

The effectiveness *in vitro* of *Trichoderma harzianum* against *Ophiostoma piceae* and the influence of trichomil treatment on germination of acorns and growth of oak seedlings

Elena Foffova

Forestry Research Institute Zvolen
Research Station
Liptovský Hrádok

1. Introduction

Species of the genus *Ophiostoma* (*Ceratocystis sensu lato*) are often associated with oak decline and they also commonly occur on oak seeds. Surovec (4) found them on 5% of all examined acorns in the years 1982-1988 but in some seed samples up to 25% of acorns were infected. They cause ophiostomopsis or black rot of acorns and are able to decay the primary shoots of germinated seeds. Surviving seedlings create several weak shoots; these shoots show growth depressions and discolorations of leaves (4,5 and 6).

Trichoderma species are well-known biocontrol fungi which are intensively studied in agriculture. Attempts were made to use them to control soil born fungi in forest nurseries (1 and 2).

Prihoda (3) found *Trichoderma viride* to be a hyperparasite of *Ophiostoma* sp. on cut surfaces of oak stems. He considered the possibility of using *Trichoderma* as possible biological treatment for tree stumps in declined oak stands.

The antagonistic activity of *Trichoderma harzianum* against *Ophiostoma piceae* and its influence on germination of treated acorns and seedlings growth were examined in the presented work.

2. Materials and methods

The influence of *Trichoderma harzianum* strains on *Ophiostoma piceae* isolates was studied *in vitro* by dual cultivation of both fungi on agar plates. The following types of media (Malt extract agar MA and potato dextrose agar PDA) were used for this purpose:

T1 (from College of Agriculture in Prague),

T11 (own isolate),

TS (from biotechnological centre of the cooperative farm in Blatnice), being the most effective in previous laboratory and in field trials with conifers.

Three of the applied *Ophiostoma piceae* strains (VCH, N, KO) were isolated by Frauknechtova (Forestry Research and Game Management Institute of Prague) from damaged trees in declined oak stands in Bohemia.

Each of the dually cultivated fungi was inoculated with a disc (8 mm diameter) of a 14-day-old culture and placed at opposite sides of the agar plate, 10 mm from the edge. Single cultures of each strain were used as controls and for evaluation of growing rates of the colonies. Each variant was replicated twice. Every day the growth of colonies, creation of inhibition zones and other symptoms of antagonistic activity were evaluated.

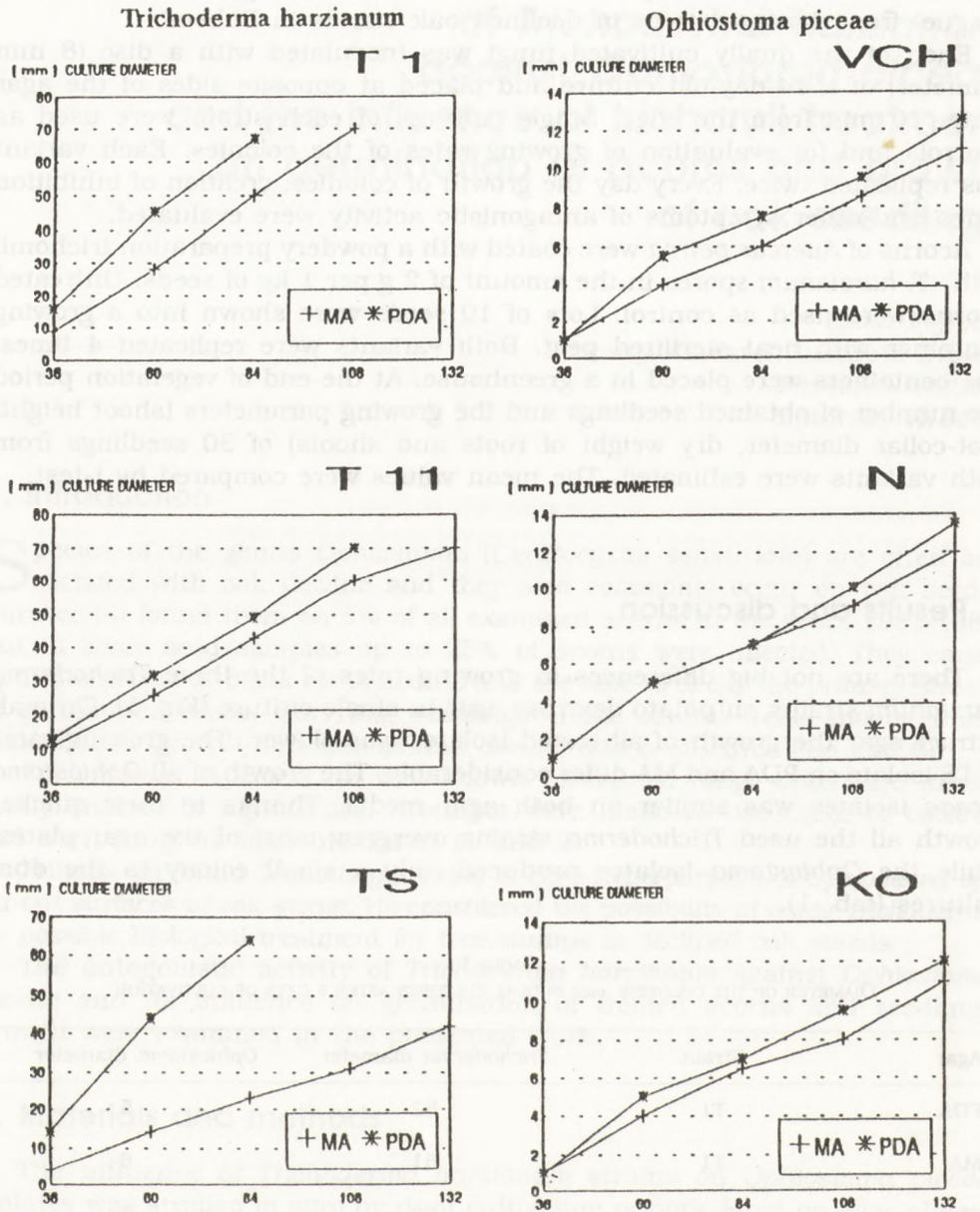
Acorns of *Auercus petrea* were coated with a powdery preparation trichomil with *T. harzianum* spores in the amount of 2 g per 1 kg of seeds. Untreated acorns were used as control. Lots of 10 seeds were shown into a growing container with heat sterilized peat. Both variants were replicated 4 times. The containers were placed in a greenhouse. At the end of vegetation period the number of obtained seedlings and the growing parameters (shoot height, root-collar diameter, dry weight of roots and shoots) of 30 seedlings from both variants were estimated. The mean values were compared by t-test.

3. Results and discussion

There are not big differences in growing rates of the three *Trichoderma harzianum* strains on potato dextrose agar in single culture (Fig. 1). On malt extract agar the growth of all tested isolates was slower. The growing rates of TS isolate on PDA and MA differ considerably. The growth of all *Ophiostoma piceae* isolates was similar on both agar media. Thanks to their quicker growth all the used *Trichoderma* strains overgrew most of the agar plates, while the *Ophiostoma* isolates produced only a small colony in the dual cultures (tab. 1).

TABLE 1
DIAMETER OF THE COLONIES (mm) IN DUAL CULTURES AFTER 5 DAYS OF CULTIVATION

Agar	Strain	<i>Trichoderma</i> diameter	<i>Ophiostoma</i> diameter
PDA	T1	52	5
MA	T1	51	6
PDA	T11	50	7
MA	T11	50	7
PDA	TS	52	5
MA	TS	46	11



PDA - Potato dextrose agar; MA - Malt extract agar

Fig. 1. Growing rates of the tested strains in single culture.

No inhibition zones were created between the tested species, when the cultures were in direct contact. *Trichoderma* strains did not permit further growth of *Ophiostoma* colonies and especially T1 isolate on both media and TS isolate on PDA very quickly overgrew the cultures of *Ophiostoma* and abundantly sporulated on them. The considerable suppressive activity indicates the potential ability of *T. harzianum* to control *Ophiostoma* fungi.

There were no significant differences in shoot height, root-collar diameter and in root and shoot dry weight of seedlings from treated and untreated acorns, but germination of treated seeds proved to be significantly lower (tab. 2).

TABLE 2
GERMINATION OF SEEDS AND AVERAGE OF GROWTH PARAMETERS OF SEEDLINGS
FROM UNTREATED ACORNS AND ACORNS TREATED WITH TRICHOMIL

	Average		t-test	Sig. level
	treated	untreated		
Germination of seeds	88%	89%	2,83	0,030
shoot height	79 mm	73 mm	1,49	0,142
root-collar diameter	2,6 mm	2,6 mm	0,09	0,932
dry weight of shoots	0,234g	0,201g	1,26	0,213
dry weight of roots	0,696g	0,796g	0,91	0,615

Similar negative effects on the germination of pine and spruce seeds were observed in sterilized peat nursery substrate after inoculation with *T. harzianum*. But in a substrate artificially contaminated with *Fusarium oxysporum*, the effect of *T. harzianum* was protective (Foffova – unpubl.).

The considerable suppressive activity *in vitro* indicates the potential ability of *T. harzianum* to control *Ophiostoma* fungi. It is necessary to verify these results *in vivo*, by trials with biological treatment of naturally and artificially infected acorns.

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Literature

1. Červinková H., (1990), in: Fellner R., Studium dalších houbových chorob v lesních školkách (predevším padání semenáčku) a metody obrany. Research report VULHM, Jilovište – Strnady, 28-70.
2. Duda B., Sierota Z. H., (1987), Eur. J. For. Path., 17, 110-117.
3. Prihoda A., Heško J., Surovec D., Leontový R., (1987), Vedecké Práce VULH vo Zvolene 36, 33-56.
4. Surovec D., (1990), Ph. D. Thesis. VULH, Zvolen, 145.
5. Šafranskaja V. N., (1954), Les. Chozjajstvo, 7(11), 69-71.
6. Urošević B., Jančarik V., (1957), Práce VULH ČSR 13, 93-124.

Skutecnost *Trichoderma harzianum* w badaniach *in vitro* przeciw *Ophiostoma piceae* i wpływ traktowania trichomilem na kiełkowanie żółędzi i wzrost siewek dębu

Streszczenie

Rozwój kultur patogenicznego grzyba *Ophiostoma piceae* wyizolowanych z chorych drzew w zamierających drzewostanach dębowych był skutecznie hamowany przez *Trichoderma harzianum* w testach na pożywce agarowej.

Traktowanie żółędzi przez preparat trichomil w formie pudru zawierającego zarodniki *Trichoderma harzianum* obniżało kiełkowanie żółędzi w sterylizowanym torfle i nie wpływało znacząco na wzrost siewek.

Adres dla korespondencji:

Elena Foffova, Forestry Research Institute, Research Station, 03-301 Lip-tovsky Hradok, Czecho-Slovakia.