Reaction of micromycetes to antagonistic organisms and fungicides in substrate

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The species composition of micromycetes and their spreading in greenhouse substrates, reaction to the chemical component propamocarb hydrochloride (previcur 607 SL) and interaction with antagonistic microorganisms *Streptomyces griseoviridis* and *Trichoderma harzianum* were studied. Both chemical and biological treatments changed the number and composition of micromycetes in the substrates. From an infected and non-treated substrate there were isolated and identified 52 fungi species belonging to 28 genera, mostly to the genera *Alternaria, Aspergillus, Cladosporium, Fusarium, Mucor, Penicillium, Pythium, Rhizoctonia* and *Verticillium*. The systemic fungicide previcur suppressed most efficiently the development and spreading of micromycetes in the substrate. The antagonists *S. griseoviridis* and *T. harzianum* suppressed many pathogenic fungi species. The similarity and disparity coefficient by Sörensen of microflora in comparison with the non-treated substrate was reliable and reached in tests with previcur 30.38%, with *S. griseoviridis* 38.04% and with *T. harzianum* 43.02%.

Key words: micromycetes, Sörensen coefficient, *Streptomyces griseoviridis, Trichoderma harzianum*, previcur

INTRODUCTION

Various fungi species can function in the greenhouse substrate; a considerable part of them are pathogenic and conditionally pathogenic fungi [1, 2]. The poor species composition of soil microorganisms causes ecosystem instability in the greenhouse. There is an opinion that the pathogenic properties of fungi depend to a large degree on environmental conditions [3, 4].

As the application of fungicides often produces a harmful effect on ecosystems, searching for microorganisms-antagonists to plant pathogens and introducing them into agroecosystems is one of the solutions in modern plant protection. There are many reports on the application of potential microbial antagonists to control soil-borne pathogens [5–8]. Among other fungi, strains of *Trichoderma harzianum* were recommended as agents for biological control of plant diseases [9, 10]. Kortemaa et all. [11] studied the effect of soil-spraying time on root-colonization ability of antagonistic *Streptomyces griseoviridis*.

The purpose of this study was to determine the quantitative and qualitative composition of micromycetes living in infected and treated greenhouse substrates.

MATERIALS AND METHODS

In tests, a highly infected and not disinfected (non-treated) greenhouse substrate was treated by chemical and biological means. The substrate in vegetative boxes (0.6 x 0.4 x 0.1 m) was poured by the systemic fungicide previcur 607 SL (propamocarb hydrochloride) at a rate of 9.6 ml/0.024 m^3. In other treatments the substrates were sprayed with the biological fungicides Mycostop (active substance *Streptomyces griseoviridis*, min. 10^8 c.f.u./g), rate – 0.12 g/0.024 m^3 and pure culture of *Trichoderma harzianum* (min. 10^8 c.f.u./ml), 0.12 g/0.024 m^3, and with a pure culture of *Trichoderma harzianum* (min. 10^8 c.f.u./ml) 1 l/0.024 m^3.

Fungi were isolated before and after substrate treatments. For mycological analysis the following agar media (pH 4.0–4.5) were used: malt extract, potato and Czapek. The cultural and morphological peculiarities of fungi were studied employing microscopy methods and identification was performed according to different manuals (Domsch, Gams,

1970, 1980; Ellis, 1976; Arx, 1981 and others) at the Institute of Botany, Laboratory of Biodeterioration Research. Percentage similarity of the complexes of fungal species was evaluated using the T. Sörensen coefficient [2].

RESULTS AND DISCUSSION

From a non-treated substrate there were isolated and identified 52 fungal species belonging to 28 genera and *Mycelia sterilia* (Table). Prior to substrate treatment there were 49.200 propagules/1g of dry soil. After the treatment with the fungicide previcur, the concentration of colony-forming units was 26.700 from 1 g d. m. of soil and 37.400–38.300 c.f.u./1 g d. m. of soil in combinations with antagonistic organisms *S. griseoviridis* and *T. harzianum*.

Form the non-treated substrate the following fungal genera were isolated most frequently: *Aspergillus fumigatus, Aureobasidium pullulans, Chaetomium globosum, Cladosporium herbarum* and various species of *Fusarium, Mortierella, Penicillium* and *Trichoderma*. Such frequently isolated fungi as *Penicillium decumbens, Penicillium expansum, Penicillium paxilli, Penicillium spinulosum, Humicola grisea* and *Rhizopus stolonifer* var. *stolonifer (= R. nigricans)* can cause intensive maceration of root tissues [2, 12]. Also typical soil-borne fungi, *Mortierella* spp. and *Trichoderma* spp., were abundant in the investigated substrate. In many cases pathogenic *Botrytis cinerea, Fusarium oxysporum, Fusarium solani, Fusarium solani var. argillaceum, Phoma betae, Phoma lingam, Pythium debaryanum, Rhizoctonia solani, Thielaviopsis basicola* and *Verticillium albo-atrum* were isolated

Table. **Fungi isolated from substrates under different kinds of treatment**

Fungus species	Before treatment	After treatment		
		Previcur	*Streptomyces griseoviridis*	*Trichoderma harzianum*
1	*2*	*3*	*4*	*5*
Acremonium alternatum Link ex Gray	+	+	+	+
Acremonium cerealis (P. Karst.) C. H. Dickinson	+	–	+	+
Acremonium murrorum (Corda) S. Hughes	+	–	+	+
Acremonium strictum W. Gams	+	–	+	+
Alternaria alternata (Fr.) Keissl. (= *A. tenuis* Nees)	+	+	+	–
Alternaria sp.	+	–	–	–
Arthrinium phaeospermum (Corda) M. B. Ellis	–	–	+	–
Aspergillus clavatus Desm.	+	+	+	+
Aspergillus fumigatus Fresen.	+	+	+	+
Aspergillus niger van Tiegh.	+	–	+	+
Aspergillus sp.	+	–	–	–
Aureobasidium pullulans (de Bary) G. Arnaud	+	–	–	+
Blastomyces sp.	+	–	–	–
Botrytis cinerea Pers. ex Fr.	+	+	+	–
Cephalosporium curtipes Sacc.	+	–	–	–
Chaetomium globosum Kunze	+	–	–	–
Chaetomium piluliferum J. Daniels (= *Botryotrichum piluliferum* Sacc. et Marchal)	+	–	–	–
Chloridium chlamydosporis (Beyma) S. Hughes	–	–	+	–
Chrysosporium pannorum(Link) S. Hughes	+	–	+	+
Chrysosporium sp.	–	–	–	+
Cladosporium cladosporioides (Fresen.) de Vries	+	+	+	+
Cladosporium herbarum Link ex Fr.	+	–	+	+
Doratomyces microsporus (Sacc.) F. Morton et G. Sm.	+	+	+	+
Fusarium oxysporum Schltdl. emend W. C. Snyder et H. N. Hansen	+	+	–	–
Fusarium solani (Mart.) Appel et Wollenw.	+	–	–	–
Fusarium solani (Mart.) Appel. et Wollenw. *var. argillaceum* (Fr.) Bilai	+	–	–	–
Fusarium sp.	+	–	–	–
Geotrichum candidum Link ex Pers.	+	–	+	+
Gliocladium viride Matr.	+	–	+	+
Gliocladium sp.	+	–	–	+

Table continued

1	*2*	*3*	*4*	*5*
Gymnoascus reessii Baran.	+	–	+	–
Humicola grisea Traaen	+	–	–	–
Mortierella higrophgila Linnem.	–	+	+	–
Mortierella horticola Linnem.	+	+	–	+
Mortierella hyalina (Harz) W. Gams	–	–	+	+
Mortierella isabellina Oudem.	+	+	+	+
Mortierella lignicola (Martin) Gams et Moreau	–	–	+	–
Mortierella pusilla Oudem.	+	–	+	+
Mortierella vinacea Dixon-Stew.	+	–	+	+
Mortierella sp.	–	+	–	–
Mucor hiemalis Wehmer	+	+	+	–
Mucor plumbeus Bonord.	+	+	–	+
Mucor sp.	–	+	–	–
Myceliophthora vellerea (Sacc & Speg.) Oorschot	–	+	–	–
Mycotypha africana Novak et Backus.	–	+	+	–
Olpitrichum macrosporum (Farl.) Sumst. (= *O. carpophilum* G. F. Atk.)	+	–	–	–
Paecilomyces fumosoroseus (Wize) A. H. S. Brown & G. Sm.	+	–	–	–
Paecilomyces sp.	–	–	+	–
Penicillium canescens Sopp	+	+	–	–
Penicillium capsulatum Raper et Fennell	+	–	–	+
Penicillium decumbens Thom	+	–	+	+
Penicillium expansum Link ex Gray	+	+	–	–
Penicillium lividum Westling	+	+	+	+
Penicillium nigricans (Bainier) Thom	+	+	–	+
Penicillium oxallicum Currie and Thom	+	–	+	+
Penicillium palitans Westling	+	–	+	+
Penicillium paxilli Bainier	+	+	+	+
Penicillium spinulosum Thom	+	+	+	+
Penicillium sp.	+	–	–	–
Phoma betae A. B. Frank	+	+	–	–
Phoma lingam (Tode ex Fr.) Desm.	+	+	+	–
Phoma sp.	+	–	–	+
Pleurothecium recurvatum Höhn.	–	–	+	–
Pythium debaryanum Hesse	+	–	–	–
Pythium sp.	+	–	–	–
Rhizoctonia solani Kühn	+	+	–	–
Rhizopus stolonifer (Ehrenb. ex Fr.) Vuill. var. *stolonifer* (= *R. nigricans* Ehrenb.)	+	–	+	+
Rhizopus sp.	+	–	+	+
Streptomyces sp.	–	–	+	–
Syncephalis nodosa van Tiegh.	–	–	+	–
Thielaviopsis basicola (Berk. et Br.) Ferr.	+	+	–	+
Torula sp.	+	–	+	+
Trichoderma harzianum Rifai	+	+	+	+
Trichoderma viride Pers. ex Gray (= *T. lignorum* (Tode) Harz)	+	–	+	+
Trichoderma sp.	+	+	+	+
Verticillium albo-atrum Reinke et Berthold	+	–	–	–
Verticillium dahliae Kleb.	–	+	–	–
Verticillium nigrescens Pethybr.	–	–	+	+
Verticillium sp.	+	–	–	–
Volutella ciliata Alb. et Schwein. Ex Fr.	+	–	+	+
Zygosporium sp.	–	+	–	–
Mycelia sterilia	+	+	+	+
Total of isolates	65	32	46	41
Total number of species	52	27	40	34

from a non-treated substrate. These species are known as agents of root infection of many crops.

The activity of some organisms is depressed partly or completely, others can adapt and grow [4]. Microorganisms functioning in a propamocarb-contaminated substrate were found and 27 fungi species belonging to 17 genera and *Mycelia sterilia* were identified. The systemic fungicide previcur affected many of soil-borne micromycetes. The disinfected substrate contained by 25 fungal species less than the non-treated one. There is an opinion that when a number of saprotrophic organisms remain, parasitic fungi such as *Botrytis cinerea, Fusarium oxysporum, Phoma betae, Phoma lingam* and *Rhizoctonia solani* can spread without competition.

From a substrate treated with *S. griseoviridis* and *T. harzianum* there were isolated and identified respectively 40 and 34 fungal species ascribed to 23 and 17 genera. In this case the complex lignin-cellulose prevailed, ant it destroyed micromycetes from the genera *Trichoderma, Aspergillus, Mortierella* and *Mucor*. The antagonistic organisms *S. griseoviridis* and *T. harzianum* were more aggressive against such species as *Fusarium oxysporum, Fusarium solani, Fusarium solani* var. *argillaceum, Phoma betae, Pythium debaryanum, Rhizoctonia solani* and *Verticillium alboatrum*.

It should be noted that all substrates contained the following fungal species: *Acremonium alternatum, Aspergillus clavatus, Aspergillus fumigatus, Cladosporium cladosporioides, Doratomyces microsporus, Mortierella isabellina, Penicillium lividum, Penicillium paxilli, Penicillium spinulosum* and *Trichoderma harzianum.* These micromycetes are abundant in soil and destroy various organic compounds. These fungi adapt to extreme nutrition conditions and can survive chemical and physical factors [12].

The obtained data show that different microorganism species and their compositions can function in variously treated substrates. The similarity and disparity coefficient by Sörensen of microflora in comparison with the non-treated substrate is reliable: in tests with previcur it reached 30.38%, with *S. griseoviridis* – 38.04% and with *T. harzianum* – 43.02%.

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MIKROMICETŲ REAKCIJA Į ANTAGONISTINIŲ ORGANIZMŲ IR FUNGICIDO POVEIKĮ SUBSTRATE

S a n t r a u k a

Ištirtas mikromicetų paplitimas ir jų rūšinė sudėtis šiltnamio substrate, jų reakcija į propamokarbo hidrochloridą (previkuras 60,7% v.t.) ir sąveika su antagonistiniais mikroorganizmais *Streptomyces griseoviridis* ir *Trichoderma harzianum*. Tiek cheminės, tiek biologinės apsaugos priemonės turėjo poveikį mikromicetų skaičiui ir sudėčiai substrate. Iš užkrėsto ir neapdoroto substrato izoliuota ir identifikuota 52 grybų rūšys, priklausančios 28 gentims. Vyravo *Alternaria, Aspergillus, Cladosporium, Fusarium, Mucor, Penicillium, Pythium, Rhizoctonia* ir *Verticillium* genčių rūšys. Dirvos mikroorganizmų vystymąsi ir paplitimą labiausiai slopino sisteminio veikimo fungicidas – previkuras. Antagonistai *S. griseoviridis* ir *T. harzianum* slopinančiai veikė daugelį patogeninių dirvos grybų. Gautas patikimas substratų mikrofloros panašumo ir skirtumo koeficientas. Lyginant su neapdorotu substratu, dezinfekuotame previkuru jis siekė 30,38%, įterpus *S. griseoviridis* – 38,04% ir *T. harzianum* – 43,02%.

Raktažodžiai: mikromicetai, Siorenseno koeficientas, *Streptomyces griseoviridis, Trichoderma harzianum*, previkuras