HELIA, **19**, Nr. 24, p.p. 79-86, (1996) UDC 632.4:633.854.78

HYPHAL INTERFERENCE OF *Diaporthe/Phomopsis Helianthi* WITH RELATED SPECIES

Dubravka Franić-Mihajlović[1], Jelena Vukojević[2], Nevena Mitić[3]

[1] *The "Siniša Stanković" Institute for Biological Research. 29 Novembra 142. 11060 Belgrade. Yugoslavia.*
[2] *Institute of Botany and Botanical Garden, Faculty of Biology, University of Belgarade, Yugoslavia.*
[3] *Center for Agriculture and Technological Research, Zaječar, Yugoslavia.*

Received: October 4.1995.
Accepted: July 19.1996 .

SUMMARY

The possibility for application of hyphal interference as an additional method for confirmation of the specificity of the *Phomopsis helianthi* isolated from *Helianthus annuus* has been examined. With this aim, inter-relationships of 13 fungal isolates, members of the *Diaporthe/Phomopsis* complex have been studied. Microscopic investigations of the demarcation zones between colonies of the same and different isolates of *Phomopsis/Diaporthe*, revealed the phenomenon of the hyphal interference between different isolates. The results obtained suggest that hyphal interference could serve as an additional parameter for a more reliable determination of fungal specificity.

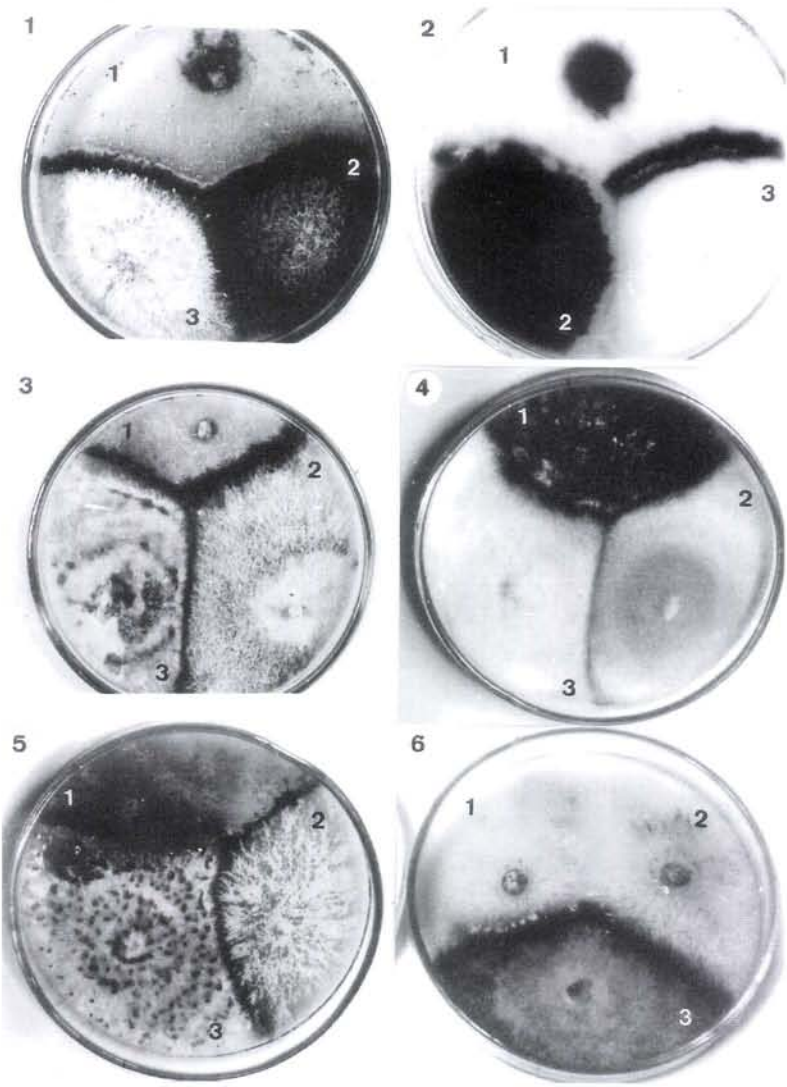
Key words: Hyphal interference, *Phomopsis/Diaporthe* complex, demarcations lines.

INTRODUCTION

Studies of the isolates in the complex *Diaporthe/Phomopsis* aimed at the distinction of different species is of great importance from both theoretical and practical points of view, since these fungi are known for their extremely high phytopathogenetic potential, frequently attacking crops of considerable economic importance. *Diaporthe/Phomopsis helianthi*, a sunflower pathogen, described as a new species on the basis of detailed investigations lasting for many years, belongs to this complex.

During the examinations of inter-relationships of related fungi from *Diaporthe/Phomopsis* complex, numerous authors reported the appearance of dark demarcation lines not only between the colonies of different species, but also between the colonies of the same species, but of different types, when cultured on the same substrate (Webber and Gibbs, 1984; Ploetz and Shokes, 1986; Brayford, 1990a; Muntañola-Cvetković et al., 1990).

Figures 1-6. Demarcation lines formed between neighbouring colonies of different Phomopsis/Diaporthe species, growing on PDA



Figures 1, 2. Surface and reverse of P. helianthi (1), Phomopsis sp. from Daucus carota (2), P. pulla (3) cultures. The front formed in the immediate vicinity of the colonies contact makes any direct contact between the hyphae impossible

Figure 3. P. helianthi (1), Diaporthe sp. from Arctium lappa (2) and P.longicolla (3)

Figure 4. P. helianthi (1), Phomopsis sp. from Achillea millefolium (2) and Arctium lappa (3)

Figure 5. P. helianthi (1), Phomopsis sp. from Cirsium arvense (2) and P. longicolla (3)

Figure 6. P. helianthi (1, 2) and Phomopsis sp. from Artemisia vulgaris (3)

Faced with the continuous problem of identification and clear separation of the species within the extremely heterogeneous complex *Diaporthe/Phomopsis*, we decided to determine in this work whether the phenomenon of hyphal interference could be applied as an additional parameter for the confirmation of the specificity of *Phomopsis helianthi* isolated from *Helianthus annuus*.

MATERIAL AND METODS

Fungal isolates used throughout the present work were as follows: *Phomopsis helianthi* (H1.P.) isolated from *Helianthus annuus*, *Phomopsis pula* (HED.P.) isolated from *Hedera helix*, *Phomopsis longicolla* (GLM.P.) isolated from *Glycine max*, *Phomopsis* sp. isolated from *Achillea millefolium* (ACH.P.), *Arctium lappa* (LAP.P.), *Artemisiae vulgaris* (ART.P), *Cychorium intybus* (CYC.P.), *Cirsium arvense* (CIR.P.), *Daucus carota* (DAU.P.), *Lactuca serriola* (LAS.P.), *Xanthium italicum* (XIT.P.) and *Diaporthe helianthi* (H1.D.) isolated from *Helianthus annuus* and *Diaporthe* sp. isolated from *Arctium lappa* (LAP.D.).

Different combinations each consisting of three fungal isolates were inoculated in Petri dishes containing potato-dextrose-agar (PDA) substrate (Booth, 1971). The three isolates were inoculated equi-distant from the others in all possible combinations in the same Petri dish. In this way 50 different combinations were prepared.

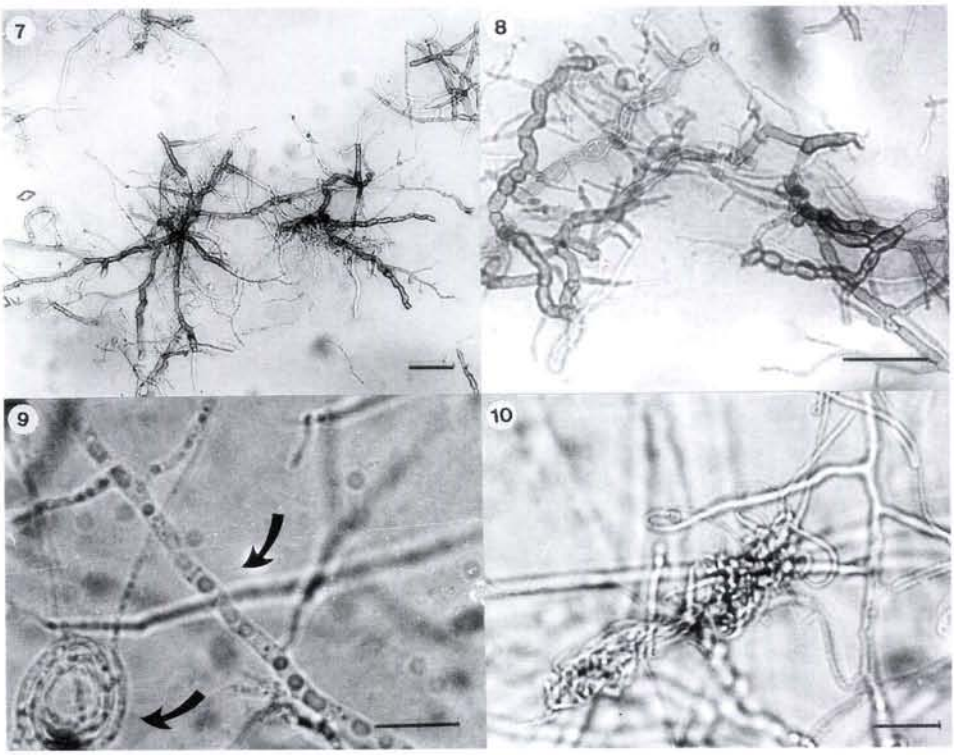
The cultures grown under laboratory conditions (daylight, 22±2°C) were successively examined after 5, 8 and 11 days of age.

The isolates were designated by the codes consisting of abbreviated name of the host plant, stage the isolate was prepared from (*Phomopsis* or *Diaporthe*) and the year of isolation.

RESULTS

In experiments when the three *Phomopsis* isolates were placed in the same Petri dish, the formation of dark demarcation lines between the colonies was frequently observed. These demarcation lines appeared in 100% of the cases between the *Phomopsis* isolates examined originated from different host plants. At the site of contact, or even in the immediate proximity of the contact between the colonies of different isolates, a dark front, distinct also from the reverse of a Petri dish was formed (Figures 1, 2). In the case of the following isolates: H1.P.89 - XIT.P.91; H1.P.89 - DAU.P.90; H1.P.89 - HED.P.88 and HRD.P.88 - DAU.P.90, this front was formed in advance of the contact between the colonies, thus making impossible any direct contact between the hyphae (Figures 1, 2). In the other isolates the front was formed at the site of contact of the neighboring colonies (Figures 3-6). Microscopic examinations of the fronts revealed altered hyphae

Figures 7-10. Microscopic examinations of demarcation lines



Figures 7,8. P. helianthi and Diaporthe sp. from Arctium lappa. Dark pigmented and thickened hyphae. BAR 20mm

Figures 9,10. P. helianthi and Phomopsis sp. from Cirsium arvense. Vacuolated hyphae, phenomenon of coils. BAR 5 mm.

which were thickened, vacuolated and more intensively pigmented. Also, coiling of the hyphae in the shape of waves or loops was observed (Figures 7-10).

When three isolates originating from the same host plant were inoculated in the same Petri dish, demarcation lines were formed between LAP.P.89 and LAP.D.90, LAS.P.91 and LAS.P.92; XIT.P.90 and XIT.P.91 and also between XIT.P.91 and XIT.P.92 (Figure 11). In the remaining combinations, colonies of the same species growing on the same substrate made contact and were intertwined, sometimes discontinuing further growth after the contact, but no visible demarcation lines were formed (Figure 12). On the microscopic preparations of the contact zones, none of the signs characteristic for the phenomenon of hyphal interference mentioned above were seen.

In all 50 combinations, the isolates of *P. helianthi* (H1.P.89, H1.D.91, H1.P.92, H1.D.92.) formed black lines in the contact zone with the other isolates examined (Figures 1-6), but demarcation lines were never formed at the site of contact between the two *P. helianthi* isolates (Figure 6). The mycelia of different *P. helianthi* isolates grew in the same Petri dish opposite to one another, making contact, intertwining and forming a completely homogenous mycelium.

We find the results of experiments when perithecia of *Diaporthe* isolates from *Arctium lappa* and *Helianthus annuus* were inoculated very closely, opposite to one another in the same Petri dish, especially interesting (Figure 13). In this case two types of mycelia were formed: one more aggressive and identical in appearance to *Diaporthe* isolate from A. lappa and the other identical to *P. helianthi*. After 7 days, black reproductive structures appeared on *P. helianthi* mycelium which is characteristic for this species. After maturation, the pycnidia contained only b-conidia. On the mycelia of *Phomopsis* sp. from *A. lappa*, black pycnostromatic structures with elongated rostrums containing a-conidia were formed after 10 days. These results are concordant with the characteristics of this species. Between these two types of mycelia, a very clear black demarcation line was formed. Examinations of both morphological and physiological characteristics of these colonies during the following 30 days did not reveal any mixing of the two species.

DISCUSSION

Hyphal interference represents a type of antagonism which can appear at the sites of hyphal contact or in their vicinity in different fungal species growing on the same substrate (Ikediugwu and Webster, 1970). At the site of contact, the cells cease to grow, losing at the same time turgescency, while the cytoplasm becomes vacuolated and granulated. As a consequence, cell membrane permeability may change, leading to the hyphal death. Examinations of the black demarcation lines formed between the colonies of different *Phomopsis* isolates revealed the phenomena corresponding to hyphal interference as described by

Ikediugwu and Webster (1970). The appearance of loops, waves and hyphae coiling around one another in the case of contact, agrees well with the observations of Denis and Webster (1971) who reported the coiling of *Trichoderma hyphae* around hyphae of other fungi at the site of contact. This phenomenon definitely results from the inter-relationship of the two fungi which are in contact, since it did not occur when the hyphae of the other fungi were replaced by plastic strands of the same diameter. In this case, hyphae of *Trichoderma* overgrew plastic strands without any difficulty.

According to Webster (1970), hyphal interference can be of great significance, if the hypothesis that it represents the most obvious and the most clear form of interspecific competition is true. As suggested by Deacon (1984), demarcation lines do not form between genetically identical colonies or monokaryons and thus, they represent genetically based isolation mechanisms which prevent the

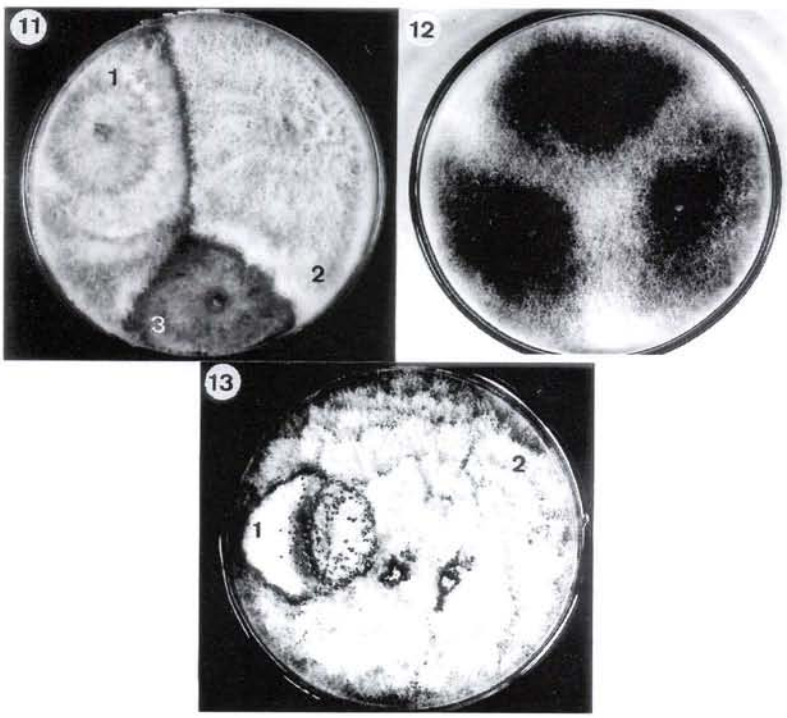


Figure 11. Demarcation lines between the three isolates originating from Xanthium italicum, on PDA. Type 1 (1), type 2 (2), type 3 (3)

Figure 12. Phomopsis sp. (3 colonies) isolated from Cichorium intybus

Figure 13. Colonies of P. helianthi (1) and Phomopsis sp. from A. lappa (2) formed by inoculation of perithecia very close, opposite to one another, after 30 days of culture

mixing of dikaryons. During the present study, in three cases, demarcation lines were observed between similar isolates originating from the same host plant. However, all these isolates were prepared from plants growing at different localities and in different seasons. These results resemble the data of Brayford (1990a, b), who reported the formation of line zones between the colonies of the two morphological groups of the *Phomopsis* isolate from *Ulmus* spp., as well as between the colonies of the same morphological group. This author interpreted the appearance of the lines between the colonies of the same morphological group as an intraspecies antagonism. As already mentioned, in our experiments, demarcation line between the isolates of the same species were formed in three cases. It is worth mentioning that the isolates from *A. lappa* and *X. italicum* (in which the formation of demarcation lines was observed later) were classified in two, i.e., three separate type groups, due to morphological differences and the differences in the species of reproductive structures, as well as in the type of spores. Isolates from *Lactuca serriola* were not morphologically different, but the differences with regard to fructification ability were evident. If we accept the hypothesis that the phenomenon of demarcation line formation results from the incompatibility between genetically different colonies, then their appearance between different types within the same *Phomopsis* species could suggest the genesis of new species.

As demonstrated previously (Muntañola-Cvetković et al., 1990) P. helianthi expressed the symptoms characteristic for the phenomenon of hyphal interference in relation to all other isolates of the complex *Diaporthe/Phomopsis*, thus confirming once more its specificity within this complex.

On the basis of the results presented in this work it can be concluded that hyphal interference could be successfully applied as an additional method for a more appropriate test of the specificity of the fungal isolates.

ACKNOWLEDGEMENT

It is a pleasure to thank Professor Dr. Maria Muntañola-Cvetković for her great help and very useful sugessestions during the investigation.

The authors gratefully acknowledge support from the U. S.- Yugoslav Joint Fund for Scientific and Technological Cooperation, Agency USDA, Project No. JF-953-71.

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INTERFERENCIA HIFAL DE *Diaporthe/Phomopsis helianthi* CON ESPECIES RELACIONADAS

RESUMEN

La posibilidad de aplicación de interferencia hifal como un método adicional para la confirmación de la especialidad de las especies *Phomopsis helianthi* aisladas de *Helianthus annuus* ha sido examinada. Con este objetivo, la relación de 13 aislamientos fúngicos, miebros del complejo *Diaporthe/Phomopsis* ha sido estudiada.

Las investigaciones microscópicas de las zonas de demarcación entre asilamientos diferentes, asi como entre los mismos aislamientos de *Phomopsis/Diaporthe*, revelaron el fenómeno de la interferencia hifal entre diferentes aislamientos. Los resultados obtenidos sugieren que la interferencia hifal podria servir como un parámetro adicional para una determinación mas real de la especificidad de especies fúngicas.

L'INTERFÉRENCE HYPHALE DE *Diaporthe/Phomopsis Helianthi* AVEC LES ESPÉCES APPARENTÉES

RÉSUMÉ

La possibilité d'utiliser l'interférence hyphale comme une méthode supplémentaire pour confirmer la spécificité de l'espéce *Phomopsis helianthi* isolée dans *H. annuus* a été analysée. Dans ce but, les réactions de 13 isolats du champignon, appartenant au complexe *Diaporthe/Phomopsis* ont été étudiées. Des examens microscopiques des zones de confrontation entre isolats différents ou identiques, révélent un phénoméne d'interaction hyphale entre les différents isolats. Les résultats obtenus suggérent que l'interférence hyphale pourrait être utilisée comme un paramétre supplémentaire pour une détermination plus sûre de la spécificité de l'espéce du champignon.