Review

Mycoparasitic Fungi *Trichoderma* spp. in Plant Protection

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Abstract

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Trichoderma harzianum is a worldwide soilborne anamorphic fungus. It is a facultative parasite of a wide spectrum of fungi, but can also live as a saprophyte. The manifestation of its antagonism displays as support competition, parasitism and antibiosis. Some species of the genus *Trichoderma* can be utilised in plant protection because of their mycoparasitic and other properties. The biofungicide Supresivit containing conidia of *T. harzianum* is registered in the Czech Republic. A commercially prepared mixture of the biopreparation and granulated mineral fertiliser is a novel way to introduce it into the plant environment. To make optimal use of biological control methods it is necessary to integrate them in a complete system for the control of plant diseases. It is quite possible to combine two or more biological control agents, but these could also be used together with certain chemicals to improve disease control.

Keywords: biological control; soil-borne fungi; antagonism; rhizosphere; interaction; *Botrytis cinerea*

Hosts of mycoparasitic *Trichoderma harzianum*

Trichoderma harzianum is a worldwide soilborne anamorphic fungus. It is a facultative parasite of a wide spectrum of fungi (some of them are named in Table 1), but can also live as a saprophyte. The manifestation of its antagonism displays as support competition, parasitism and antibiosis.

The type of host fungus influences the efficiency to detect different mycoparasites. *Rhizoctonia solani* was most efficient for *Trichoderma* spp., *Botrytis cinerea* for *T. aureoviride* in soil samples. But no single host was suitable to consistently detect any single mycoparasite (Mulligan & Deacon 1992).

Various strains of *T. harzianum* and *T. viridae* were effective, though at different levels, against some fungal pathogens of caraway as listed in Table 1 (Ondřej 1997a).

The arbuscular mycorrhizal fungus *Glomus intraradices* was also damaged by *T. harzianum*. Its hyphae proliferated abundantly on the spore surface and penetrated the thick host wall.

In spite of the increasing amount of research devoted to the antimicrobial activity of *Trichoderma* spp. against plant pathogens, the possibility that these antagonistic fungi also may interfere with other components of the soil rhizosphere has not been studied to any extent (Rousseau *et al.* 1996).

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Table 1. Hosts of *Trichoderma harzianum*

Hosts of *Trichoderma harzianum*	References
Bipolaris sorokiniana	Ondřej (1997a)
Botrytis cinerea	De Meyer *et al.* (1998), Ritieni *et al.* (1997), Schirmböck *et al.* (1994), Ondřej (1997b), O'Neil *et al.* (1996), Elad *et al.* (1993), Míša (1997)
Cephalosporium gramineum	Martyniuk (1995)
Colletotrichum gloeosporioides	Ondřej (1997a)
Colletotrichum lindemuthianum	Bigirimana *et al.* (1997)
Fusarium culmorum	Michalíková & Michrina (1997), Koutecká & Dušková (1998)
Fusarium culmorum f.sp. *vasinfectum*	Sivan & Chet (1989)
Fusarium graminearum	Ondřej (1997a)
Fusarium oxysporum f.sp. *cucumerinum*	Lifshitz *et al.* (1986)
Fusarium oxysporum f.sp. *melonis*	Sivan & Chet (1989)
Fusarium oxysporum f.sp. *pisi*	Koutecká & Dušková (1998)
Fusarium solani	Koutecká & Dušková (1998)
Fusarium sp.	Roháčik *et al.* (1991)
Gaeumannomyces graminis v. *tritici*	Martyniuk (1995)
Glomus intraradices	Rousseau *et al.* (1996)
Mycocentrospora acerina	Ondřej (1997a)
Phomopsis diachenii	Ondřej (1997a)
Phytophthora cactorum	Koutecká & Dušková (1998)
Phytophthora cinnamomi	Koutecká & Dušková (1998)
Phytophthora nicotiana	Koutecká & Dušková (1998)
Phytophthora parasitica	Koutecká & Dušková (1998)
Phytophthora sp.	Dušková (1995b); Thrane *et al.* (1997)
Pythium aphanidermatum	Sivan *et al.* (1984)
Pythium graminicola	Lo *et al.* (1996)
Pythium sp.	Lifshitz *et al.* (1986)
Pythium ultimum	Dušková (1995b); Migheli *et al.* (1998); Thrane *et al.* (1997)
Rhizoctonia batati-cola [syn. *Macrophomina phaseolina* (Tassi) Goid.]	Vyas & Vyas (1995)
Rhizoctonia cerealis	Koutecká & Dušková (1998)
Rhizoctonia solani	Lo *et al.* (1996); Benhamou & Chet (1993); Vavřač *et al.* (1997); Papavizaz *et al.* (1982); Koutecká & Dušková (1998); Ondřej (1997a)
Rhynchosporium secalis	Kulichová & Gregušková (1997)
Sclerotinia homoeocarpa	Lo *et al.* (1996)
Sclerotinia sclerotiorum	Ondřej (1997a)

Antagonism of *Trichoderma harzianum*

The manifestation of antagonism displays as support competition, parasitism and antibiosis.

In the interactions between *T. harzianum* and *Fusarium culmorum*, a causal agent of winter wheat fusarioses, two fundamental modes of relationships were determined. The penetration of host hyphae

and formation of appressoria are combined with morphological changes of both host and antagonist. There is abundant sporulation of the mycoparasite, and a loss of pigment synthesis in *F. culmorum*. Production of the antibiotic principle is probably located in the tips of growing hyphae, and components responsible for the antagonism are secreted at the points of contact with the pathogen (Michalíková & Michrina 1997).

Laing and Deacon (1991) observed that *T. harzianum* inhibited host hyphae of *Pythium* spp. by production of diffusible compounds, but did not penetrate them. There was no pre-contact inhibition or tropism, but susceptible host hyphae stopped growing soon after contact – faster in contacts between host tips and sub-apical regions of parasitic hyphae than vice-versa.

Coiling of hyphae of *T. harzianum* around its host *Rhizoctonia solani* was an early event preceding hyphal damage. Contact between the two fungi was mediated by a fine, extracellular matrix originating from cells of *R. solani*. This matrix was rich in galactose residues. Chitin breakdown occurred gradually, suggesting a continuous production of chitinases by the antagonist (Benhamou & Chet 1993).

Reactions of single isolates of the soil-borne pathogens *Rhizoctonia solani* and *R. cerealis*, *Fusarium oxysporum*, *F. solani* and *F. culmorum*, *Phytophthora nicotiana*, *P. cinnamomi*, *P. parasitica* and *P. cactorum* to *T. harzianum* were different. Elevated mycelial layers, pigmented or colourless zones and mycelium intergrowth were produced where the mycelia met. Both originally inoculated fungi could be observed on any dish in 3 weeks, although one of them was clearly growing over the other. The presence of *T. harzianum* in the medium influenced the ratio of single isolate growth rates, mainly in *Rhizoctonia* spp. and *Fusarium* spp. (Koutecká & Dušková 1998).

Some isolates of *Rhizoctonia solani* tolerant to benomyl were resistant against *T. harzianum* and *T. viride* (Ondřej 1997b).

Considerable differences in lytic-degradation activity were observed between different strains of *T. harzianum* and *T. viridae* and eight fungal pathogens of caraway (Ondřej 1997a). Degradation of the mycelium of the phytopathogen was visible 6, 8 or 9 days after inoculation, depending on the strain of *Trichoderma*. Mycelium of *Mycocentrospora acerina* was coagulated and vacuolised by strains 1SU and 4THA, whereas it was corroded in interactions with strains 2NI, 3TDX and 5TVI. Hyphae of *Botrytis cinerea* were twined and penetrated by strains 1SU and 4THA, but conidia were not damaged. The strains 2NI, 3TDX and 5TVI lysed the cell walls of *B. cinerea* and due to the interaction the conidia of the pathogen aborted and lost the ability to germinate.

Interaction between *T. harzianum* and the arbuscular mycorrhizal fungus *Glomus intraradices* involves the recognition and local penetration by the antagonist of mycorrhizal spores through local hydrolysis of wall polymers, active proliferation of antagonist cells in mycorrhizal hyphae and release of the antagonist through moribund hyphal cells with multiple perforations (Rousseau *et al.* 1996).

The molecular basis of biocontrol has not been explained yet, but a synergistic mechanism between antibiosis, competition and mycoparasitism is hypothesised. Moreover, *Trichoderma* species are also characterised by the production of antibiotic peptides. The β-glucan synthetase activity on isolated plasma membranes of *Botrytis cinerea* was inhibited by peptabols (trichorzianin A and B) of *T. harzianum* (Ritieni *et al.* 1997).

Mycoparasitism is considered to be an important mechanism of biological control and probably depends on the production of lytic enzymes including chitinases, β-1,3-glucanases, and proteases. A correlation between the production of chinolytic enzymes and the suppression of fungi containing chitin as the main cell wall constituent has been demonstrated for many *Trichoderma* species. Chitinases from *Trichoderma* inhibited *in vitro* spore germination and tube elongation of a variety of fungi except *Pythium ultimum*, which does not contain chitin as a major cell wall component (Migheli *et al.* 1998).

When *T. harzianum* mycelia, grown on glucose as the sole carbon source, were transferred to fresh medium containing cell walls of *Botrytis cinerea*, the enzymes chitinase, β-1,3,-glucanase and protease were formed. The parallel formation and synergism of hydrolytic enzymes and antibiotics may have an important role in the antagonistic action of *T. harzianum* against fungal phytopathogens (Schirmböck *et al.* 1994).

In *T. harzianum* isolate T3, known to control *Pythium* damping-off of cucumber seedlings, the presence of *Pythium ultimum* induced 1,3-β-endoglucanase and cellulase in sphagnum peat moss culture. Germination of encysted zoospores and

elongation of germ tubes of a plant pathogenic *Pythium* isolate were inhibited by low concentrations of the purified enzymes. On the other hand, *P. ultimum* stimulated the germination of *Trichoderma* conidia (Thrane *et al.* 1997).

T. harzianum isolate ThB1 produced antibiotic substances at 7–25°C that inhibited the mycelial growth of *Fusarium* sp. Specific reactions of different species were found. Physiological and biochemical processes changed with changes in temperature and thus influenced the reaction to the antibiotic substances. The fast growth and colonisation of the substrate are important properties of isolate ThB1 (Roháčik *et al.* 1991).

An extracellular filtrate from cultures of *T. harzianum* inhibited linear growth of *Pythium aphanidermatum* by 83%, compared to 8% inhibition by a culture filtrate of *T. hamatum*. Conversely, substances excreted by *P. aphanidermatum* into the growth medium enhanced the linear growth of *T. harzianum* by 34%, but not that of T. *hamatum* (Sivan *et al.* 1984). Single mycoparasitic species of the genus *Trichoderma* differ in in their ability to parasitise different isolates of phytopathogenic fungi.

The activity of a bioagens is strongly influenced by environmental conditions. One of the important factors is temperature. T. *hamatum* was effective as a seed treatment at soil temperatures between 17–34°C (Harman *et al.* 1981). *T. harzianum* was most effective above 20°C and at relative humidity between 80% and 97% (Elad *et al.* 1993). Pea seeds germinated at low temperature (22°C) were better protected when coated with conidia of T. *hamatum* than with those of *T. harzianum*, but this was not the case at 30°C (Sivan *et al.* 1984).

When seeds coated with conidia of *T. harzianum* were sown at temperatures unfavourable for growth of this agent, the incidence of *Pythium* or *Rhizoctonia* damping-off was the same as in the untreated controls (Baker 1986).

Laboratory tests showed that at a mean temperature of 12°C in a greenhouse, the formulated conidia of *T. harzianum* required up to 96 h to germinate, while conidia of *Botrytis cinerea* and *Mucor piriformis* isolated from greenhouse strawberries required 11 h and 16 h, respectively. Spraying the strawberries growing under these conditions with the strain of *T. harzianum* did not affect the marketable yield of strawberries (Hjeljord 2000).

Soil pH can be adjusted to provide a favourable environment for the activity of biocontrol agents. *Trichoderma* spp. are more effective and their conidia germinate better at lower soil pH (Baker 1986). Under *in vitro* conditions, though, it is able to grow, sporulate and attack other fungi even at higher pH from 6 to 8. Some differences in antagonistic properties between strains of *T. harzianum* and *T. longibrachiatum* appeared to be correlated with different demands on pH (Prokinová 1988a). Domsch *et al.* (1980) found pH 3.7–4.7 to be optimal for maximal biomass production of *T. harzianum*.

Soil and rhizosphere-competence of *Trichoderma harzianum*

The species of *Trichoderma* genus are not particularly rhizosphere-competent.

If strains of *T. harzianum* were grown in Czapek-Dox broth without saccharose but with cellulose or xylan as sole source of carbon, the rhizosphere-competent mutants produced significantly higher biomass than the rhizosphere-incompetent wild types. Both mutants and wild types did not readily grow on glucose, galactose, cellobiose or xylose as sole source of carbon. The ability of the mutants to grow more rapidly on complex carbon substrates, typical of those found on root surfaces, and to increase biomass when simple sugars were added along with the cellulose substrate could be of ecological significance and a characteristic of rhizosphere-competence (Ahmad & Baker 1988).

Biocontrol agens can be induced by mutation to colonize rhizospheres and thereby provide protection against root-infecting pathogens (Baker 1986). Ahmad and Baker (1988) used *T. harzianum* that originally was rhizosphere-incompetent to produce mutant strains that were tolerant to benomyl. When the fungicide was added to soil, the mutants were rhizosphere-competent and colonised entire root surfaces up to 10^6 CFU/g of rhizosphere soil. Unexpected was that the benomyl-tolerant mutants were rhizosphere-competent even when the fungicide was absent from the soil.

T. harzianum strain 1295-22 colonised the rhizoplane seed coat and phylloplane of creeping bentgrass (Lo *et al.* 1998).

Mendel-Castro and Alexander (1982) suggested a method for establishing rhizosphere-incompetent microorganisms on roots by adding mancozeb to the soil.

The two original *T. harzianum* strains T12 and T95 colonised the entire length of maize and cotton roots, but strain 1295-22 derived from fusing

protoplasts of auxotrophic mutants of the prototrophic strains T12 and T95 was more effective in colonising the middle sections of the roots than either parental strain. All three *T. harzianum* strains increased the root elongation rate of both crops. Seed treatment with the new strain 1295-22 resulted in the longest roots in both crops. The average root elongation rate of maize was greater than that of cotton. When untreated seeds were sown in the soil columns, *Trichoderma* spp. could be isolated from the rhizosphere soil of only very few root segments (Sivan & Harman 1991).

T. harzianum appeared to colonise healthy roots only superficially, whereas the mucilage of the root hairs and of distal parts of wounded areas or broken parts of the roots were extensively colonised (Thrane *et al.* 1997).

When the population densities of members of the soil microflora were determined, no quantitative and qualitative differences were observed between soils infested with *T. harzianum* and the controls (Windham *et al.* 1986).

Addition of conidia of *T. harzianum* to soil significantly reduced the rate of chlamydospore germination of both *Fusarium culmorum* f.sp. *vasinfectum* and *F. oxysporum* f.sp. *melonis*. These fungi were also significantly inhibited in the rhizospheres of cotton and melon. Addition of glucose and asparagine or of an excess of seedling exudates increased the germination rate of the *Fusarium* conidia and eliminated the inhibition. Seed treated with *T. harzianum* and planted in a constantly humid soil showed a high population density of the antagonist on the developing rhizosphere. Roots of plants from treated seeds had lower levels of *Fusarium* sp. in their rhizosphere than those from untreated seeds. The greatest density of *T. harzianum* and the largest reduction in levels of *Fusarium* were detected on the lower 4 cm of the roots. The level of *Fusarium* in the rhizosphere was inversely proportional to the number of conidia of *T. harzianum* applied to the soil. On the other hand, as the concentration of the pathogen in soil increased, *T. harzianum* counts on root segments decreased. *T. harzianum* had little effect on the survival of *Fusarium* spp. in nonrhizosphere soil. Inhibition of germination may, therefore, have resulted from competition (Sivan & Chet 1989).

Many of the most efficient antagonists are potentially good competitive saprophytes. *T. harzianum* survives well in a soil substrate and is know to be a cellulose decomposer. Incorporation of this substrate into soil selectively increased the population density of *T. harzianum* at or above the level of approximately 10^6 CFU/g required to induce suppression of *Rhizoctonia* in soil. An example of this phenomenon may have been found in the field; after undecomposed corn stalks were incorporated into soil, extremely high population densities of *T. harzianum* were observed (Baker 1986).

This phenomenon could be used to advantage in zero tillage systems to improve both soil quality and health of crops.

Plant Protection with *Trichoderma* spp.

Some species of the genus *Trichoderma* can be utilised in plant protection because of their mycoparasitic and other properties (Table 2). Strains which are effectively able to suppress plant pathogens in a sufficiently wide spectrum of environmental conditions, are rhizosphere-competent and have a positive effect on growth and development of plants, are collected and maintained.

Interactions between various isolates of soil-borne pathogens (*Rhizoctonia solani* and *R. cerealis*, *Fusarium oxysporum*, *F. solani* and *F. culmorum*, *Phytophthora nicotiana*, *P. cinnamomi*, *P. parasitica* and *P. cactorum*) and mycoparasitic *T. harzianum* influenced the expression of isolate pathogenicity to cucumber plantlets under *in vitro* conditions. Seeds placed directly on the colonies of *T. harzianum* were protected from isolates with low and intermediate pathogenicity, the colonies of which caused damage to seedlings. Isolates with highest pathogenicity were growing through *T. harzianum* mycelium and exhibited offensive growth, caused mortality of germinating plants, often including those that were placed on the mycelium of the mycoparasitic fungus.The highly pathogenic *Phytophthora parasitica* stimulated plant growth when combined with *T. harzianum*, unlike the plants growing on only *T. harzianum* (Koutecká & Dušková 1998).

Some strains of *Trichoderma* sp. applied to roots of carnations were significantly effective as biofungicides (Fruzyńska-Jóźwiak & Mańka 1996).

Application of conidia of *T. harzianum* or *T. koningii* to pea seeds reduced the incidence of preemergence damping-off induced by *Pythium* sp. The germination of sporangia of the *Pythium* sp. or chlamydospores of *Fusarium oxysporum* f.sp. *cucumerinum* in the spermosphere of peas treated with isolates of *Trichoderma* was comparable to that of the untreated controls (Lifshitz *et al.* 1986).

Table 2. *Trichoderma* spp. in plant protection

Trichoderma spp.	Pathogen	Crop	Reference
T. harzianum	*Fusarium oxysporum* f.sp. *cucumerinum*	pea seeds	Lifshitz *et al.* (1986)
	Pythium aphanidermatum	peas, cucumbers, tomatoes, peppers and gypsophila	Sivan *et al.* (1984)
	Pythium sp.	pea seeds	Lifshitz *et al.* (1986)
T. koningii	*Fusarium oxysporum* f.sp. *cucumerinum*	pea seeds	Lifshitz *et al.* (1986)
	Gaeumannomyces graminis var. *tritici*	wheat	Simon (1989)
	Pythium sp.	pea seeds	Lifshitz *et al.* (1986)
T. viride	*Fusarium oxysporum* f.sp. *cucumerinum*	pea seeds	Lifshitz *et al.* (1986)
	Pythium sp.	pea seeds	Lifshitz *et al.* (1986)
Trichoderma sp.		roots of carnations	Fruzyńska-Jóźwiak & Mańka (1996)

A bran/peat preparation of a *T. harzianum* isolate, applied to either soil or rooting mixture, effectively controlled damping-off induced by *Pythium aphanidermatum* in peas, cucumbers, tomatoes, peppers and gypsophila. Disease reduction of up to 85% was obtained in tomatoes (Sivan *et al.* 1984).

The growth of radishes was significantly better if *T. harzianum* was added to the soil (Baker 1986).

Heat-stable mycelial extracts of the nonpathogenic fungus *Trichoderma longibrachiatum* induced resistance in tobacco seedlings to the pathogen *Phytophthora parasitica* var. *nicotianae*, which did not involve a hypersensitive response. Resistance could not be induced with a mycelial extract prepared in the same manner from *P. parasitica* (Chang *et al.* 1997).

T. harzianum reduced plant rot, but increased root rot of forced tulips (Greff *et al.* 1992).

Extremely high population densities of *Trichoderma* spp. made the fungus pathogenic to corn seedlings. However, the fungus can function for its rampant activity as a biocontrol agent very well (Baker 1986).

Interaction between *Trichoderma harzianum* and plants, and growth stimulation of plants

Enhanced plant growth resulting from admendments of soil with *Trichoderma harzianum* and *T. koningii* was investigated to determine if increased growth could be attributed to a direct effect of these *Trichoderma* spp. on the plant or to a secondary effect due to control of minor plant pathogens. Addition of *Trichoderma* spp. to autoclaved soil increased the rate of emergence of tomato and tobacco seedlings over that of the controls. Eight weeks after planting, root and shoot dry weights of tomato and tobacco were increased 213–275% and 259–318%, respectively, over the controls. None of the other microorganisms reinfesting autoclaved soil decreased plant growth when added to autoclaved soil. Radish plants grown with *T. harzianum* T-95 were larger than radish plants grown under similar conditions without the agent. The rate of seed germination was increased compared with controls if the seeds were separated from the *Trichoderma* spp. by a cellophane membrane. The fungi probably produced a growth-regulating factor that increased the rate of seed germination and dry weight of shoots and stems (Windham *et al.* 1986).

The stimulation of aboveground parts and root systems of plants by the presence of *T. harzianum* has been observed very frequently (Dušková 1995b).

The level of increased tomato growth induced by *T. harzianum* or *T. koningii* was further enhanced when soil fertility was increased (Windham *et al.* 1986).

The biological control activity of *T. koningii* and *T. harzianum* against *Pythium* seed rot and preemergence damping-off of pea was increased by adding various compounds to seed treatments. Compounds promoting *T. koningii* were generally ineffective in promoting the biological control activity of *T. harzianum*. Organic acids were most promotive of the activity of *T. koningii*, whereas polysaccharides and polyhydroxy alcohols were most promotive of *T. harzianum*. There was no re-

lationship between the ability of the compounds to support *in vitro* growth and proliferation of *Trichoderma* strains in the spermosphere and the increased biological control activity by the antagonist (Nelson *et al.* 1988).

The effectiveness of treatment of pea with *T. harzianum* against *Pythium ultimum* and *Rhizoctonia solani* was significantly influenced by properties of the pea cultivars and quality of the soil substrate. The level of control of *Fusarium oxysporum* f.sp. *pisi* is better with pea cultivars resistant to this fungus than with susceptible ones (Dušková 1995a).

Biological preparations based on *Trichoderma harzianum*

The biofungicide Supresivit is prepared in the Czech Republic and was registered in 1994, first for use on peas. It contains conidia of *T. harzianum* at a number of 1.4×10^{10}/g of the preparation. The effective strain was created by protoplasm fusion of two natural strains and is characterised by rhizosphere-compatibility, tolerance to mancozeb and dicarboximide fungicides, fast growth and high mycoparasitic activity (Okrouhlá 1993). The strain does not produce antibiotics or gliotoxin. It does not inhibit the nodule bacteria of legumes and mycorrhizal relations. It is licensed for use on wheat, maize, oil-rape, vegetables, ornamental plants, ornamental and forest trees against damping-off and other fungal diseases.

The effectiveness of Supresivit was found to be better in a peat substrate with pH 5.5 than in more alkaline soils. Seed treatment with the biopreparation was better than soil application. Supresivit can be used on peas in suitable cultivars and soils (Dušková 1995a).

The possibility of an effective use of biological control in plants was particularly good in gardening because planting conditions can be easily altered. Supresivit controlled the spread of infection but did not have a curative effect. The treatment worked best under optimal planting conditions (Dušková 1991). Application of Supresivit into soil before planting was able to stop the growth of *Phytophthora* spp. and *Pythium ultimum* (Dušková 1995b).

The treatment of plants by Supresivit very often stimulated the growth of both aboveground parts and root systems of plants (Dušková 1995b).

The efficacy of Trichonitrin, a Slovak preparation containing conidia of *T. harzianum*, is comparable to the efficacy of the chemical seed treatment fungicide VITAVAX 200 FF. Active growth of *T. harzianum* hyphae, which are able to saprophytically colonise the seed surface, growing roots and hypocotyls, is presumption of applying the mechanisms of competition, antibiosis and parasitism. This preparation stimulated the growth of plants. Its high biological efficacy is based on the combined actions of the named mechanisms (Roháčik *et al.* 1994).

Seed coating with Trichonitrin before sowing protected seeds and young plants of spring barley against primary infection by *Rhynchosporium secalis*. The efficacy of Trichonitrin was 15–47%, that of VITAVAX 200 FF was 32–51%. The level of control by Trichonitrin thus corresponded favourably to that of the chemical fungicide containing carboxine and thiram (Kulichová & Gregušková 1997).

Isolate 1295-22 of *T. harzianum* is the basis of commercial biopreparations. It vigorously colonises the rhizosphere and is able to suppress phytopathogenic fungi. The isolate was tested for its effect on diseases evoked by *Rhizoctonia solani*, *Sclerotinia homoeocarpa* and *Pythium graminicola*; all were significantly reduced (Lo *et al.* 1996).

Integration of biological control and other control mechanisms

For optimum effect of biological control it should be part of an integrated system that also involves other means to fight diseases. More than one bioagent can be used in combination, but biopreparations can also be used together with chemicals to improve control.

A mixture of the beneficial rhizobacteria *Pseudomonas fluorescens* and the biopreparation Trichonitrin containing conidia of *T. harzianum* showed considerable effectiveness to suppress damping-off disease of cucumbers caused by *Rhizoctonia solani*. Some treatment variants were rather inconsistent in their suppressive effect in three separate experiments; this was due to the complex interactions between plants and all microorganisms involved. In some treatments, a single fungal antagonists gave a higher suppression than mixtures. In treatments of cucumber with another bioformulation with *T. harzianum* conidia, Trichodex, alone or mixed with *P. fluorescens*, slower growth of roots and/or toxic effects on plants were observed (Vavřač *et al.* 1997).

The interaction between three isolates of *Trichoderma* (*T. harzianum* T3, *T. longibrachiatum* and *Trichoderma* spp.) and three isolates of bacteria

(*Bacillus subtilis*, *Pseudomonas putida* and an unidentified one) and their effectiveness on cucumber seedlings was tested by Prokinová (1988b). The results of experiments conducted under *in vitro* conditions did not clearly corresponded with the results of greenhouse experiments. The role of plants in the environmental interactions was probably also important.

Tolerance to agrochemicals was often found if *Trichoderma* spp. were grown on media amended with fungicides. Especially on those with high concentrations of fungicides there was unexpected fast growth compared with that at lower concentrations. This was most frequent with vinclozolin, iprodione, bitertenol and triadimefon, but also benomyl and carbendazim. The easily obtained and stable tolerance to fungicides suggests that antagonistic *Trichoderma* isolates may be suitable for an integrated control program against fungal plant diseases (Vyas & Vyas 1995).

Lifshitz *et al.* (1985) found that the control of *Rhizoctonia* damping-off of radishes by both seed treatment with the biocontrol agent *T. harzianum* and a soil mix of benadomil was additive.

Biological control of soilborne plant pathogens has not been fully implemented because of several unresolved problems. One of them is the use of chemical fungicides for seed treatment which is indispensable as it is one of the cheapest ways to control seedborne and to some extent soilborne diseases. Therefore, strains of mycoparasites more tolerant to fungicides are sought. The simultaneous application of *Trichoderma viride* or *T. harzianum* with carbendazim, captafol or aldrin gave excellent and significant control of dry root rot of soybean caused by *Rhizoctonia batati-cola* [syn. of *Macrophomina phaseolina* (Tassi) Goid.]. This joint application of a mycoparasitic organism and chemical fungicide offered better results than using either fungicide or mycoparasite alone (Vyas & Vyas 1995).

Easily obtained mutant strains of mycoparasites tolerant to some fungicides can be employed even with a chemical that is fungitoxic to the wild type of the agents. Papavizaz *et al.* (1982) inoculated a propagative medium with a benomyl-tolerant isolate of *T. harzianum* and obtained approximately 50% control of carnation cutting rot induced by *Rhizoctonia solani*. A similar level of control was achieved by adding benomyl to the rooting hormone solution which was applied to the cuttings before propagation. When both types of treatment were used, 100% control was achieved.

Howell (1991) and Tronsmo (1991) demonstrated that it is possible to obtain a synergistic effect of *Trichoderma virens* or *T. harzianum*, respectively, combined with low doses of fungicides in biocontrol of *Pythium* in cotton or *Botrytis cinerea* on apple fruits, respectively.

A strong synergistic effect was observed in the inhibition of *Pythium sp.* and *Phytophthora* sp. cyst germination by a combination of the endo-1,3-β-glucanase purified from *T. harzianum* and the fungicide Fongarid (furulaxyl). *Pythium ultimum* was at least 10 times more sensitive to the fungicide if it was used together with the purified endo-1,3-β-glucanase or cellulase (Thrane *et al.* 1997).

Commercially prepared mixtures of the biopreparation Supresivit and granulated mineral fertilisers present a new way to introduce the mycoparasite into the plant environment. This method of application had the positive effect of raising yields of the main field crops (winter wheat, spring barley, winter rape, maize and potatoes) by 5% during 4-year field experiments. The qualitative parameters of the crops were slightly better (gluten content of winter wheat, oil content of winter rape, starchiness of potatoes) or coincident (fatty acids of winter rape, protein level of spring barley, gluten index of winter wheat) in comparison with the yields on check plots (Brožová *et al.* 2002).

Use of a biopreparation based on *Trichoderma harzianum* conidia to treat aboveground parts of plants

From the known ability of *T. harzianum* to suppress growth of *Botrytis cinerea*, a widespread pathogen of plants, the use of such biopreparations to control aboveground parts of plants was developed. It was observed that the mycoparasite is able to find sources of nutrition on the plant surface and to defend the plant tissue against attack by some fungal pathogens.

Biocontrol of *B. cinerea* with *Trichoderma* spp. is generally believed to result from direct interaction of the biocontrol agent with the pathogen, or from a *Trichoderma*-induced change in environmental conditions that affect the development of *B. cinerea*. In tomato, lettuce, pepper, bean and tobacco, *T. harzianum* T39 application at sites spatially separated from *B. cinerea* inoculation resulted in a 25–100% reduction of grey mould symptoms, caused by a delay or suppression of spreading lesion formation. Given the spatial separation of

both microorganisms, this effect was attributed to the induction of systemic resistance by the bioagent. *T. harzianum* T39 soil application 7 days before *B. cinerea* challenge significantly reduced grey mould severity in tomato, lettuce and pepper although the biocontrol agent was not detected on the leaves of these plants. A combination of leaf and soil treatment in bean was even more effective and reduced the total lesion diameter by about 35% compared to the control. Generally, more than 1 day was needed for *T. harzianum* T39 soil treatment to induce host plant resistance. In tobacco, *B. cinerea* was only effectively controlled when inoculated one or more days after *T. harzianum* T39 treatment (De Meyer *et al.* 1998).

Simultaneous inoculation and preinoculation with *T. harzianum* gave good control of *B. cinerea* (50% and 90% disease reduction, 10 days after inoculation). The rate of rooting was not reduced by the biocontrol agent once infection was established. However, sporulation by *B. cinerea* was specifically reduced on the rooting stem pieces (O'Neil *et al.* 1996).

A mixture of *T. harzianum* with a dicarboximide fungicide resulted in up to 96% control of cucumber grey mould. At the end of the season, 85% of the population of *B. cinerea* was found to be resistant to dicarboximide fungicides. Although not significant, the mixture of *T. harzianum* with vincolin controlled the disease on fruits and stems slightly better than each of the treatments alone. *T. harzianum* alone did not reduce fruit infection but reduced stem infection by 46%. Populations of *T. harzianum* on fruits were one tenth of that on leaves of cucumber. They remained high after the second and third spray (Elad *et al.* 1993).

The good effect of the biopreparation Supresivit on grey mould of grape by foliar spray was found by comparing the efficacy of biological and chemical fungicides. Treatment with the biopreparation had a positive influence on the sensory quality of wine (Míša 1997).

Even in treatment of the phylloplane it is essential to allow for suitable conditions for the mycoparasite. Suppression of *B. cinerea* incidence by *T. harzianum* on tomato stem pieces was significant at 10°C and higher temperatures up to 26°C (O'Neil *et al.* 1996).

Three commercial *Trichoderma* products (Trichodex, Binab TF WP and Rootshield) and the laboratory strain *T. harzianum* P1 were sprayed weekly onto greenhouse-grown strawberry plants during the flowering period in an attempt to reduce fruit disease caused by *B. cinerea* and *Mucor piriformis*. None of the treatments affected the marketable yield of strawberries. Laboratory tests showed only slow formation of *Trichoderma* conidia at the mean temperature of the greenhouse (12°C). The *Trichoderma* strains were not only unable to germinate as quickly as *B. cinerea* and *M. piriformis* at the ambient temperatures of the greenhouse, but the commercially formulated conidia had also lost the capacity to germinate on and effectively colonize nutrient-poor natural substrates (Hjeljord *et al.* 2000).

However, during 4 years of field experiments in the USA, *T. harzianum* delivered by bumble bees or honey bees provided better *Botrytis* control on strawberries than that applied as a spray. In addition, the bee-delivered *T. harzianum* provided the same or better level of control of *Botrytis* as commercial fungicides applied at bloom (Kovach *et al.* 2000).

T. harzianum T39 was not only effective against *B. cinerea*, but worked against more that one pathogen. Soil treatment reduced anthracnose symptoms caused by *Colletotrichum lindemuthianum* in bean (Bigirimana *et al.* 1997) and also reduced white mould in lettuce (De Meyer *et al.* 1998).

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Souhrn

Brožová J. (2004): **Mykoparazitické houby** *Trichoderma* spp. **v ochraně rostlin**. Plant Protect. Sci., **40**: 63–74.

Trichoderma harzianum je zástupce anamorfních hub běžně se vyskytující v půdním prostředí v mnoha oblastech. Stejně jako řada hub tohoto rodu je fakultativním mykoparazitem schopným saprofytické výživy na běžných substrátech a aktivně napadajícím hyfy širokého spektra hub. Antagonismus se může projevovat kompeticí výživy,

parazitismem a antibiózou. Vzhledem ke svým mykoparazitickým vlastnostem k řadě houbových patogenů rostlin a dalším vhodným vlastnostem je snaha využít některé druhy rodu *Trichoderma* k ochraně rostlin. Jsou vybírány a šlechtěny takové kmeny, které v dostatečně širokém spektru životních podmínek účinně potlačují původce rostlinných chorob, obsazují rhizosféru rostlin a mají kladný vliv na jejich růst a vývoj. Biofungicid Supresivit, jehož účinnou složkou jsou konidie *Trichoderma harzianum*, je vyráběn v ČR. Pro rozšíření využívání biologických způsobů ochrany je nutné zapojit je do celkového systému ochrany pěstovaných plodin. Ukazuje se, že lze dobře kombinovat více prostředků biologické ochrany, ale že mohou být zároveň používány i některé chemické přípravky, které svými účinky účinnost ochrany dále zvyšují a lépe tak zabraňují napadení rostlin původci chorob.

Klíčová slova: biologická ochrana; biopreparáty; půdní fytopatogenní houby; rhizosféra; interakce; *Botrytis cinerea*

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