RESPONSES OF SUNFLOWER PLANTS TO DIFFERENT PHOMOPSIS ISOLATES. I. FROM S U N F L O W E R

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ABSTRACT

Phomopsis helianthi was consistently isolated from diseased sflw. plants, and its teleomorph Diaporthe found abundantly on overwintered sflw.stem debris during the past 4-yr. Other Phomopsis isolates were occasionaly obtained from sflw. debris which differed from P. helianthi in several morpho-physiologic features. Plants of the inbred line S-200 (V-8931) were inoculated with each of these isolates in field conditions and in the greenhouse. A realtionship was found between pl. responses and inoculations techniques employed. Only D. helianthi ascospores gave positive results when inoculated using non-wounding methods. When the host was wounded and the inoculum inserted in the tissues, the cultures identified as P. sojae did not manifest symptoms of the disease. The behaviour of the isolates from the other 3 collections suggests that a parasitic mechanism other than that of P. helianthi exists in this case. The rarity of these isolates and the fact that their natural ingress into the plants could not be demonstrated exclude them as causative agents of the Phomopsis outbreaks in the Yugoslav sunflower fields.

INTRODUCTION

The widespread presence of the holomorph Diaporthe helianthi Munt.-Cvet. et al. in the major Yugoslav sflw. region was noted in a previous study (Muntañola-Cvetković et al. submit.). It was also stated that four Phomopsis collections differed from P. helianthi Munt.-Cvet. et al. in several morpho-physiologic characteristics, and no life-history connections could be established between the isolates of these four collections and P. helianthi. These isolates were designated as P.2 (from an overwintered sflw. stem fragment), which was similar to P.sojae Lehm.; P.3 and P.8 (from overwintered stem fragments); and P.7 (from a lesion on the stem of a senescent pl. whose aspect differed from the typical lesions produced by P.helianthi) and were collectively identified as Phomopsis sp.

The aim of the present work was to determine the pathogenicity of all these isolates on sflw. and their role in the epiphytotic Phomopsis outbreaks which have occurred in Yugoslavia during the past years.

MATERIAL AND METHODS

FUNGAL CULTURES. Besides D.helianthi , representative isolates of each collection were tested for pathogenicity on sflw. Cultures of P.sojae and D. phaseolorum (Cooke & Ellis) Sacc. var. caulivora Athow & Cald., isolated from soybeans in Yugoslavia, were also included for comparison in certain tests. The fungi to be tested were cultivated on Potato Dextrose Agar (PDA) or Malt Agar (MA, at laboratory light- and temperature-conditions.

Pathogenicity tests. Plants of the inbred line S-200 (V-8931), which had shown great susceptibility to P.helianthi were tested in the

field (breeding nursery) and in the greenhouse. Environmental conditions in the greenhouse were automatically controlled by an INDAL microprocessor. Plants were grown at 20° ± 2°C during a 12 h period of 25000-40000 lx, and at 16° ± 2°C during the 12 h dark period. The light was supplied by 1000 W Philips G/92/2 tubes. R.H. varied from 57-86%. The inoculated pl. were 6-wk old (budding stage, 15-16 leaves). The inoculations were performed with: a) ascospores obtained from mature perithecia formed on overwintered debris (14^4 ascospores/ml); b) conidia obtained from pycnidia formed on 25-dy col. on PDA or MA; c) mycelial mats cut into 5 mm diam discs from the edge of 7-dy col. cultivated at 25°C on PDA. The plants sprayed with suspensions of ascospores or conidia were kept in a humid atmosphere for 48h after inoculations. The inoculations by wounding techniques were performed by inserting mycelial mats into wounds (about 5 mm^2 towards the pith) made in the stems with a dissecting scalpel, between the 9th and the 10th leaf. The inoculated parts were wraped in cotton-wool soaked with ster. water or agar pieces. Inoculations were carried out during mid-May and mid-June 1982, and from May to August 1983, on a minimum of 10 pl./isolate/test. The behaviour of the inoculated pl. was followed daily, and measurements of the lesions were recorded every 5 dy. The results were followed during 1 month, and in some cases 40 dy. Data was statistically processed.

RESULTS

The severity of the symptoms provoked by a given isolate often varied from plant to plant. However, essential differences were observed among the tested fungi with regard to canker size, number of leaves affected, and, in particular, to the rate at which the disease progressed.

The pathogenic course in field tests was more rapid than in the greenhouse (Table 1 and Figs. 1 and 2).

INOCULATIONS WITH ASCOSPORE SUSPENSIONS. The first symptoms appeared between 10 and 15 dy, at the blade margins, with darkening of the foliar veins, which was most conspicuous at the underside of the leaf. The parasite further penetrated into the main vein, and from there to the petiole. When the infection reached the stem, the upper leaves lost their green colour and necrotic lesions appeared which later might become confluent. Conidiomata filled with only β-conidia were abundantly produced on the necrotic lesions which appeared on the stems during the later phases of pathogenesis. Thus, wounding was not needed in the case of D. helianthi.

INOCULATIONS BY AQUEOUS SPRAY OF CONIDIA. It was not possible to demonstrate the pathogenicity of the isolates under study in the tests conducted when non-wounded plants were sprayed with conidia. Although some, very rare, plants inoculated with P.helianthi β-conidia manifested symptoms of the disease by 20-25 dy, it was not possible to demonstrate whether the lesions were really provoked by these conidia. All the other unwounded plants sprayed with P.2, P.3, P.7, and P.8 α- or α- and β-conidia aqueous suspensions remained healthy, just as did the plants sprayed with D. phaseolorum var. caulivora ascospores and with conidia of P. sojae isolated from soybeans.

Table 2. Responses of the susceptible sunflower inbred line S-200 (V-8931) to the inoculation of representative *Phomopsis* isolates from sunflower plants and debris collections by wounding technique (30 dy after inoculation)

Isolates	Field experiments						Greenhouse experiments					
	Statistical parameters				Pycnidia	Conidia	Statistical parameters				Pycnidia	Conidia
	Range *	$\bar{x}$	σx	Cv			Range	$\bar{x}$	σx	Cv		
P. helianthi	125-180	151.0	20.5	13.5	abundant	only β-	70-120	96.5	18.6	14.3	abundant	only β-
P. sojae (from soybeans)	-	-	-	-	-	-	-	-	-	-	-	-
P.3	210-300	272.3	19.4	7.1	many	α-	Dead plants					
P.7	29-42	34.2	14.6	39.2	-	-	38-54	43.6	14.2	32.6		
P.8	190-340	267.4	16.5	6.2	-	-	Dead plants					
P. sojae (from soybeans)	-	-	-	-	-	-	-	-	-	-	-	-
D. phaseolorum var. *caulivora* (from soybeans)	-	-	-	-	-	-	-	-	-	-	-	-
Controls	-	-	-	-	-	-	-	-	-	-	-	-

* (Range refers to the lesion length in mm; $\bar{x}$ = average; σ = standard deviation; Cv = coef. of variation)

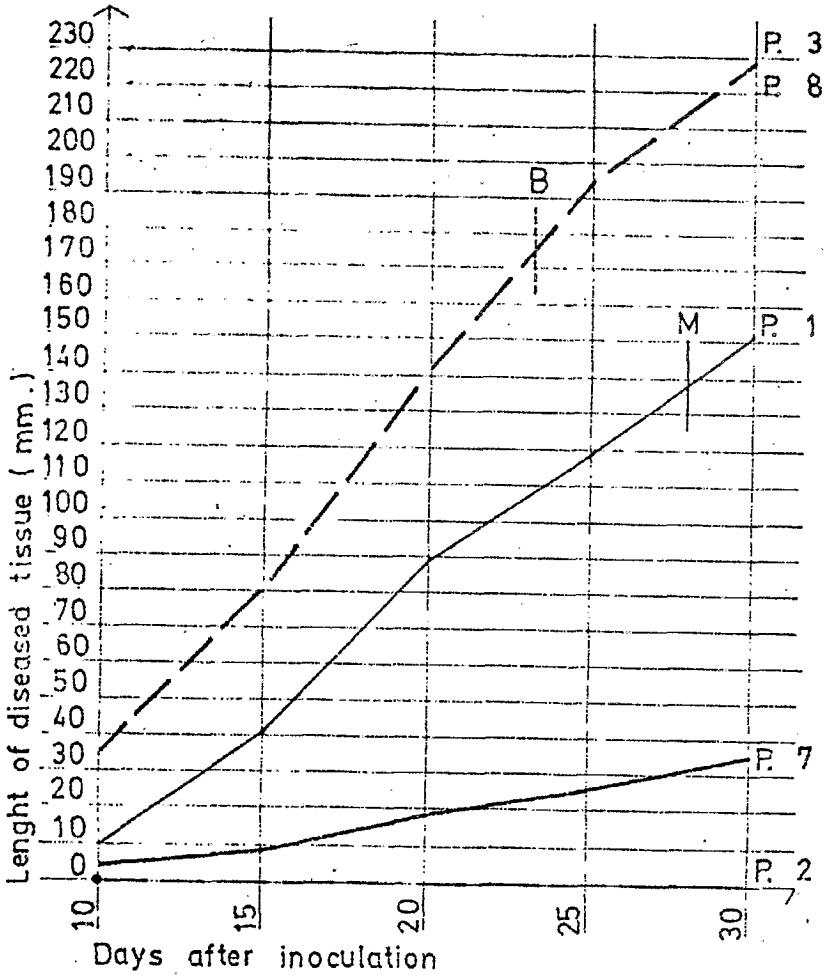

Fig. 1

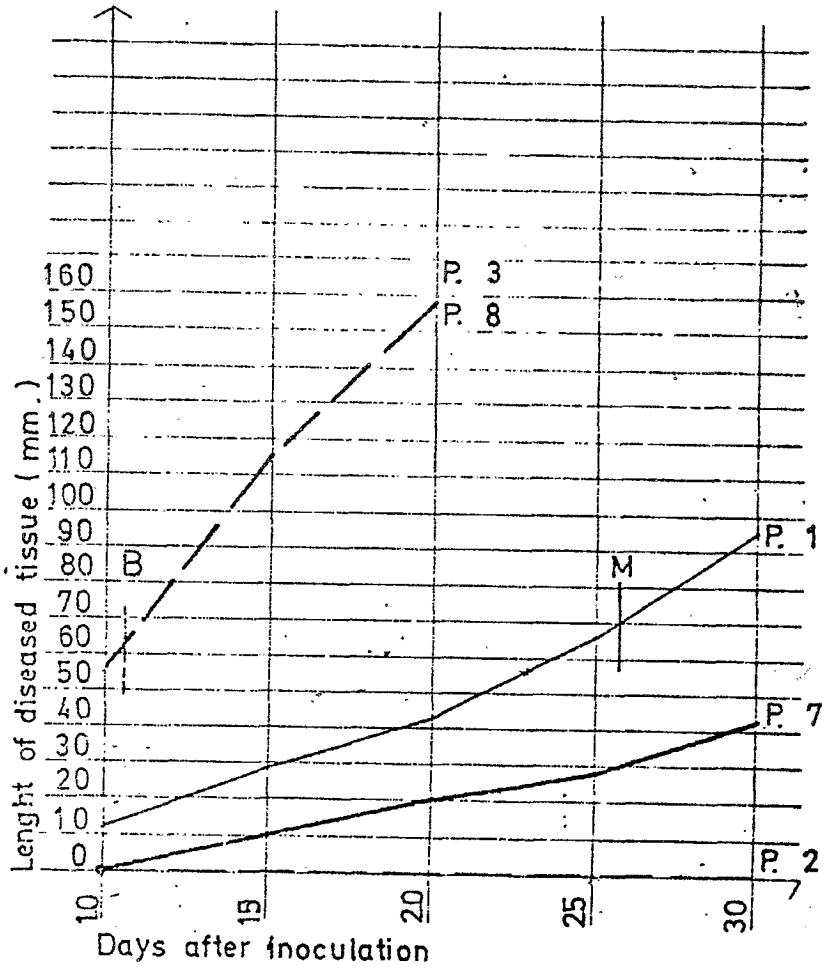

Fig. 2.

Figs. 1 and 2. Rate of lesion extension after wounding inoculation of sunflower stems under field and greenhouse conditions, respectively. P.1= P. helianthi; P.2= a P. sojae-like fungus isolated from sunflower overwintered stem debris; P.3, P.7, and P.8= isolates from sunflower debris collectively identified as Phomopsis sp. B= Plant breakage due to tissue disintegration at the inoculation point. M= plants become moribund.

INOCULATIONS USING WOUNDING TECHNIQUES. Various reactions were obtained when the inoculum was introduced into the host tissues by wounding methods.

The external factors, especially temperature, did not greatly affect the course or rapidity of pathogenesis of P.3 and P.8 isolates in the field. Plant breakage began 20-30 dy after inoculation due to disintegration of wounded tissues. Within a distance of 100 mm from the inoculation point, all the tissues were transformed into a wet-rot, dark mass, in which only mechanical fibers were distinguished. The plants wilted at this point. Pycnidia filled with α-conidia were only found in the case of P.3 field-inoculations, but not on the plants tested in the greenhouse.

In contrast, the pathogenic course of P. helianthi in experimental inoculations using wounding techniques depended considerably upon a complex interaction of external factors, especially temperature and humidity. High temperatures at the initial stages of the fungal ingress and proliferation delayed the pathogenic process, because the growth of P. helianthi is hampered at temperatures above 27 C. On the other hand, high temperatures provoked dramatic changes in the later stages, when the parasite had colonized the conductive tissues. Under these conditions a greater amount of water is required due to the increased leaf transpiration. Since the vessels are obstructued, the flow becomes interrupted and the plants collapse. Pycnidia filled with only β-conidia were abundantly produced on the cankers of the inoculated plants in the breeding nursery and in the greenhouse.

P.7 cultures were less destructive. Symptoms were initially similar to those provoked by P. helianthi, but the necrotic process was primordially limited to the cortex. The subcortical extension of the invading process so evident in the case of P. helianthi was not observed here. The necrotic spots surrounding the inoculation court did not surpass 40 mm in 30 dy, and tissues situated 4-5 mm away from the spot margin remained healthy. Chlorotic and necrotic lesions affected only some leaves above the inoculated point. Pycnidia were not produced on the necrotic surfaces.

Regardeless of external conditions, P.2 never induced pathologic changes on the inoculated sunflowers. Symptoms of the disease were neither observed in the plants inoculated with P. sojae, nor with D. phaseolorum var. caulivora isolated from soybeans. Wounded control plants inoculated with ster. water or nutrient agar did not manifest pathologic changes.

DISCUSSION

The results obtained in this study underscore the significance of the morpho-physiologic differences previously reported among several groups of isolates from sunflower plants and debris (Muntañola-Cvetković et al., submitted).

Although the pathogenic processes were faster in field experiments than in those of the greenhouse, the curves representing these processes indicate a similar behaviour in both cases for each group of isolates. Moreover, the curves illustrate different degrees of virulence among the groups when the respective isolates were inoculated on sunflower plants under the same conditions. Light quality and abrupt changes in other environmenatl conditions to which field-growing plants are subjected may activate systems conducive to pycnidium formation. This would explain why these reproductive structures were more frequently found on field-inoculated plants than in those tested in the greenhouse.

A relationship was found between plant responses and inoculation techniques employed.

The fact that D. helianthi ascospores gave positive results when inoculated by non-wounding techniques suggests that a host-fungus relationship exists in this case. P. helianthi β-conidia did not reproduce the disease because they do not commonly germinate. The lack of polysaccharide reserves in these structures has been studied by Muntañola-Cvetković et al. (submitted).

A natural ingress of P.3 and P.8 could not be demonstrated; however, once introduced into the wounded host tissues these isolates were even more destructive than P. helianthi. The failure to obtain positive results when unwounded plants were inoculated with germinable α-conidia, and the rapid plant decay when the inoculum was inserted into the wounded tissues suggest a parasitic mechanism other than that of D. helianthi. The behaviour of P.7 isolates was similar to that of P.3 and P.8 in this respect. Varying degrees of virulence were however observed among these isolates which were collectively referred to as Phomopsis sp. in a previous paper (Muntañola-Cvetković et al. (submitted). The inability of these isolates to freely penetrate into living sunflower plants is a key feature; it may explain their rarity in the material collected and exclude them as causative agents of the Phomopsis outbreaks in Yugoslavia.

D. phaseolorum var. caulivora and P. sojae from soybeans, and the P. sojae -like fungus P.2, must likewise be eliminated as possible inducers of these outbreaks, since plants inoculated with these fungi did not develop pathologic signs, regardless of the techniques employed.

CONCLUSIONS

1) Only D. helianthi ascospores reproduced the disease when inoculations were carried out using non-wounding methods;

2) No consistent responses were obtained when plants were inoculated with P. helianthi β-conidia;

3) The seldom isolated cultures identified as Phomopsis sp. provoked several types of reactions when the host was wounded and the inoculum inserted into the plant tissues;

4) Considering: a) the extensive presence of P. helianthi and its teleomorph Diaporthe and the low frequency of other Phomopsis isolated from sunflower in Yugoslavia; b) the origin of these other isolates (two from dead fragments and the third from a semi-dead plant); c) the fact that pathologic processes did not develop in unwounded plants sprayed with conidial suspensions but did result from inoculations by violent, unnatural interventions, the present study corroborates that the holomorph D. helianthi is the sole responsible agent of the Phomopsis outbreaks which have caused severe damage in the major Yugoslav sunflower fields during the past years.

REFERENCES

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