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Diaporthe (Phomosis) SP., A NEW PATHOGEN OF COCKLEBUR (*Xanthium italicum Moretti*) AND OF SUNFLOWER (*Helianthus annuus L.*)[1]

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SUMMARY

In July 1988, we have observed symptoms on *Xanthium italicum Moretti*, similar to those provoked by *Phomopsis helianthi Munt.-Cvet.* on sunflowers. The causative agent of these symptoms on *X. italicum* is *Diaporthe (Phomopsis) sp.* that we have arbitrarily named *Phomopsis (Diaporthe) "xanthium"*.

This publication describes the anamorphic and telomorphic forms of this parasite, and gives the first results concerning its biological cycle on *X. italicum*.

The peculiarity of this fungus is to be equally pathogenic on sunflowers (results proved by ascospores tests), and to provoke on the stems of this plant identical symptoms to those developed by *P. helianthi*. Then, what is the relation between *P. "xanthium"* and *P. helianthi*?

The comparison of their anamorphic forms brings them together. But, on the other hand, their telomorphic forms present quite important differences. However, one of the *P. helianthi* biotypes isolated by Yang (1983) is characterised by typical traits of *D. helianthi* and of *P. "xanthium"*. Considering these various observations, and the fact that the *Xanthium* and *Helianthus* genera are both native to the American continent, we are presently working on the following hypothesis: could *Phomosis (Diaporthe).helianthi* be a form of *Phomopsis (Diaporthe)."xanthium"* adapted to a new host (the sunflower)?

INTRODUCTION

The pathogic fungus of *Helianthus annuus L.* (*Asteracea*, tribe of *Heliantheae*), *Phomosis (Diaporthe) helianthi Munt.-Cvet.*, has been studied since 1980. So, we have in hand numerous results concerning its morphological and biological properties (Muntanola-Cvetković et al., 1981, 1985; Aćimović and Štrasser, 1982; Marić et al., 1982; Herr et al., 1983; Yang et al., 1984; Fayret and Assemat, 1987).

During observations on the Danubian flora, we have noticed on another *Asteraceae* of *Heliantheae* tribe - *Xanthium italicum Moretti* - symptoms similars to those provoked by *P. helianthi* on sunflowers. The causative agent for these symptoms on *Xanthium italicum* belongs to the *Phomopsis* genus.

For all these different reasons (similarity of symptoms, belonging of these two parasites to the *Phomopsis* genus, taxonomic position of the *Helianthi* and *Xanthium*

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genera) we have decided to study this new parasite, its possible pathogenicity on the sunflower and its taxonomical relation to *P. helianthi*.

MATERIAL AND METHODS

1- Stains isolation (July-August 1988): *Xanthium italicum* infected leaves coming from 34 places in Vojvodina (Yugoslavia) have allowed the constitution of a 25-isolate collection (P1 to P25). The isolations have been made according to the method described by Altman (1966) on potatoe-dextrose-agar (PDA) and have been cultivated at 23°C ±2°C in the dark. In November 1988, infected stems have been harvested in the same location. From this material kept in natural conditions we have constituted a 53 ascospore isolate collection.

2- Nutrient media: For the composition and the preparation of the media we have refered to the work by Smith and Onions (1971). Concerning Fayret's medium we have introduced a cold sterilization of amino acids and of vitamins.

3- Biometrical study:

* Stem fragments bearing mature perithecia have been fixed in the Bouin's solution and dehydrated in graded series of alcohol, then embeded in paraffin. Sections 5 μm thick have been cut out transversally to the perithecium level (microtom MC2). After being mounted in Canada Balsam 50, identical preparations to these presented in Figure 11 have been measured (magnification 16 x 10, F=10).

**Pycnidia*: a similar study is being carried out.

*Asci: 100 measurements have been carried out (magnification 16 x 40, F=1,515).

*Ascorpores and condia: we have used the immersion technique (magnification: 16 x 100, F=0,628, impresion oil nD(20)=1,515). Only the ascopores freed from their asci have been measured.

4- Pathogenicity tests: they have been carried out according to the following method:

* Spraying of ascopore solution: the preparation of suspension has been realized after Mihaljčević (1984a). The plants wrapped in a plastic foil after spraying have been put 72 hours in growth chamber (20°C-/+ 2°C, 16 hours day / 8 hours night, light intensity 2500 lux, relative humidity 100%),
* mycelin mats on the apical part of the leaves (Bertrand and Tourvielle, 1987),
* mycelin mats on severed petioles (Bertrand and Tourvielle, 1987),
* mycelin mats directly inserted in wounded stems (Mihaljčević et al., 1984a).

5- Vegetable material: *Xanthium italicum* has been produced from seeds harvested in October. To suppress the dormancy of the seeds gathered from October to February, it has been necessary to stock them at 5°C during a minimum of 21 days. The plants have been grown on a mixture of compost and river sand (50%-50%), in plastic pots (30 x 20 x 10 cm) with 6 or 9 plants in each pot. In the greenhousse, the average temperature was 25°C-/+ 5°C, 16 hours day, 8 hours night, light intensity: 35000 lux.

Concerning the tests on the sunflower we have used three lines sensitive to *P. helianthi* L-1, L-3, CMS-20. Cultivated on compost, they have undergone growing conditions similar to these described above.

Statistical processing has been carrided out on the SYSTAT program (SYSTAT INC., 1987) kindly provided by Dr. Mihaljčević. Confidence intervals have been measured with a 5% risk.

RESULTS

1- Symptoms observed on *Xanthium italicum Moretti*:

The first symptoms appeared on the edges of foliar limbs. There is a tissue discoloration which presents a light dehydration. Then this zone darkens, necroses take a roughtly triangular shape, very typical. One of the corners of this triangle is centred on the main foliar vein (Fig. 1). They expand toward the petiole, perceeded by a light green, sometimes golden yellow margin (Fig. 1). The first phase leads to the petiole colonization, a transversal section of which shows a darkening of the vascular system. At this stage, the foliar limb is completly parched (Fig. 2).

On the stem there appear dry cankers, reddish brown, circular to elliptical, centred on the initially infected petiole (Fig 4). A longitudinal section of this zone reveals that a darknening of the vascular system preceeds the necroses visible on the epidermis. The different necroses can merge to form a large ring around the stem. From the petiole insertion point, the parasite also destroys medular parenchyma by making cavities. These two phenomena provoke a sharp plant withering, which frequently breaks under the action of wind or various impacts. In spring, the infected plants can be recognized by the silver-grey to reddish-brown colour of the epidermis.

2- First observations of the anamorphic stage:

The isolations carried out from infected leaves and stems have allowed to observe, under pure culture, white colonies, roughly circular, oily looking (Fig. 6) and composed of septate mycelium. Aerial mycelium and substrated ones are to be found in variable proportions. On the fourth day of culture, outlines of pycnidia differentiate under the shape of globulous or subglobulous structures, colourless. After the wall melanization and the occurrence of one or several ostioles, liquid exudates, colourless to greenish–beige appear (sixth/seventh day - Figs. 7, 8). These exudates like the pycnidia body, are rich in beta conidia, hyaline cells, filiform, with one of their tips bent (Fig. 9). The daily observation of the pycnidia content from the fifth to the fifteenth day of culture on PDA has not allowed the observation of alpha conidia on the isolates of our collection. At the stage of developing, the mycelium allows to differentiate two isolate types, the first one keeping this whitish colouring on a light beige substrat, the second one producing golden drops and a brown-dark brown substrate (Fig. 6). These two types have been observed either pure or mixed. We have been able to link these morphological particularities of mycelium to morphological variability observed at the level of pycnidia (work in progress).

These morphological elements, according to the classification criteria proposed by Barnett and Hunter (1972), indicate the *Phomosis* genus.


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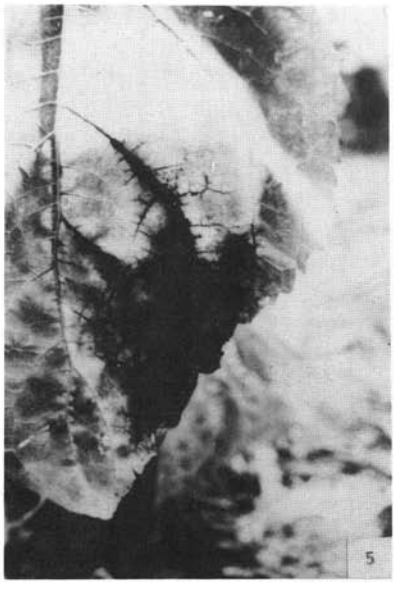
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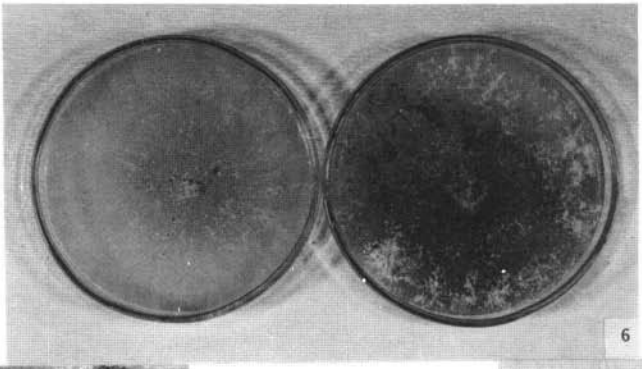
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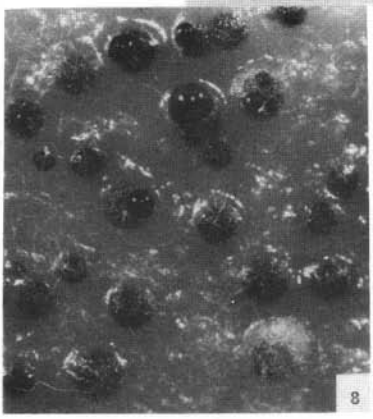
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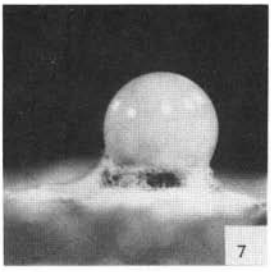
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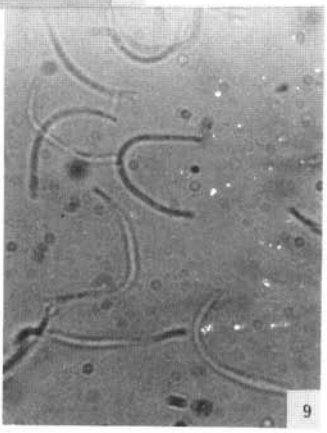
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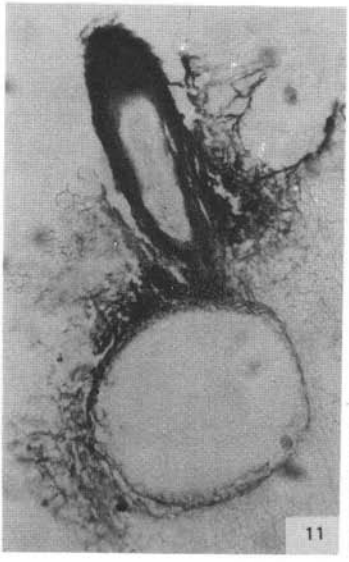
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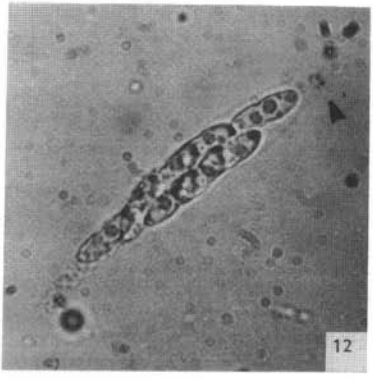
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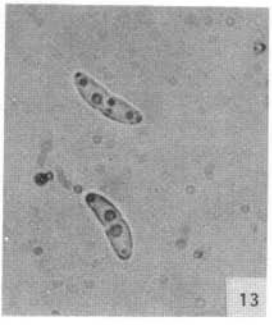
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Fig. 1: *Phomopsis "xantium"*, natural infection: leaf symptom (arrow: limb tissues discoloration).
Fig. 2: *idem*: petiole colonisation.
Fig. 3: *idem* artificial infection with ascospores: symptome on leaves.
Fig. 4: *Phomopsis "xantium"*, natural infection: stem cankers.
Fig. 5: *Diaporthe (Phomopsis) helianthi Munt.-Cvet.*: symptoms on leaf du to a natural infection.
Fig. 6: *P. "xanthium", two mycelial froms found in PDA.*
Fig. 7, 8: *idem*, pycnidia on PDA (arrow: exudat, x32).
Fig. 9: *idem*, beta conidia (from natural host, x1600).
Fig. 10: *D. "xantium"*, mature perithecia from natural infected stem of *Xantium italicum Moretti* (x32).
Fig. 11: *idem*, Perithecium munted in Canada balsam (x51,2)
Fig. 12: *idem*, asci (arrow: location of the apical chitinoide ring, x1600).
Fig. 13: *idem*, ascospores (x1600).
Fig. 14: symptoms provoked by *Phomopsis (Diaporthe) "xantium"* on sunflower leave (arrow: colonised vein).
Fig. 15: *idem*, colonisation of the petiole.
Fig. 16: *idem*, necroses on the sunflower stem.
Fig. 17: *Phomopsis helianthi*: stem canker (natural infection).

In order to be clearer, we have decided to arbitrarily name the anamorphic stage of this parasite as *Phomopsis "xanthium"*.

On natural host, the pycnidia develop on the necrotic tissues of the stem. The pycnidia are brown-dark brown, globulose, variable (work in progress). However, on five infected stem samples (October 1988 to February 1989) coming from 17 different locations, we have noticed:

* an abundant production of beta conidia morphologically identical to those produced on PDA,
* the absence of alpha conidia.

Table 1 presents the measurements of beta conidia produced on natural host.

3- First observations made on the telomorphic stage:

On naturally infected stems harvested at the end of August and put in humid chamber at 23°C, we have observed, after 30 days of incubation, perithecia caracteristic for the *Diaporthe* genus.

The globose bodies set in the cortical tissues are topped by a beak carrying an ostiole (Fig. 10). The outside tissues are black and are not supported by any real stromatic formation.

The perithecial cavity contains elongated asci presenting an apical chitinoid ring (Fig. 12). Each ascus produced eight two-celled ascospores, hyaline, the cytoplasm of which contains two fatty and very refringent drops (Fig. 13). The ascorspores germination can be observed after 2 or 3 hours at 23°C. The infected stems coming from various places have identically produced perithecia, confirming that the perithecial induction takes place before the winter period. Afterwards, the time necessary for perithecial development decreases progressively. In March-April, it is only 2 or 3 days at 23°C. The perithecia, asci and ascorpores' biometical characteristic are given in Table 1. The morphological characteristic of the ascospore cultures (53 isolats) leads us to the same conclusions drawn above.

Table 1. Dimensions of beta conidia form *Phomopsis "xanthium"*, perithecia, asci and ascopores from *Diaporthe "xanthium"* (from natural host). L = length in μm, l = width in μm

	Beta conidia		Perithecia		Asci		Ascospora	
	L	l	L	l	L	l	L	l
n	1000		50		100		1000	
Mean	16.6	0.8	307	253	52.0	8.3	11.3	2.9
M min	7.9	0.3	200	180	36.0	4.8	9.3	2.5
M max	24.5	1.8	400	320	64.8	14.4	13.6	3.6
s	2.9	0.2	46	34	5.6	1.5	0.6	0.2
$M-2\sigma/\sqrt{n}$	16.5	0.8	289	239	5.0	8.0	11.2	2.9
$M+2\sigma/\sqrt{n}$	16.8	0.9	324	266	53.0	8.6	11.3	2.9

For all the 88- isolate collection the telomorphic form has been systematically and abundantly observed on the medium (PDA). The perithecia appear either directly on the bottom of Petri dishes, or in the medium under the pycnidia. In both cases, they are morphologically identical to the perithecia produced on the natural host. They contain

ascospores. A study is in progress to determine the ascospore size, its germination and pathogenic power on *X. italicum*.

The Fayret's medium is favorable for the development of the telomorphic from which will arbitrarly be named *Diaporthe "xanthium"*.

Table 2. Detail of experimentation concerning the *Helianthus annuus L.*'s inoculation with *Phomopsisi "xanthium"* (method of ascospores)

Date of inoculation	Sunflower lines	Stage plant/inoculation	% necrosed plants	Time of incubation*
9.12.1988	L–1	star bud	83	39
5.01.1989	L–3	bud 3.5 cm	79	29
16.01.1989	CMS–20	begin. flow.	90	22
24.03.1989	L–1	8 leaves	85	46
13.3.1989	L–1	bud 1 cm	98	33

* in days

4- Pathogenicity tests:

* Pathogenicity of *Phomosis (Diaporthe) "xantium"* on *Xanthium italicum Moretti*:

The inoculation by ascospores suspension has allowed us to reproduce the whole of the infection process observed under natural conditions. After 20 days of incubation, we have obtained typical foliar symptoms (Fig 3) which have progressed into necroses on the stem with a 100% success rate. A pyncidia and perithecia formation has occurred on the necrosed tissue. The used reisolations have reproduced colonies characteristic for *P. "xanthium"* in pure culture.

Beta conidia suspension on unwounded plants has not given any results. Repetitions of these manipulations are in progress.

Whith the mycelium tests on leaves, we have not be able to induce the infection process. After 45 days of incubation, 2 to 3 cm of the leaf were necrosed but the main vein has not been colonized. The necrosed zone was outlined by a conspicuous bright yellow margin.

The mycelium mats on petioles and wounded stems have provoked the formation of cankers and fructifications of pyncidia rich with beta conidia, then perithecia containing ascospores. As indicated in Table 3 the stem method is the more drastic of these two methods (cf. detail in the next paragraph).

* Pathogenicity of *Phomopsis (Diaporthe) "xanthium"* on sunflower:

In the field (August 1988), we have used the tree mycelial tests at the budding stage, 3-5 cm (line l-1).

The inoculation on leaves has ended by the same phenomenon observed with *P. "xanthium"* on *X. italicum* (necroces limited to a few centimeters, main foliar vein uncolonized, bright yellow margin). On the other hand, the petiole and the stem methods have allowed the formation of necroses wholly identical to these provoked by *Phomosis (Diaporthe) helianthi* on sunflowers. Morphologically it has not been possible to differenciate the pyncidia on the infected tissues from those produced by *P. "xanthium"* on *X. italicum* or from those of *P. helianthi* on sunflowers. They exclusively contain beta condia. The pure cultures issued from re-isolation of *P. "xanthium"* on sunflowers have kept their specificity, i.e., golden mycelial exudate for the isolates possessing this feature.

In the greenhouse, during winters 1988 and 1989, we have carried out inoculations by spraying *Diaporthe "xanthium"* ascospores. Details of the experimentation are summed up in Table 2. The lenght of incubation has been calculated in relation to the occurence of stem necroses and not in relation to the occurence of the first foliar symptoms. As it is, because of their atypical aspect, the symptoms have been difficult to observe,more difficult than the symptoms developed by *P. helianthi* on sunflowers.

On green leaves we have not observed any necrotic phenomena whatsoever. Some atypical necroses have formed on leaves, presenting symptoms of senescence (yellowing of limbs). The colonization of the vein has taken place (Figs. 14, 15) but the necroses on the limb were diffused, not preceded by a tissues discoloration or by a yellow margin. At the same time on plants inoculated with *D. helianthi* ascospores we have witnessed the typical development of foliar necroses. Similar tests are presently being carried out to confirm these observations.

In spite of atypical foliar symptoms, *P. "xanthium"* has developed stem cankers indistinguishable from the ones produced by *P. helianthi* on sunflowers (Figs 16, 17). This infection process has lead to the formation of pycnidia - containing beta conidia - and perithecia. The ascospores of the first *Phomosis (Diaporthe) "xanthium"* generation on sunflowers are being the object of a very precise biometric study (in progress).

Defined this way, the incubation period varies according to the host plants physiological stage at the time of inoculation. The shortest delay corresponds to the begining of the flowering stage.

* Test of pathogenicity of *Phomosis (Diaporthe) helianthi* on *Xanthium italicum Moretti* (Table 3):

Table 3. Results of the inoculation of *Xanthium italicum* (x.i) with *P.helianthi* (PH), using the wounded stem method and severed petiole method (1 = enght in mm)

	Inoculation on stem (1)			Inoculation on petiole (1)		
	PH/X.i	PX/X.i	Con.	PH/X.i	PX/X.i	Con.
Mean	14.7	226.3	0	2.6	56.5	0
σ	23.5	46.3	0	7.2	10.7	0
n	24	24	6	30	60	6
Confidence interval	5/23	207/244	–	0/5.2	30/84	–
Coefficient of var. (%)	158	20	–	279	18	–
% plants with symptoms	25	100	0	16	75	0
% destroyed plants	0	100	0	0	18	0

Inoculation by mycelial mats on the stem: the percentage of plants infected by *P. helianthi* is very low (25%) as compared with *P. "xanthium"* (100%). Besides, the lenght of the necroses is not a point in favour of typical infection process (14.7 mm on average-confidence interval 5-23 mm) which is confirmed by the absence of completly destroyed plants. *P. "xanthium"*, the pathogenicity of which has been proved by ascospores test, has destroyed 100% of plants with necroces of 226.3 mm on average (confidence interval of 208.6, 6-244.6).

When we examine the results obtained with the mycelial tests on petioles it is clear that the stem tissues have not been significantly colonized by *P. helianthi* (necroses of

2.6 mm on average, confidence interval 0-5 mm). *P. "xanthium"* has developped cankers of 56.5 mm on average (confidence interval 29.5-83.5 mm).

Mycelial tests on leaves: as we have demonstrated for *P. "xanthium"* on *X. italicum* and on sunflowers, *P. helianthi* has not been able to reproduce typical symptoms on *X. italicum*.

Ascospores tests: these tests which can alone confirm the pathogenicity of *P. helianthi* on *X. italicum* are now being made.

In conclusion: the ascospores tests realized allow us to confirm:

* *Phomosis (Diaporthe) "xanthium"* is pathogenic of *X. italicum*,

* this parasite is also pathogenic of *H. annuus*.

DISCUSSION

Barnet and Hunter (1972) define the *Phomopsis* genus according to the structure of pyncidia containing alpha and beta conidia (sometimes c conidia) on one hand and acoording to the imperfect form of the *Diaporthe* genus on the other.

The pyncidia of the parasite isolated on *X. italicum* correspond to the morphological criteria described by Barnett and Hunter but until now have not produced alpha conidia on natural host or on PDA. Only beta conidia have been observed. Before we give a definite verdict on the capacity of this parasite to produce or not alpha conidia, we have to test media prepared from host plant (stems, leaves, seeds). These media have exceptionally provoked alpha conidia production with *P. helianthi* (Muntanola-Cvetković et al., 1985).

We can affirm, however, the beloning of our parasite to the *Phomopsis* genus because in pure culture on PDA we have observed the systematic pasage of the anamorphic form to the telomorphologic stage (*Diaporthe*). This perfect form has been characterized by the presence of pseudostromata (in pure culture) and by the morphology of its perithecia (according to Nitsch in Muntanola-Cvetković et al., 1981a).

About the taxonomy, we also have to precise the significance of the two mycelial forms observed on PDA. The noted difference has been kept during successive subculturing. A comparative study will allow to define their morpho-physiological characteristics and their biological cycle (existence of distinctive telomorphic forms).

According to the progress of our bibliographical reseach concerning the mycoflora of *Xanthium spp.*, only three representatives of the *Phomopsis* genus are known on these weeds. Voros (in Ubrizsy and Voros, 1968) discribes *Phomopsis xanthii Voros* on *Xanthium strumarium L.*, a parasite which is characterized by its alpha conidia (7.2-9.0 x 2.2-2.7 μm), beta conidia (18.0-23.5 x 0.7-0.9 μm) and the absence of perfect form on the natural host. Mihaljčević and Muntanola-Cvetković (1984b) have isolated on *X. italicum* and on *X. strumarium*, two *Phomopsis* species with the following characteristic (in Mihaljčević and Muntanola-Cvetković, 1984b):

	from *X.italicum*	fom *X.strumarium*
alpha conidia	(+)	+
beta conidia	–	–
perithecia:		
on host	–	–
on media	–	–

+ = present; (+) = occasionaly present; – = absent

These three parasites do not have any known perfect form on natural host and medium. They produce alpha condia in variable proportion. Those two observations allowed to differentiate them without hesitation - so it seems to us - from our parasite. However we are presently checking the morpho-physiological properties of these isolates.

The specifity of *P. "xanthium"* will be proved after testing its pathogenicity on differents hosts, in particular on the two species of *Xanthium* present in Yugoslavia (*X. strumarium* and *X. californis L.* according to Koljazinski, personal communication, 1989).

The second aspect of this publication concerns the pathogenicity of *P. "xanthium"* on *H. annuus*.

According to Koch's postulate, we have proved the pathogenicity of *P. "xanthium"* on cultivated sunflowers. Then, what is the relation between *P. "xanthium"* and *P. helianthi*?

There are some common points between these two fungi:

- the symptoms provoked by these two parasites on their respective natural hosts are similar on leaves, petioles and stems. The confrontation of the description concerning *P. helianthi* (Muntanola-Cvetković et al., 1981, 1985; Herr et al., 1983; Yang et al., 1984; Fayret and Assemat, 1987) with our own documents is sufficient to prove it (Figs. 1, 3, 5). In both cases, it seems that these fungi synthesize a toxin which provokes a limb discoloration and the formation of a margin between necrosed and healthy tissues.
- The infection process leaf-petiole-stem admitted for *P. helianthi* (Petrov et al., 1981; Bertrand and Tourvielle, 1987) is identical to the one developed by *P. "xanthium"* on *X. italicum*. The similarity is underlined by Mihaljčević (1984b) who remarks on numerous *Phomopsis spp.* which feature the absence of visible symptoms during the vegetative phase of their host. This remark also applies to the two previously quoted isolates found on *X. strumarium* and *X. italicum*.
- One of the two mycelial forms of *P. "xanthium"* seems morphologically very close to the one of *P. helianthi*. A comparative study is necessary, pariculary with respect to this form on malt-agar which, according to Muntanola-Cvetković (1981, 1985) allows to characterize *P. helianthi*.
- The two parasites would produce exclusively beta conidia. Concerning *P. helianthi*, the isolates of Herr (1983) and Fayret (1987) contradict Muntanola-Cvetković's conclusion (1988) on this point. As underlined in Yang (1983) and Herr (1983), it is important to determine if this character is controlled genetically or by ecological factors.
- The similarity of pyncidia is logical in as much as these two parasites belong to the *Phomopsis* genus. Besides, the dimension of the fructification on *P. helianthi* differs in function of their developing stage and of other factors (in Muntanola-

Cvetković et al., 1988). It is probable that *P. "xanthium"*'s pycnidia are subject to the same phenomenon which makes all biometrical comparisons uncertain.

- The main differences noticed between *P. helianthi* and *P. "xanthium"* concern their telomorphic stage.
- The perithecial induction in *D. "xanthium"* on *X. italicum* takes place ealier than the one of *D. helianthi* on sunflowers. In the humid chamber, *D. "xanthium"* develops mature perithecia as soon as the end of August, beginning of September. If the perithecial induction in *D. helianthi* occurs during autumn (Muntanola-Cvetković et al., 1981, 1985; Herr et al., 1983; Mihaljčević et al., 1984) the maturation in humid chamber takes place only in February-March (same authors). On the sunflower, *Phomopsis (Diaporthe) "xanthium"* keeps this feature. Indeed, on the sunflower stems inoculated at the same time by *P. "xanthium"* and by *P. helianthi* and harvested in October, the perithecia of *D. "xanthium"* have appeared in November and those of *D. helianthi* at the end of March. In natural conditions, the *D. "xanthium"* fructifications come to maturity on *X. italicum* in March-April (according to climatic conditions) when a new generation of plant has germinated. The same synchronism exists for the *D. Helianthi- H. annuus* couple (Muntanola-Cvetković et al., 1988).
- The ascospore size: there is a hightly significant difference (student's test: t.obs=49,23; ddl=998; n=500) between the *D. "xanthium"* ascospores (11,7 x 2,9 μm; 9,3-16,6 x 2,5-3,6 μm) and those of *D. helianthi* (13,4 x 3,3 μm; 10,7-16,5 x 1,7-3,9 μm). Our measures are in concordance with the ones of Muntanola-Cvetković (1981) and Fayret (1987).

The induction of the *D. "xathium"* form on PDA is a striking feature which differentiates it from the majority of *P. helianthi* isolates (Muntanola–Cvetković et al., 1981, 1985; Herr et al., 1984; Fayret and Assemat, 1987). On the other hand, Yang (1984), in the United States has found on the sunflower one isolate of *P. helianthi* which like *P. xanthium* easily produces perithecia on PDA. According to Muntanola–Cvetković (1985), this isolate can be considered as a special *P. helianthi* biotype. Then among the species *Phomopsis (Diaporthe) helianthi Munt.–Cvet.*, isolates appear, native to the USA, which have one of the characteristic of *P. "xanthium"*. These observations, considering the American origin of *Xanthium* and *Helianthus* genera, have lead us to consider the following working hypothesis: could *P. helianthi* be a form of *P. "xanthium"* adapted to a new host (sunflower)? In this perspective we have already produced and studied two generations of *P. "xanthium"* on sunflowers.

CONCLUSION

The pathogenic agent responsible for the symptoms observed on *Xanthium italicum Moretti* belongs to the *Phomopsis* genus. It has perfect *Diaporthe* stage. We have temporarily named it *Phomosis (Diaporthe) "xanthium"*.

Althought incomplete, these studies undertaken to characterize it allow us to conclude the following.

- The conidial stage presents two types of mycelia, one of them is whitish on beige substrate, the other produces golden mycelial exudate with a brown substrate. On the host and media their pycnidia would only produce beta conidia.
- The ascospores (11.7 × 2.9 μm) constitute the primary inoculum in the absence of pathogenicity of beta conidia. The perithecial induction takes place on the natural host very early (August, September) but the maturing of perithecia corresponds to the development of a new host generation. The infection pathway occurs as follows: necrosis on the leaves, colonization of the petioles, and cankers on the stems. The cankers bear in the course of vegetation pycnidia rich in beta conidia.

First comparisons with other pathogens belonging to the *Phomosis* genus and present on *Xanthium spp.* indicate that we have probably determined a new pathogen on *Xanthium italicum*.

The particularity of *Phomosis (Diaporthe) "xanthium"* is its equal pathogenicity on *Helianthus annuus L.*. The symptoms it provokes on sunflower stem are in all points identical to those due to *Phomosis (Diaporthe) helianthi Munt.-Cvet.*. These two parasites do not produce alpha conidia, and one of the mycelial type of *P. "xanthium"* is wery similar to *P. helianthi*. Their telomorphicl forms oppose them: the perithecial induction is very early with *D."xanthium"* in relation to *D.helianthi* (on *X.italicum* as well as on sunflowers), they significantly differ in the size of ascospores and *D."xanthium"* has an easy production of perithecia on PDA. This last feature nevertheless brings our parasite near the biotype of *P.helianthi* isolated by Yang in the United States. And considering the fact that *Xanthium* and *Helianthus* are both native to the North American continent, we are presently working on the following hypothesis: could *Phomosis helianthi* be an adapted form of *P."xanthium"* on a new host, *Helianthus annuus*?

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Diaporthe (Phomopsis) SP., NOUVEAU PARASITE DE *Xanthium italicum Moretti*, PATHOGENE D' *Helianthus annuus L.*

Carriere, J.B., Petrov, M.

En juillet 1988, nous avons observé des symptomes sur *Xanthium italicum Moretti* identiques à ceux provoqués par *Phomopsis helianthi Munt.–Cvet.* sur tournesol. L'agent pathogéne responsable de ces symptomes sur *X.italicum* est un *Phomopsis (Diaporthe) sp.* nommé arbitrairement *Phomopsis (Diaporthe) "xanthium"*.

Cette publication décrit les formes anamorphes et telemorphes de ce parasite et présente les premiers résultats concernant son cycle biologique sur *X.italicum*.

La particularité de ce champignon est d'être également pathogéne sur tournesol (résultat prouve par tests ascospores), et de provoquer sur cette plante des symptomes sur tige identiques à ceux developpes par *P.helianthi*. Quelle est alors la relation entre *"P.helianthi* et *P."xanthium"*?

La comparaison des formes anamorphes de ces deux parasites les rapproche. Par contre, la comparaison de leurs formes télémorphes les oppose. Cependant, un des biotypes de *P.helianthi* isolé par Yang (1983), posséde des caracteristiques typiques de *D.helianthi* et de *D."xanthium"*. Ces diverses observations, compte tenu de l'origine Nord Americaine des genres *Xanthium* et *Helianthus* nous ont conduit a considerer l'hypothése suivante: *Phomopsis (Diaporthe) helianthi* serait il une forme de *Phomopsis (Diaporthe) "xanthium"* adaptée à un nouvel hote (le tournesol)?

Diaporthe (Phomopsis) SP., NUEVO PATOGENO DE *Xanthium italicum Moretti* Y DE GIRASOL (*Helianthus annuus L.*)

Carriere, J.B., Petrov, M.

En Julio de 1988 hemos observado en *Xanthium italicum* Moretti, síntomas similares a los producidos por *Phomopsis helianthi* Munt-Cvet. en girasoles. El agente causal de estos síntomas en *X. italicum* es un *Diaporthe (Phomopsis)* s. p. que hemos denominado arbitrariamente *Phomopsis (Diaporthe) "xanthium"*.

Esta publicación describe anamorfo y telemorfo de este parásito y ofrece los primeros resultados de su ciclo biologico en *X. italicum*.

La particularidad de este hongo reside en ser igualmente patogénico en girasoles (según los ensayos de inoculación con ascoporas), y en que produce en los tallos de esta planta sítoma idénticos a los que desarrolla *P. helianthi*.

La comparación de sus anamorfos los sitúa en un mismo grupo. Pero, por otro lado, los teleomorfos presentan diferencias bastantes importantes. Sin embargo, uno de los biotipos de *P. helianthi* aislado por Yang (1983) se caracteriza por características típicas de *D. helianthi* y de *P. "xanthium"*. Considerando estas observaciones y el hecho de ser tanto *Xanthium* como *Helianthus* géneros nativos del continente americano, trabajamos actuelmente en la siguiente hipótesis: podría ser *Phomopsis (Diaporthe) helianthi* una forma de *Phomopsis (Diaporthe) "xanthium"* adaptada a un nuevo huésped (el girasol)?