# VERIFICATION OF THE INHIBITORY EFFECTS PROVOKED BY SEVERAL ACTINOMYCETES ON THE GROWTH OF THE FUNGUS *PHOMOPSIS HELIANTHI* AND CONGENERIC SPECIES

M. Muntañola-Cvetković, Nevena Mitić and Jelena Vukojević

The "Siniša Stanković" Institute for Biological Research, 29 Novembra 142, 11060 Belgrade 38, and Institute of Botany, Faculty of Sciences, Takovska 43, 11000 Belgrade, Yugoslavia.

The reproducibility of the results obtained in 1990 and 1991 on the differential inhibitory effects of four *Streptomyces* species and one unnamed *Streptoverticillium* on several *Phomopsis* cultures was investigated. The present study was enlarged with new fungal cultures which were isolated from *Phomopsis* conidiomata and *Diaporthe* ascomata found on sunflowers, soybeans and several common weeds. The experiments were performed either with freshly isolated cultures or with cultures that had been stored for two and three years at 4°-8°C. The results showed that: the inhibitory potential of the actinomycetes had not decreased after a 2-yr storage on PDA slants in the refrigerator; the sensitivity of the old fungal isolates had not changed during this period; the reactions of the new isolates were in agreement with those of the species groups recognized in previous screenings; the pathogen of the sunflowers *Diaporthe/Phomopsis helianthi* Munt.-Cvet. *et al.* showed significant differences with respect to *D.arctii* (Lasch) Nitschke (*Phomopsis arctii* (Lasch) Traverso), the species that constitues the core of an ill-defined and heterogenous assemblage into which the congeneric fungus on *Helianthus* was tentatively included in the past.

An *in vitro* screening test based on the effects of some selected actinomycetes was used in previous investigations (1, 2, 3 ), as a complementary parameter in distinguishing the pathogen *Diaporthe/Phomopsis helianthi* Munt.-Cvet. *et al.* from congeneric cultures isolated from several plants growing in the same region of Voivodina. The aim of the present study was to investigate the reproducibility of the results obtained after a two- and three-year storage period of the actinomycete cultures on potato-dextrose-agar (PDA) slopes at 4°-8°C. Besides several fungal isolates that had been already investigated in 1990 and 1991, new cultures were included in the 1992 study.

## Material and Methods

About 150 fungal isolates were tested for their sensitivity toward the vicinity of four *Streptomyces* species (*S. albidoflavus, S.albus, S.diastaticus, S.* sp.) and an unnamed *Streptoverticillium*. The fungal cultures were recovered from *Phomopsis* pycnidia and *Diaporthe* perithecia found on naturally infected growing plants or in overwintered plant parts belonging to the following species: *Achillea millefolium* L., *Arctium lappa* L., *Cichorium intybus* L., *Glycine max* (L.) Merr., *Helianthus annuus* L., *Lactuca serriola* L., *Sonchus arvensis* L., *Xanthium italicum* Moretti, and *X. strumarium*. The present investigations were performed either with freshly isolated fungal and actinomycete cultures or with cultures that had been stored for two and three years in the refrigerator with periodical transfers. The procedures were the same as those employed in 1991. Five-d old actinomycete cultures were point-inoculated at the center of PDA inverted plates. The fungi were transferred four days later to three symmetrical positions in the plates, using mycelial tips of 7-d old colonies. Cultivation was performed under the following daily conditions: 8h fluorescent white light, 4,500 K / 12h dark; light intensity 27 $\mu$mol/sec/m$^2$; temperature 26° ± 2°C. The distances between the margins of the fungal and the actinomycete colonies were measured in mm as shown in Fig.1, placing the

inverted Petri dishes on a light-box. Records were taken at 4-d intervals during a 45-d period.

## Results

Twenty colour photographs presented at the 13th International Sunflower Conference in Pisa, Italy, illustrate part of the results obtained. A selection of the data recorded in 1991 and 1992 for two actinomycetes (*S. albus* and *S. albidoflavus*) and five groups of fungi isolated from four host plant species have been summarized in Graphs 1 and 2. The following can be deduced: a good reproducibility of the results; the inhibitory potential of the actinomycetes did not decrease after a 2-yr storage on PDA slants at 4°-8°C; the sensitivity of the old fungal isolates was not altered during this period; the reactions of the new isolates were in agreement with those of the species groups recognized in previous screenings. The slight differences recorded in 1992 with respect to 1991 are due to a change in the temperature of the cultivation room: 24° ± 2°C in 1991 and 26° ± 2°C in 1992. The higher temperature favoured the growth of the actinomycetes and enhanced their bioactivity; however, the correlation between the actinomycetes and the fungi did not change. The actinomycetes could be ranked by their inhibitory potential and the fungi by their sensitivity following the same scale recorded in previous studies. It was so verified that the phenomena of sensitivity may complement the data concerning the morphological characters exhibited by the fungi under study when grown under standardized conditions. With scanty aerial mycelium, small stromata and the most dramatic reactions to the growth-inhibitory effects of the actinomycetes, *P. helianthi* clearly differed from the cultures isolated from *A. lappa*, soybeans and others, of vigorous growth, large stromata, and moderate or scarce reactions to the activity of the actinomycetes.

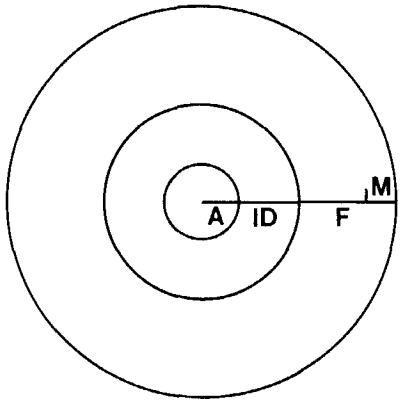
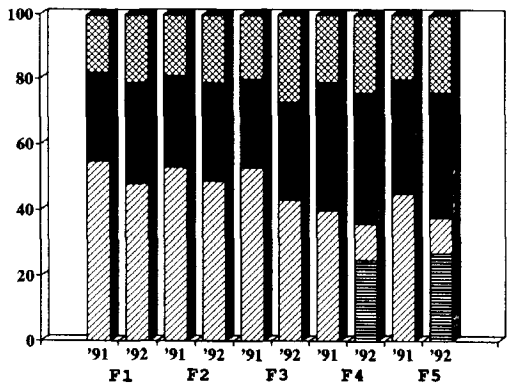
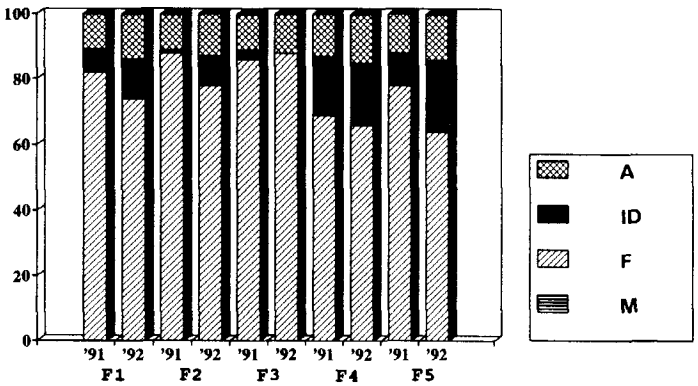



Fig. 1. Schematic representation of a plate with three *Phomopsis* colonies grown under the inhibitory effects of an actinomycetes. **A**, Actinomycete colony radius; **ID**, Distance between the actinomycete and the fungal colony margins. **F**, Distance between the colony front or internal margin of the fungal colony and the edge of the plate. **M**, Distance between the external margin of the fungal colony and the edge of the plate.

Graph 1




Graph 2




Graphs 1 and 2. Effects of the antagonistic activity of *S. albus* (Graph 1), and *S. albidoflavus* (Graph 2), against five fungal isolates: F1, *P. longicolla* Hobbs, isolate SO3.P.89 from soybeans; **F2**, *Phomopsis* isolates LAP.P.89 and LAP.P.92.J.Pm from *A. lappa*; **F3**, Type-1 isolates, XIT.1.91.J, from *Xanthium* spp.; **F4**, Type-2 isolates, XIT.P.90, from *Xanthium* spp.; **F5**, *P. helianthi*, H1.D.89 and H1.D.D.Rs isolates from ascomata found on overwintered sunflower stems. Captions **A**, **ID**, **F**, and **M**, the same as in Fig. 1.

## Conclusions

The pathogen of the sunflowers *Diaporthe/Phomopsis helianthi* showed significant morphological and inhibition differences with respect to *D. arctii* (Lasch) Nietschke (*P. arctii* (Lasch) Traverso), that form the core of an ill-defined and heterogenous assemblage into which Wehmeyer (4) tentatively included the congeneric fungus on *Helianthus*.


## Acknowledgments

The authors gratefully acknowledge the financial support of the U.S.-Yugoslav Joint Fund for Scientific and Technological Cooperation, Agency USDA, Project N° JF-953-71. They are also grateful to Dr. Miroslav Mihaljčević, who collected the plant material.


## References


(1) Franić-Mihajlović,D., Vukojević,J. & Muntañola-Cvetković,M. (1990). Differential sensitivity toward actinomycete antifungal metabolites in *Diaporthe/Phomopsis* species. Arhiv biol. nauka, Beograd, **42**, 31P-32P.

(2) Muntañola-Cvetković,M.,Bojović-Cvetić,D., Franić- Mihajlović,D. & Vukojević, J. (1990). Hyphal interference and antagonism among *Diaporthe* or *Phomopsis* species and sensitivity toward antifungal metabolites. 4th International Mycological Congress. Abstracts. p. 86.

(3) Muntañola-Cvetković,M., Vukojević,J. & Mihaljčević,M. New approaches in distinguishing between *Phomopsis* isolates from different host plant species. *(Submitted)*

(4) Wehmeyer, L.E. (1931). The genus *Diaporthe* Nitschke and its segregates. Univ. Mich. Press. Sci. ser. **9**, 1-349.