ABSTRACT OF HABILITATION THESIS

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MORPHOLOGICAL AND GENETIC DIVERSITY OF FUNGI FROM THE GENUS *PHOMOPSIS* (*SPHAEROPSIDALES, FUNGI IMPERFECTI*) AND ANALYSIS OF FACTORS LIMITING THEIR OCCURRENCE

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Key words: *Phomopsis* spp., orchard plants, diversity, antagonistic microorganisms, chitosan, fungicides

Fungi from the genus *Phomopsis* are common pathogens of various plants species. The majority of *Phomopsis* spp. are asexual but some of them have teleomorphs within genus *Diaporthe*. So far more than 800 species and subspecies have been described but they are slightly differentiated from the point of view of colony morphology and spore dimension. That is why identification of *Phomopsis* isolates within genus is difficult.

The major aims of the work were: 1) to study the possibility of colonizing apparently healthy *Vitis vinifera* mother canes by *Phomopsis viticola* on commercial plantations in different regions of Poland, 2) to estimate the harmfulness of this pathogen to grapevine cuttings produced from the plantations, 3) to indicate some microorganisms and chemicals limiting growth and development of the pathogen. The additional aim was to differentiate some isolates of *Phomopsis* spp. based on the classical and molecular methods.

The studies considered eight isolates of *P. viticola* originating from grapevine, six isolates of *P. mali* obtained from apple, pear and cherry trees and two isolates of each of the following species: *P. archerii*, *P. juglandina*, *P. juniperovora* as well as two unidentified isolates originating from blueberry, walnut, thuja and hazel. They were characterized on the basis of culture colour and mycelium growth rate on PDA medium, appearance of pycnidia, the time required for sporulation and morphology of spores. The ability of the isolates to infect grapevine canes was studied *in vitro* using artificial inoculation. Their genetic variability was studied with RAPD technique. Protection activity of *Trichoderma* spp., *Gliocladium* spp., *Pseudomonas fluorescens*, *Bacillus* sp. and chitosan was estimated on the basis of successful infection number on grapevine canes and *P. viticola* reisolation from these canes. The

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chemicals selected represent different mode of action on the pathogen and they were tested *in vitro* at six various concentrations of active ingredients.

It was shown that colony morphology and spore dimension of the studied *Phomopsis* isolates were only slightly differentiated. The highest number of grapevine canes with symptoms of necrosis was observed after inoculation with *P. viticola*. Some canes were infected also by *P. mali*, while *P. archeri* and *P. juglandina* caused disease symptoms only occasionally. The grapevine canes inoculated with *P. juniperovora* and with isolates from hazel remained healthy. Basing on similarity coefficients values, two main groups of strains were distinguished. Group I contained species other than *P. viticola*, and group II contained strains only of this fungus.

Small differentiation in mycelial growth and morphology of *Phomopsis* spp. spores indicate the difficulties in correct and quick identification of these species within genus. Clusters of strains from grapevine within one group of similarity and results of artificial inoculation suggest pathogenic specialization of *P. viticola* to this plant. The results of RAPD analysis which confirmed differentiation within *Phomopsis* strains demonstrated with conventional methods, indicate that conventional and molecular methods are complementary.

Moreover, it appeared that *P. viticola* was able to colonize healthy grapevine canes and next caused disease symptoms on the cuttings during callusing and soon after planting. Isolation of this pathogen from apparently healthy canes which may be used for vegetative reproduction, points to the possibility of the pathogen spread with symptomless cuttings onto new plantations.

Among microorganisms tested, *Trichoderma viride, T. koningii* and *T. harzianum* were the most active in canes protection against infection by *P. viticola*. It was found that chlorothalonil, flusilazol and thiophanate-methyl caused the decay of all *P. viticola* spores in each studied concentration. After using mancozeb, cyprodinil, azoxystrobin and chitosan a strong inhibition of spore viability was also observed, especially at concentrations of 100 mg a.i. on 1 cm^3 and above. It seems that among the chemicals tested mancozeb and chitosan may have a wider application in *P. viticola* control in Polish conditions. It results both from common usage of mancozeb in programmes of grapevine protection against various phytopathogens in the world and from its ability to inhibit the spore viability of native *P. viticola* strains. What concerns chitosan, its lack of harmfulness of to living organisms gives the possibility for use in ecological and integrated pest management programmes of plant protection, which is crucial from the point of view of environmentally friendly grapevine protection.

Research papers constituting the habilitation thesis

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