy sporulation.

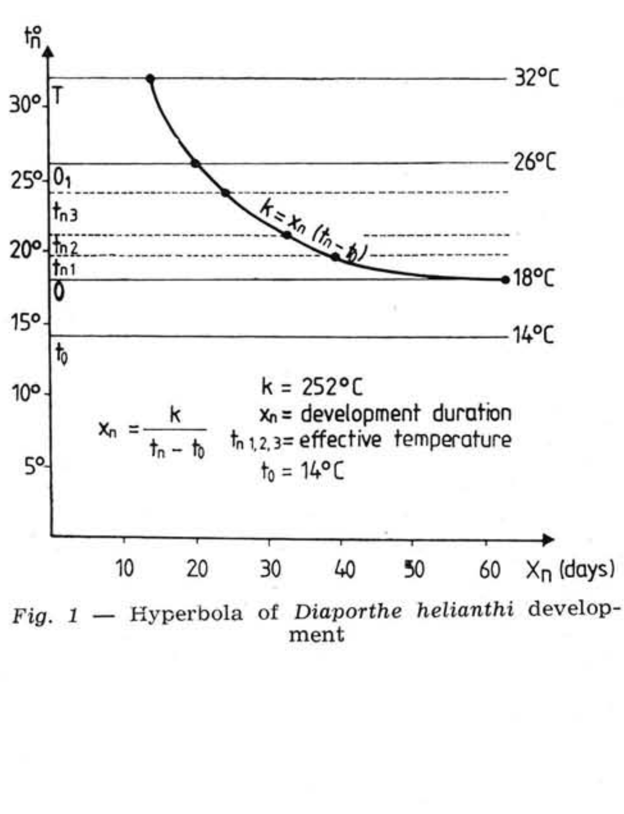

Fig. 1 — Hyperbola of *Diaporthe helianthi* development

Table 2

Dynamics of setting and maturation of *Diaporthe helianthi* **perithecia on sunflower stems kept under various field conditions, 1983**

Variants	March decade			April decade			May decade			June decade		
	I	II	III	I	II	III	I	II	III	I	II	III
At soil surface :												
V_1 vertical stems	—	—	—	—	—	—	—	—	—	—	±	±
V_2 horizontal stems	—	—	—	—	—	—	—	±	+	+	+	+
Stems buried at :												
V_3 5 cm	—	—	—	—	—	±	±	±	+	+	0	0
V_4 10 cm	—	—	—	—	—	±	±	±	0	0	0	0
V_5 15 cm	—	—	—	—	—	±	±	0	0	0	0	0
V_6 20 cm	—	—	—	—	—	±	±	0	0	0	0	0
V_7 25 cm	—	—	—	—	—	±	0	0	0	0	0	0
V_8 30 cm	—	—	—	—	—	±	0	0	0	0	0	0
T°C	2.9	4.8	11.2	11.4	10.1	15.7	15.4	20.2	19.3	19.8	16.2	19.8
P mm	—	—	1.2	13.1	5.2	0.5	1.4	1.8	63.3	6.6	76.6	22.7
R H %	74.3	57.0	65.2	78.5	77.8	64.2	67.4	60.2	77.6	66.4	50.3	73.4

— = lack of perithecia.
± = perithecia under setting.
\+ = mature perithecia.
0 = rotten stems.

Table 3

Dynamics of setting and maturation of *Diporthe helianthi* **perithecia on sunflower stems kept under various field conditions, 1984**

Variants	April decade			May decade			June decade			July decade		
	I	II	III	I	II	III	I	II	III	I	II	III
At soil surface :												
V_1 vertical stems	—	—	—	—	—	—	—	—	±	±	±	+
V_2 horizontal stems	—	—	—	—	±	±	±	±	+	+	+	+
Stems buried at :												
V_3 5 cm	—	—	—	—	±	±	+	+	0	0	0	0
V_4 10 cm	—	—	—	—	±	±	+	0	0	0	0	0
V_5 15 cm	—	—	—	—	±	±	+	0	0	0	0	0
V_6 20 cm	—	—	—	—	±	±	0	0	0	0	0	0
V_7 25 cm	—	—	—	—	±	±	0	0	0	0	0	0
V_8 30 cm	—	—	—	—	±	0	0	0	0	0	0	0
T°C	8.7	9.4	6.2	12.8	17.0	18.6	19.0	17.0	19.0	17.7	22.0	18.3
P mm	29.1	23.0	28.2	1.0	24.5	18.1	68.1	9.0	24.0	36.1	18.0	20.0
R H %	76.0	76.0	80.0	70.0	77.0	78.0	69.0	78.0	72.0	77.0	74.0	75.0

— = lack of perithecia.
± = perithecia under setting.
\+ = mature perithecia.
0 = rotten perithecia.

In 1984, the first perithecia developed only in the second decade of May to the variants buried deeper, their maturation taking place during the second decade of June. On the buried vegetal residues, the pathogen remained viable until the first decade of June. Furthermore, the attacked sunflower stem fragments were decomposed under the influence of biotic and abiotic soil factors and consequently the parasite disappeared (Table 4). These results confirm the ones obtained by V ö r ö s (personal communication) who considered a good measure the deep burial of the residues of the attacked sunflower plants in order to exhaust the natural inoculum in soil.

Examining the meteorological conditions in 1983 and 1984 (Tables 2 and 3) it could be observed that at the end of May and beginning of June there were heavy rainfalls, therefore the main part in perithecia maturation was played by temperature. Really, the lower temperature values which were characteristic to April—May—June period of 1984 negatively influenced the development duration (X_n) of the parasite thus delaying the time of ascospores occurrence and causing infection. No doubt that the disease incubation was more or less dependent upon the environmental temperature, a suggestion supported by the thermic feature of the parasite, as well. The subsequent works have to make clear this relation and the role of humidity in disease development.

Figure 2 shows the dynamics of ascospores collected on blades with vaseline which also

Table 4

Setting and maturation of ***Diaporthe helianthi*** **perithecia on sunflower stems under laboratory conditions, 1984**

Variants	January obs. after days	February obs. after days	March obs. after days	April obs. after days	May obs. after days	June obs. after days	July obs. after days
	0 10 20 30	0 10 20 30	0 10 20 30	0 10 20 30	0 10 20 30	0 10 20 30	0 10 20 30
V_1 vertical stems	— — — —	— — — —	— — — —	— ± ± +	— ± + —	— ± + —	± + +
V_2 horizontal stems	— — — —	— — + —	— — ± +	— ± + +	± + + —	± + + —	+ + +
Stems buried at :							
V_3 5 cm	— — ± +	— ± ± +	— — ± +	— ± + +	± + + —	+ + + —	0 0 0
V_4 10 cm	— — ± +	— ± ± +	— ± + +	— ± + +	± + + —	0 0 0 —	0 0 0
V_5 15 cm	— — ± +	— ± ± +	— ± + +	— ± + +	± + + —	0 0 0 —	0 0 0
V_6 20 cm	— — ± +	— ± ± +	— ± + +	— ± + +	± + + —	0 0 0 —	0 0 0
V_7 25 cm	— — ± +	— ± ± +	— ± + +	— ± + +	± + + —	0 0 0 —	0 0 0
V_8 30 cm	— — — ±	— ± ± +	— ± + +	— ± + +	± + + —	0 0 0 —	0 0 0

— = lack of perithecia.
± = perithecia under setting.
+ = mature perithecia.
0 = rotten stems.

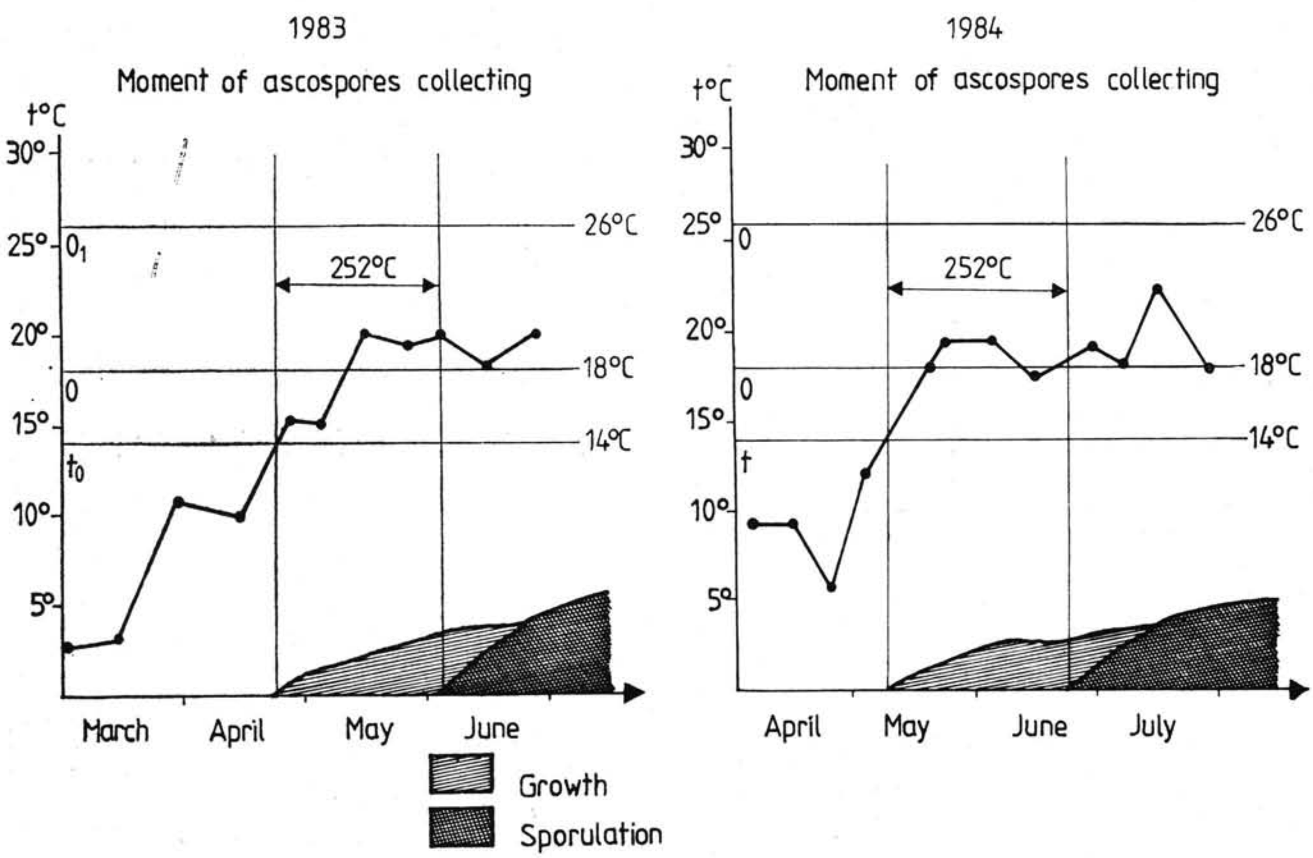

Fig. 2 — Setting and maturation of perithecia of *Diaporthe helianthi*

substantiates the dependence of the development duration of *Diaporthe helianthi* upon temperature. If in 1983 the low thermic threshold of development (t_o) was attained in the third decade of April, the constant K=252°C being attained in the first days of June, when the first ascospores were collected, in 1984, t_o was obtained in the second decade of May, the first ascospores being captured at the end of June. This was due to the low temperatures registered during that period too, which caused a delay in achieving the thermic constant (K=252°C).

CONCLUSIONS

The meteorological conditions favouring the development of *Diaporthe-Phomopsis* in the recent years have enabled the extent of stem canker to almost all sunflower cropping regions of Romania. However the pathogen existence has not always been accompanied by significant crop damages.

Phomopsis helianthi had the low thermic threshold at 14°C, the sporulation threshold (O) at 18°C with its optimum (O_1) at 26°C, and the maximum thermic threshold (T) at 32°C.

Estimated by means of Blunck's formula, the development constant of the parasite *Diaporthe helianthi* was 252°C. The development duration ranged between 18 and 63 days, depending on the moment when the constant K is achieved and this could be graphically expressed by a hyperbola.

The occurrence of perithecia on the residues of the attacked sunflower stems located at soil surface or buried at different depths was dependent upon temperature and the time of achieving the thermic value of the parasite development, which also was supported by collecting the first ascospores at the beginning of June in 1983 and at the end of the same month, in 1984.

By approximating the moment of the first ascospore onset therefore of plant infection with *Diaporthe helianthi*, the chemical treatment for prevention and control of sunflower stem canker can be successfully warned.

REFERENCES

Acimović M. and Straser Nada, 1981, *Phomopsis sp. a new parasite in sunflower,* Helia 4 : 43—58.

Baicu T., Gutenmacher I., Alexandri Al., Popa I., Maier I., 1985, *Rezultate de producție obținute prin aplicarea unui complex de măsuri pentru combaterea pătării brune și frîngerii tulpinilor de floarea-soarelui,* A IX-a Conferință Națională de Protecția Plantelor — București, 5—6 septembrie 1985.

Ciurea Alexandra, Hulea Ana, Rafailă C., 1983, *Ciuperci parazite nou semnalate la floarea-soarelui,* Producția Vegetală, Cereale și Plante Tehnice XXXV, 1, p. 32—34.

Herr L. J., Lipps P. E., Watters B. L., 1983, *Diaporthe Stem Canker of Sunflower,* Plant Disease 8 : 911—913.

Ignatov B., 1984, *Problemi s bolestite po slincio-Gleda,* Rastitelna zascita 10, 19—21.

Iliescu H., Csép N., 1982, *Starea fitosanitară a culturilor de floarea-soarelui din Cîmpia de Vest în anul 1981,* Rev. Prot. Plant., 10 (1) : 91—92.

Iliescu H., Csép N., Ivancea Valeria, Stoica V., Păcureanu Maria, 1983 (a), *Evoluția bolilor produse de diferiți agenți patogeni la floarea-soarelui,* Producția Vegetală 1 : 35—38.

Iliescu H., Csép N., Craiciu Mihaela, 1983 (b), *Chemical control of sunflower cryptogamic diseases in Romania,* Helia 6 : 35—38.

Ioniță Alina, Jinga V., Ciurea Alexandra, Iliescu H., 1985, *Aspecte din ecologia ciupercii Phomopsis helianthi, principalul agent patogen implicat în pătarea brună și frîngerea tulpinilor de floarea-soarelui,* A IX-a Conferință de Protecția Plantelor — București, 5—6 septembrie 1985.

Lamarque Claudine, 1985, *Evolution permanente de la situation phytosanitaire du Tournesol en France,* Oléagineux 367 (4) : 20—24.

Marić A., 1984, *Zbornik radova o suzbijanju bolesti sunconkreta hemijskim sredstvima tokom 1983 godine u Vojvodini,* Ed. Srbija, *Beograd, 122 p.*

Mihaljčević M., Petrov Marija, Muntanola-Cvetković Maria, 1980, *Phomopsis sp. novi parazit sunconkreta u Jugoslaviji,* Savremena Poljopriveda 28 (11—12) : 481—576.

Muntanola-Cvetković Marija, 1981, *On the identity of the causative agent of a serious Phomopsis — Diaporthe disease in sunflower plants,* Nova Hedwigia 34 : 417—435.

Németh G., Princzinger G., Vörös J., 1981, *Uj napraforgo-betegseg Magyaroreszagon,* Magyar Mezögazdasag 36 (48) : 10—11.

Săvescu A. and Rafailă C., 1978, *Prognoza în protecția plantelor,* Ed. Ceres, București, 353 p.

RECHERCHES CONCERNANT LA PROGNOSE DE L'ATTAQUE DE *DIAPORTHE-PHOMOPSIS* SUR LE TOURNESOL

Résumé

Les conditions météorologiques des années 1983 et 1984 favorables à l'évolution du parasite *Diaporthe-Phomopsis* ont permis l'extension de la maladie dans presque toutes les zones de culture du tournesol en Roumanie. La présence du pathogène n'a pas été, toutefois, accompagnée par des degâts notables. Le seuil thermique inférieur (t_0) du parasite *Phomopsis helianthi* se situe à 14°C, le seuil de sporulation (0) à 18°C et l'optimum (0_1) à 26°C, le seuil thermique supérieur (T) à 32°C.

Calculée selon la formule de Blunck, la constante thermique (K) du développement du parasite *Diaporthe helianthi* est de 252°C et la durée du développement est comprise entre 21 et 63 jours, dépendant du moment où la constante K est réalisée, elle pouvant être représentée par une hyperbole.

L'apparition des périthèces sur les débris des tiges de tournesol attaqués, restés à la surface du sol ou enterrés à différentes profondeurs, est dépendante de la température et du moment de la réalisation de la

constante thermique du développement du parasite, fait également confirmé par la capture des premiers ascospores au début du juin et à la fin du même mois en 1984.

En déterminant le moment approximatif de l'apparition des premiers ascospores, donc de l'infection à *Diaporthe helianthi* des plantes, les traitements chimiques peuvent être avertis, afin de prevenir et combattre l'attaque du champignon.

INVESTIGACIONES CON RESPECTO A LA PROGNÓSIS DEL ATAQUE DE *DIAPORTHE-PHOMOPSIS* EN GIRASOL

Resúmen

Las condiciones meteorológicas favorables a la evolución del parásito *Diaporthe-Phomopsis* en los años 1983—1984 permitieron la extensión de la enfermedad en casi todas las zonas del cultivo de girasol de Rumania. La presencia del patógeno no fue sin embargo acompañada por daños importantes.

El umbral térmico inferior (t_0) del parásito *Phomopsis helianthi* se sitúa a 14°C, el umbral de esporolación (0) a 18°C, con óptimo (0_1) a 26°C, el umbral térmico superior (T) a 32°C.

Calculada conforme a la formula de Blunck, la constante térmica de desarrollo (K) del parásito *Diaporthe helianthi* es de 252°C y la duración del desarrollo está comprendida entre 21 y 63 días, en función del momento cuando se realiza la constante K, la cual puede estar representada por una hipérbole.

La aparición de los peritecios en los restos de tallos de girasol, que permanecen en la superficie del suelo a bien están enterrados a diferentes profundidades, depende de la temperatura y del momento de la realización de la constante térmica de desarrollo del parásito, hecho confirmado también por la captura de las primeras ascosporas, a principios y a finales del mes de junio de 1984.

Por determinar el momento aproximativo de la aparición de las primeras ascosporas, esto es de la infectación de las plantas por *Diaporthe helianthi*, se podrán averiguar los tratamientos químicos para prevenir y combatir el ataque de este hongo.