Efficacy of biofungicides Supresivit and Polyversum against *Phytophthora* root pathogens on European chestnut (*Castanea sativa* Mill.)

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ABSTRACT: The effect of two commercially produced biopreparations on the originators of chestnut ink disease in Slovakia – soil fungi *Phytophthora cambivora* and *Phytophthora cinnamomi* was tested in laboratory conditions. Investigations of interrelations between *Trichoderma harzianum (Pythium oligandrum*) and isolates of *Phytophthora* sp. obtained from infected tissues of *Castanea sativa* Mill. proved more important inhibitive effects for *Pythium oligandrum* (biopreparation Polyversum); efficiency 25.3% (*Ph. cambivora*) and 26.2% (*Ph. cinnamomi*). Biopreparation Supresivit (*Trichoderma harzianum*) reached the efficiency 9.1% for *Ph. cambivora* isolates and 9.2% for *Ph. cinnamomi* isolates. Significant effect of the used bioformulas for inhibition of mycelial growth of both *Phytophthora* sp. and significant difference in the efficacy of two different bioformulas were confirmed statistically.

Keywords: sweet chestnut; *Phytophthora cambivora*; *Phytophthora cinnamomi*; biological control; biopreparations; *Trichoderma harzianum*; *Pythium oligandrum*

The genus *Phytophthora* includes several pathogenic species with a wide host range. In the last years an increasing number of species have been associated with declining trees. Since the beginning of the century, in chestnut soil-borne pathogens *Phytophthora cambivora* and *Phytophthora cinnamomi* are well known as causal agents of the so called "ink disease". These two fungi cause root and collar rots, and necrotic lesions on stems of chestnuts in nurseries, plantations and forests.

At the beginning of research on the ink disease many authors pointed out the importance of development of effective control method against root rots of ornamental and fruit woody plants caused by *Phytophthora*. Chemical control provided by highly effective oomycetes-specific fungicides, such as metalaxyl and fosetyl Al, has been successful (DAVIS 1982; BENSON 1985; MARCHETI, D'AULERIO 1998) but is not always desirable because of the high cost of application, potential hazards to the environment, toxicity to crops, and the development of fungicide-resistant strains (HAMM et al. 1984; COHEN, COFFEY 1986).

Although biological control of root-disease fungi has been studied by many researchers, few studies have been made on the control of *Phytophthora* by the use of specific antagonistic hyphomycetous fungi (SHEA, BROADBENT 1983). WESTE and MARKS (1987) pointed out the importance of soil microorganisms that cause decomposition of parasitic hyphae, sporangial decline and reduction of resistant zoospores.

Some recent studies have demonstrated the reduction in *Phytophthora* root rots by the species *Aspergillus* (SZTEJNBERG, TSAO 1986; ALMASSI et al. 1996), *Gliocladium* (SMITH et al. 1990), *Myrothecium* (GEES, COFFEY 1989), *Penicillium* (SZTEJNBERG, TSAO 1986; OWNLEY, BENSON 1992; FANG, TSAO 1995) and *Trichoderma* (SMITH et al. 1990; KIM et al. 1992; CHAMBERS, SCOTT 1995).

Research on the antagonistic activity of *Trichoderma viride* to 4 soil-borne *Phytophthora* species confirmed inhibitive effects on radial growth of parasitic mycelium in all combinations antagonist-pathogen (HELLER, THEILERHEDTRICH 1994). CHAMBERS and SCOTT (1995) demonstrated antagonistic effects of *Phytophthora* sp. to *Trichoderma* isolates under *in vitro* conditions.

The addition of *Trichoderma viride* spores as a potential antagonist 10 days before the inoculation of substrate with *Phytophthora* sp. resulted in a significant improvement of the health state of cultivated plants. The biocide in dosage of 300 and 600 g/m^3 inhibited the development of the pathogen (*Phytophthora* sp.) in used substrate (ORLIKOWSKI 1995).

DAVIS et al. (1978), VROT and GRENTE (1985), MEOTTO et al. (1993) pointed up the importance of mycorrhizal symbiosis as a possible way of biological control on European chestnut, where *Leucopaxillus cerealis* showed the most satisfactory effect. BRANZANTI et al. (1999) reported the effect of four species of ectomycorrhizal fungi (*Lacccaria laccata*, *Hebeloma crustulini-*

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forme, *Hebeloma sinapizans*, *Paxillus involutus*) on the suppression of chestnut ink disease.

In Slovakia research on the importance and use of antagonistic fungi to control fungal plant diseases including root pathogens was on the increase in the early seventies. Results obtained by the application of methods using the phenomenon "fungus contra fungus" indicate possibilities of more extensive application of alternative forms in plant protection (MICHALÍKOVÁ, MICHRINA 1997a,b). DUŠKOVÁ (1992, 1995) confirmed the inhibitive effect of biofungicides by suppression of root pathogen growth.

The aim of this study was to test the ability of two biopreparations to reduce ink disease of European chestnut caused by *Phytophthora* pathogens. The present work aimed to determine the efficacy of selected biological preparations in laboratory conditions and on the basis of laboratory testing to evaluate the possibilities of application in biological control on European chestnut as a promising woody plant species cultivated in Slovakia.

MATERIAL AND METHODS

The efficacy of two biopreparations (Supresivit, Polyversum) preferred at the present time in connection with the ability to inhibit the growth of mycelial hyphae of root plant pathogens was tested in laboratory conditions.

Supresivit is a natural microbial preparation on the basis of aerial conidia (spores) of *Trichoderma harzianum* Rifai aggr. PV 2888-94 in a uniform mix with inert filler in the powdered form; 1 gram of the powder contains minimally 1.4×10^{10} of conidia with 70% germinability. Preparation with batch No. 59010334 (Fytovita, Ltd. Praha) was used for the experiment.

The active substance of the biopreparation Polyversum is oospores of *Pythium oligandrum* Drechsler in an inert filler; 1 gram contains minimally 1×10^6 to 1×10^7 of oospores with 70% germinability. Preparation of the company Biopreparáty, Ltd. Praha was used for the experiment.

To observe the compatibility of *Trichoderma harzianum* (Supresivit) and *Pythium oligandrum* (Polyversum) with obtained isolates of *Phytophthora* at *in vitro* experiments we proceeded according to the method by CHAMBERS and SCOTT (1995).

In vitro antagonistic effect was observed on malt agar (30 g of malt agar in 1,000 ml H_2O) and 5% concentration of biopreparation was used (0.5 g of biopreparation in 100 ml liquid agar).

Table 1. Average daily growth of *Phytophthora cambivora* (PCA) and *Phytophthora cinnamomi* (PCI) under the influence of biopreparations Supresivit and Polyversum

Number of replications	Supresivit		Polyversum		Control	
	Phytophthora isolate		*Phytophthora* isolate			
	PCA	PCI	PCA	PCI	PCA	PCI
1	3.5	3.0	2.5	2.0	3.5	3.5
2	3.0	3.5	3.0	3.0	3.5	4.0
3	3.0	4.0	2.0	3.0	3.0	4.0
4	3.5	4.0	3.0	3.5	4.0	4.5
5	3.0	3.5	2.5	3.0	3.0	4.0
6	2.5	4.5	2.5	3.0	3.0	4.5
7	3.0	4.0	2.0	3.0	3.0	4.5
8	3.5	3.0	2.5	2.0	3.5	3.5
9	2.5	4.0	3.0	3.0	3.0	4.0
10	3.0	4.0	2.0	3.5	3.5	4.5
11	3.0	3.5	3.0	3.0	3.5	4.0
12	2.5	3.5	1.5	3.0	2.5	4.0
13	2.5	4.0	2.0	3.5	3.0	4.5
14	3.0	3.5	2.5	2.5	3.5	3.5
15	4.0	3.5	3.0	3.5	4.0	4.0
16	3.0	3.5	2.5	3.0	3.0	4.0
17	2.5	4.5	2.0	3.0	3.0	4.5
18	3.0	4.0	3.0	3.5	3.5	4.5
19	3.0	3.5	2.5	3.0	3.5	4.0
20	2.5	4.0	2.0	4.0	3.0	4.5
Average (mm)	2.98	3.75	2.45	3.05	3.28	4.13

Average daily growth (mm)

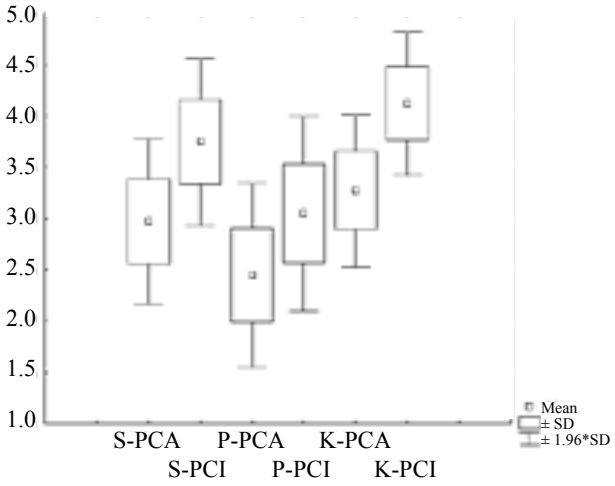

Fig. 1. Average daily growth of *Ph. cambivora* and *Ph. cinnamomi* isolates under the influence of biopreparations Supresivit and Polyversum (S – Supresivit, P – Polyversum, K – control, PCA – isolate of *Ph. cambivora*, PCI – isolate of *Ph. cinnamomi*)

Sterile chilled (37–39ºC) culture medium was supplemented with tested biopreparations in a sterile box. A segment of agar (5 × 5 × 5 mm) with pure fungal cultures obtained by cultivation on malt agar was placed on these treated solid media in Petri dishes. Cultures were subsequently incubated at 24–26ºC in darkness.

The efficacy of tested biopreparations was evaluated after 48 hours by recording the dimensions of pathogen colonies. In the process of cultivation growth rate was always recorded after 24 hours (average daily growth in mm/24 hours), over the four days. Agar media untreated with biopreparations were used for a control experiment. 20 replications were made for both *Phytophthora* isolates.

To confirm the effect of tested biopreparations on the growth of investigated fungal pathogens method ANOVA-MAIN EFFECT (STATISTICA-6) was applied. Statistical significance of variance was evaluated by non-parametric Mann-Whitney *U*-test.

RESULTS

Average daily growth (ADG) of *Ph. cambivora* isolates with the use of Polyversum ranged from 1.5 to 3.0 mm/day (2.45 mm/day on average), for *Ph. cinnamomi* isolates from 2.0 to 4.0 mm/day (3.05 mm/day on average). Values of ADG with the use of Supresivit ranged from 2.5 to 4.0 mm/day (2.98 mm/day on average) for *Ph. cambivora* isolates and from 3.0 to 4.5 mm/day (3.75 mm/day on average) for *Ph. cinnamomi* isolates. In control dishes ADG averaged 3.28 mm/day for *Ph. cambivora* isolates and 4.13 mm/day for *Ph. cinnamomi* isolates (Table 1).

By investigations of the inhibitive effect of tested biopreparations higher values of ADG were recorded for Supresivit (for both *Phytophthora* isolates); differences in average daily growth compared with growth with the use of Polyversum averaged 0.53 mm/day for *Ph. cambivora* isolates and 0.7 mm/day for *Ph. cinnamomi* isolates.

The comparison of efficacy of tested biopreparations under laboratory conditions proved more intensive inhibitive effects of *Pythium oligandrum* oospores (Polyversum). Suppression of radial growth of mycelia of pathogens showed more noticeable for the bioformula Polyversum; it confirmed lower values of average daily growth for both *Phytophthora* sp.

By the use of Supresivit biopreparation a decrease in average daily growth on average by 0.3 mm/day (efficiency 9.1%) was observed in *Ph. cambivora* isolates and on average by 0.38 mm/day (efficiency 9.2%) in *Ph. cinnamomi* isolates. Under the influence of Polyversum a decrease in ADG on average by 0.83 mm/day (efficiency 25.3%) for *Ph. cambivora* isolates and 1.08 mm/day (efficacy 26.2%) for *Ph. cinnamomi* isolates was found out. Comparison of average daily growth of *Ph. cambivora* and *Ph. cinnamomi* isolates with growth on untreated samples (control) by the use of both bioformulas is illustrated in Fig. 1.

Testing of recorded data by the method ANOVA-MAIN EFFECT in programme STATISTICA-6 confirmed significant effects of used biopreparations on suppression of growth of both *Phytophthora* sp. and

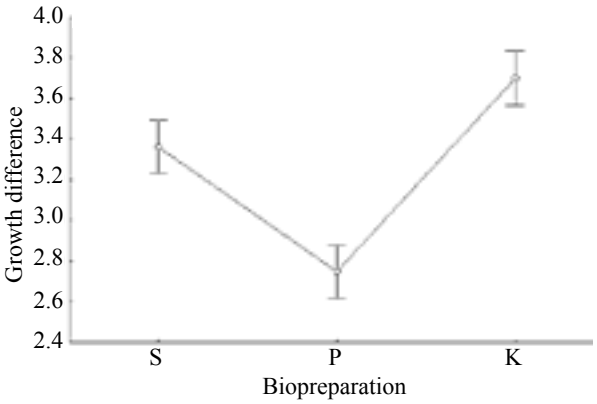

Fig. 2. Effect of biopreparations Supresivit and Polyversum on average daily growth of mycelium of *Phytophthora* isolates (S – Supresivit, P – Polyversum, K – control)

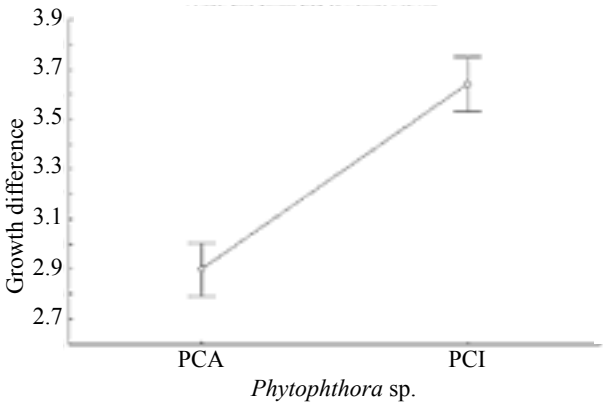

Fig. 3. Effect of *Phytophthora* isolates on average daily growth of *Phytophthora* mycelium (PCA – isolate of *Ph. cambivora*, PCI – isolate of *Ph. cinnamomi*)

significant difference in the efficacy of two different biopreparations (F = 52.77; p = 0.001) (Fig. 2). A significant difference in growth rate for both *Phytophthora* sp. (F = 93.87; p = 0.001) was also confirmed (Fig. 3).

To confirm statistical significance of differences, with respect to normal distribution of recorded values, non-parametric Mann-Whitney *U*-test was used for all observed combinations. *U*-test confirmed significant differences between the values on significance level p = 0.001 by comparing two types of preparations as well as both *Phytophthora* sp.

Variance in growth rate of *Ph. cinnamomi* under the influence of Supresivit compared with control sample was confirmed only on significance level p = 0.01.

DISCUSSION

The control of *Phytophthora* fungi presents a cardinal problem for arborists and planters of European chestnut all over the world. Classical biological control is one of several potential ways of application of living microorganisms to control the populations of phytopathogens, where the philosophy of biological control is not eradication of disease originator and its elimination from the ecosystem, but diversion of soil balance in favour of microorganisms which cause no diseases and maintenance of potential phytopathogens at a sufficient distance from cultivated plants (ANSELMI et al. 2000).

The mechanism of biological control with *Trichoderma* sp. is based mainly on mycoparasitic and aggressive competitions with pathogens. *Trichoderma* sp. can colonize substrata in the soil very quickly because of very fast growth and abundant production of spores. Mycelia of *Trichoderma* sp. can grow along and coil around the hyphae of host fungi or produce short branches that tightly surround the host hyphae. Penetration of host mycelia may or may not occur (PIETR 1997).

In broadleaved stands of Poland the phenomenon of hyperpathogenicity is proposed as another biological method to control the root pathogens on a large scale. It is known that some fungi of *Trichoderma* genus can inhibit the increment or destroy mycelium of many fungal species. Rhizomorphs found in the forest soils can be seriously damaged or completely destroyed by *Trichoderma*. This phenomenon was also confirmed by laboratory observations. The most active were *Trichoderma viride* and *T. harzianum* (OSZAKO 1997).

Positive effects of the soil microorganism *Pythium oligandrum* on stimulation of root system development and disease resistance of plants were observed lately. These effects are probably caused by correlations between the fungus and higher plant where the exchange of some useful substances takes place.

In the last years in the Czech Republic the preparation Polyversum whose active substances are oospores of *Pythium oligandrum* enjoyed more extensive applications in the system of biocontrol. This fungus colonizes the rhizosphere of higher plants and unlike most other *Pythium* sp. feeds as a mycoparasite, its nutrition comprises about 50 other phytopathogenic fungi which attack mainly roots and root collars of plants (RUBÁK 2001).

Efficacy of bioformulas in laboratory conditions is often tested by commercially produced biopreparations as well as pure cultures of individual bioagents. Their active substances are most often fungal spores that commonly occur in nature and parasitize on harmful phytopathogenic fungi. The effect of the used substrate and used biopreparation by testing of Supresivit and Polyversum on *Pythium ultimum* growth under laboratory conditions was proved. Supresivit was more effective in the control of *Pythium ultimum* growth than Polyversum (*Pythium oligandrum*) that showed a very low effect (DUŠKOVÁ 1992, 1995).

In this instance our primary concern is a possibility of eliminating the root disease occurrence in chestnut by means of suppressive effects on the fungi *Pythium oligandrum* and *Trichoderma harzianum.* By investigations of the effects of bioformulas Supresivit and Polyversum on *Ph. cambivora* and *Ph. cinnamomi* growth overall more intensive suppressive effects were observed when Polyversum was used.

On the basis of knowledge of several authors and results of our own observations, in the stage of laboratory experiments, it is possible to suppose more extensive applications of tested biopreparations directly in field conditions. Field trials will be the object of research in the near future.

Results obtained by experiments with biological preparations under laboratory conditions point to a possible way of suppression of chestnut root rots caused by *Phytophthora* by the antagonistic species *Trichoderma* and *Pythium*. Definite possibilities for the protection of this woody plant follow, with prospects for more intensive use of biopreparations in biological protection of European chestnut and generally in integrated plant protection, especially in conditions of orchards and gardens, arboreta and other dendrological objects. Intensive efforts to know and understand the effects and use of biological preparations in plant protection relate to their ability to suppress the root pathogens under the influence of agents non-toxic to plants and with minimal risk for the environment.

CONCLUSION

The present study tested two commercially produced biopreparations under *in vitro* conditions which are able to suppress *Phytophthora* infection on European chestnut.

The suppression of radial growth of hyphae of pathogenic mycelium was more marked for the biopreparation Polyversum; the use of this preparation resulted in lower values of ADG for both *Phytophthora* sp.

Investigations of interrelations between *Trichoderma harzianum* (*Pythium oligandrum*) and isolates of *Phytophthora* proved more important inhibitive effects for

Pythium oligandrum (Polyversum); efficacy 25.3% (*Ph. cambivora*) and 26.2% (*Ph. cinnamomi*).

The use of the biopreparation Supresivit led to a decrease in average daily growth on average by 0.3 mm/day (efficacy 9.1%) for *Ph. cambivora* isolates and on average by 0.38 mm/24 hours (efficacy 9.2%) for *Ph. cinnamomi* isolates.

Suppression of *Phytophthora* pathogens by the tested biological formulas confirmed the suitability of their application in biological protection of *Castanea sativa*.

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Účinnosť biofungicídov Supresivit a Polyversum proti koreňovým hnilobám gaštana jedlého (*Castanea sativa* Mill.) spôsobeným hubami rodu *Phytophthora*

ABSTRAKT: Účinnosť dvoch komerčne vyrábaných biopreprátov proti pôvodcom atramentovej choroby gaštana jedlého na Slovensku – pôdnych patogénov *Phytophthora cambivora* a *Phytophthora cinnamomi* bol testovaný v laboratórnych podmienkach. Skúmanie vzájomných vzťahov medzi *Trichoderma harzianum (Pythium oligandrum*) a izolátmi *Phytophthora* sp. získanými z napadnutých pletív *Castanea sativa* preukázalo výraznejší inhibičný účinok huby *Pythium oligandrum* (biopreparát Polyversum); účinnosť 25,3 % (*Ph. cambivora*) a 26,2 % (*Ph. cinnamomi*). Biopreparát Supresivit (*Trichoderma harzianum*) dosiahol účinnosť 9,1 % pre izoláty *Ph. cambivora* a 9,2 % pre izoláty *Ph. cinnamomi*. Štatisticky bol potvrdený signifikantný vplyv použitých biopreparátov na inhibíciu myceliárneho rastu oboch druhov *Phytophthora* sp. ako aj signifikantný rozdiel v účinnosti dvoch rozdielnych bioprípravkov.

Kľúčové slová: gaštan jedlý; *Phytophthora cambivora*; *Phytophthora cinnamomi*; biologická ochrana; biopreparáty; *Trichoderma harzianum*; *Pythium oligandrum*

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