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COLONIZATION OF ROOTS AND GROWTH STIMULATION OF CUCUMBER BY IPRODIONE-RESISTANT ISOLATES OF *TRICHODERMA* SPP. APPLIED ALONE AND COMBINED WITH FUNGICIDES

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Abstract

The ability of *Trichoderma harzianum* and *T. viride* iprodione-resistant mutants to colonize cucumber root system was examined and their effect on plant biomass production was assessed, as well as their efficiency in pathogen control when applied alone or combined with fungicides (iprodione or propamocarb).

The dosages which inhibited the radial growth of tested mutants by 50% (ID_{50}) ranged from 26 to 821 μg/ml of iprodione and from 389 to 1824 μg/ml of propamocarb hydrochloride. For mutant types, the calculated ID_{50} values were 90–40 times higher for iprodione and propamocarb hydrochloride, respectively, than for wild types.

After introduction of *Trichoderma* spp. isolates into the soil substrate an increase in dry biomass production of cucumber plants occurred. Plant growth stimulation was associated with improved colonization of the root systems by *Trichoderma* isolates. The best colonizers occupied the root systems in the range from 227 to 1159 cfu per 1 g. They also stimulated their growth by 41–106%. A synergistic effect was noticed in the case of reduction of the number of cfu of *Sclerotinia sclerotiorum* and *Fusarium culmorum* on roots after combined application of iprodione or propamocarb with tested iprodione-resistant mutants.

Key words: biological control, iprodione, *Fusarium culmorum*, propamocarb, *Trichoderma, Sclerotinia sclerotiorum*

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Introduction

Fungi from *Trichoderma* genus are well known antagonists of several plant pathogenic fungi and are commonly used as biological control agents. Numerous reports show that *Trichoderma* species are powerful antagonists of the following genera: *Fusarium*, *Pythium*, *Sclerotinia*, *Rhizoctonia* and *Gaeumannomyces*. Different mechanisms have been suggested as being responsible for this biological control, including mycoparasitism, antibiosis, competition and induction of defense responses in host plant (Benitez et al. 2004, Harman 2000, Papavizas 1985, Pietr 1997).

Some strains of *Trichoderma* can also promote plant growth by enhancing production of plant hormones, improving mineral uptake, increasing availability of biogenic elements as well as by releasing nutrients from the soil and organic matter (Altomare et al. 1999, Inbar et al. 1994, Kücük and Kivanc 2003). Stimulation of plant growth by *Trichoderma* spp. has been reported for various plant species, including bean, carnation, cucumber, lettuce, maize, pepper and tomato, both under greenhouse and field conditions (Baker 1989, Mańka et al. 1997, Bailey and Lumsden 1998, Inbar et al. 1994, Kleifeld and Chet 1992, Ousley et al. 1994).

Biological control of plant pathogens is a potential non-toxic alternative to chemical fungicides. Nevertheless, biological control rarely gives better control than a good fungicide (Elad et al. 1982, Lifschitz et al. 1985). The combination of chemical and biological agents to control plant pathogens seems to provide a solution, which can overcome the problem of fungicide resistance and can reduce the fungicide input into environment (Cook 1988, Papavizas and Lewis 1988, Elad and Shtienberg 1994, De et al. 1996, O'Neill et al. 1996).

The practical application of *Trichoderma* as biocontrol agents integrated with chemical treatments required selection of fungicide resistant isolates. The first successful selections of new *T. harzianum* and *T. viride* biotypes resistant to benzimidazoles and related fungicides as well as to several others fungicides (chlorothalonil, procymidone, iprodione, and vinclozolin) were described previously by Papavizas et al. (1982) and Abd-El Moity et al. (1982). Additionally, some of benomyl-resistant mutants were found to produce higher amounts of cellulase enzymes (Ahmad and Baker 1987, Cuevas et al. 1994).

We have selected several iprodione-resistant strains of *T. viride* and *T. harzianum* (Pietr et al. 1993 b, Stankiewicz et al. 1997). They also show strong mycoparasitic activity and high activity of extra-cellular hydrolytic enzymes (Stankiewicz et al. 1997). Here, we investigated the ability of *T. harzianum* and *T. viride* iprodione-resistant mutants to colonize cucumber root systems as well as their effect on the plant biomass production. We also focused on the efficiency of pathogen control by the *Trichoderma* isolates applied alone and combined with fungicides (iprodione and propamocarb hydrochloride).

Materials and methods

Chemicals

The following fungicides were used for study: iprodione (3-[3,5-dichlorophenol]-*N*-[isopropyl-2,4-dioxoimidazolidine-1-carboxamide – Rovral FLO 255 SC BASF, Ludwigshafen, Germany) and propamocarb (propyl 3-[dimethylamino]propylcarbamate – Previcur 607 SL, Aventis CropScience S.A, Lyon, France). Dry powders of tested fungicides were disinfected with few drops of mixture of chloroform and ethanol (1:4) and later on they were suspended in sterile water. The appropriate volumes of aqueous suspensions were added to molten potato dextrose agar (PDA) before pouring into Petri dishes.

Resistance to fungicides

The inhibitory activity of tested fungicides was determined using medium poisoning method. The effect of tested fungicides on the growth of tested strains was expressed by inhibitory dose resulting in 50% inhibition of radial growth of the mycelium (ID_{50}) on PDA. Tested fungicides were added into molten PDA after autoclaving. 1 ml of appropriate concentration of fungicide suspension in sterile 70% ethanol was added in order to achieve a final dose ranging from 0.1 to 1000 μg of active substance per 1 ml of medium. The control dishes contained organic solvent alone. The solvent was evaporated from plates in laminar box. Inoculations were done with discs (8-mm diameter) cut out from the edge of seven-day-old colony and placed in the centre of test dishes. Radial growth of the mycelium was measured for four days. Incubation was carried out at 28°C. Each experiment was carried out in three replicates. The value of ID_{50}, expressed in microgrammes per 1 ml, was calculated on the basis of regression analysis according to the probit transformation method (Sen and Srivastava 1990).

Tested strains

Wild type strains of *T. harzianum* F84 and F85 and *T. viride* Td50, and 16 of their mutants were used in this study. The mutants were selected earlier for resistance to iprodione fungicide (Pietr et al. 1993 b, Stankiewicz et al. 1997).

Experimental procedure

Root colonization and plant growth

The efficiency of root system colonization by tested strains of *T. viride* and *T. harzianum* and the associated effect on growth of cucumber plants was studied in pot experiments. The experiments were set up in plastic pots each containing 500 g of soil substrate. Greenhouse cucumber (*Cucumis sativus*) cv. ‘Skierniewicki’ was used throughout the experiments. Five seeds per pot were sown. Fungal inoculum of strains was prepared according to previously published protocol (Pietr et al. 1993 a). Final inoculum density ranged from 5×10^6 to 10×10^6 colony forming units (cfu) per 1 g of soil substrate. The experiment was carried out in four replicates over the period of five weeks (14-h light cycle, 26–28°C day and 16–18°C night). The plants were irrigated on alternate days. Pots without any treatment were set up as control. The effect of *Trichoderma* species on plant growth was estimated by measuring mean dry biomass from 10 randomly chosen plants. Plants were oven-dried for 72 h at 75°C. The results of the study are compilation of independent series of experiment carried out for each set of tested mutants and for comparison expressed in percentage of dry biomass yield of control plants.

The degree of root colonization was determined by enumeration of *Trichoderma* spp. cfu on V8 solid medium (Papavizas and Lumsden 1982) with 10 mg/l of iprodione. Root systems were gently rinsed with tap water to remove soil particles. Then roots were cut into small fragments (1-cm long) and transferred to a sterile solution of 0.1 M $MgSO_4$. Suspensions were shaken by 20 min (150 rpm) and used for plate inoculation. The test was carried out in three replicates. The data were analyzed using Student's t-test ($p = 0.05$).

Pathogen control

The aim of the second experiment was to investigate the effect of combined biological and chemical method on cucumber protection against soil-borne pathogens and on root systems colonization by *S. sclerotiorum* F110 and *F. culmorum* F1 in a pot experiment. Selected isolates of *T. viride* Td50-B35, Td50-57 and *T. harzianum* F85-257 were used in the second experiment. Three methods of protection were applied: biological protection with selected isolates, chemical protection with iprodione or propamocarb as well as combined biological and chemical method. The aim of applying a fungicide active against *Oomycetes* (propamocarb) was to study colonization ability of *Trichoderma* strains in the presence of *F. culmorum*, when pathogens sensitive to the fungicide were suppressed.

The pots were prepared as described above, incubated for five days at room temperature and then cucumber seeds were sown. Enumeration of tested *Trichoderma* strains cfu was performed as described above. Counting cfu of pathogens inhabiting root system was done by plate method on V8 medium without iprodione (roots prepared as described above). Additionally, colonies were identified on the basis of characteristic morphological features.

Biological protection. Three days after introduction of *Trichoderma* isolates, biomass of *F. culmorum* F1 or *S. sclerotiorum* F110 was added to the soil substrate. Inoculum of pathogens was prepared according to protocol of Wojtkowiak-Gębarowska (2005).

Chemical method. Soil substrate inoculated as described was watered with 0.1% iprodione suspension used against *S. sclerotiorum* F110 or 0.1% propamocarb suspension used against *F. culmorum* F1. Fungicides were applied two and 10 days after the seeds were sown.

Biological-chemical method. Soil substrate inoculated with *Trichoderma* isolates and with the pathogen was additionally watered with suspension of fungicides, as described above.

Results

For each tested wild type strain of *T. viride* and *T. harzianum* the sensitivity to fungicides was similar and varied only lightly (Table 1). The sensitivity to propamocarb expressed by reduction of radial colony growth (ID_{50}) was distinctly lower than to iprodione (Table 1). Among the tested mutants, the biggest differences were found in the case of iprodione. Calculated ID_{50} for iprodione ranged from 26 to 821 μg/ml. The inhibition of radial growth by propamocarb by 50% was observed in doses that ranged from 389 to 1824 μg/ml and the range was similar for strains of both species. Among the mutants, the most resistant to iprodione were *T. harzianum* F84-242 (ID_{50} = 821 μg/ml), *T. harzianum* F85-257 (ID_{50} = 740 μg/ml) and *T. viride* Td50-57 (ID_{50} = 602 μg/ml). The most resistant to propamocarb mutants were *T. harzianum* F84-242 (ID_{50} = 1095 μg/ml), *T. harzianum* F85-257 (ID_{50} = 907 μg/ml) and *T. viride* Td50-B35 (ID_{50} = 1029 μg/ml). The mutants were 90 times and 40 times more resistant than parent strains to iprodione and propamocarb, respectively.

Results of the first pot experiment (Fig. 1) indicated that the application of wild type *T. viride* Td50 strains to the soil substrate caused a decrease in cucumber seedlings dry matter by about 39% while wild type strains *T. harzianum* F84 and F85

Table 1

Inhibitory doses that repressed the radial growth by 50% (ID_{50}) on PDA (μg/ml)

Tested strain of *Trichoderma*	Iprodione	Propamocarb
T. viride Td50 (wild type)	4.6	25.1
T. viride Td50-22 (mutant)	479	781
T. viride Td50-35 (mutant)	613	918
T. viride Td50-40 (mutant)	283	516
T. viride Td50-57 (mutant)	632	1 018
T. viride Td50-140-4 (mutant)	535	1 824
T. viride Td50-B34 (mutant)	457	970
T. viride Td50-B35 (mutant)	602	1 029
T. harzianum F84 (wild type)	9.3	16.1
T. harzianum F84-240 (mutant)	254	1 014
T. harzianum F84-241 (mutant)	217	423
T. harzianum F84-242 (mutant)	821	1 095
T. harzianum F84-243 (mutant)	175	1 064
T. harzianum F84-244 (mutant)	458	389
T. harzianum F85 (wild type)	3.3	25.9
T. harzianum F85-247 (mutant)	139	1 450
T. harzianum F85-257 (mutant)	740	907
T. harzianum F85-260 (mutant)	298	596
T. harzianum F85-261 (mutant)	641	671

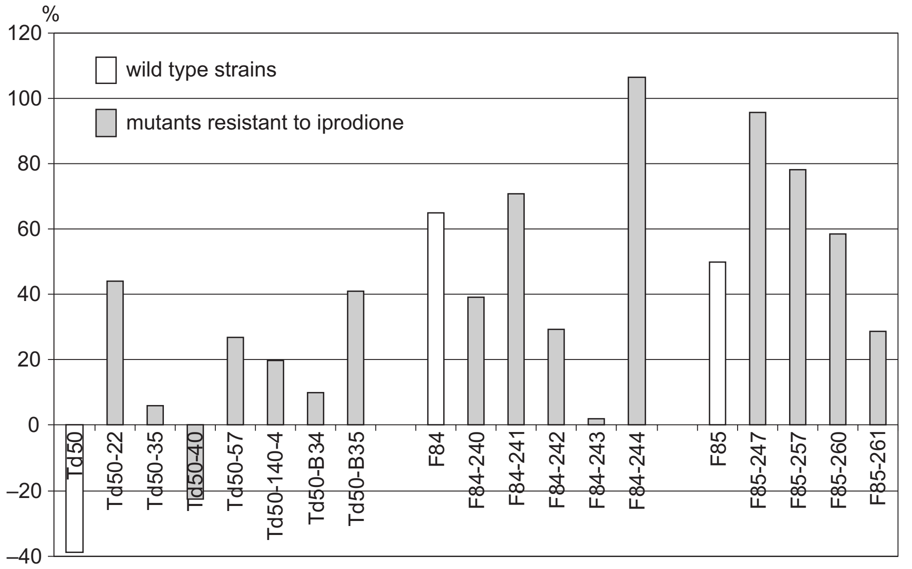

Fig. 1. Relative yield of cucumber plants dry biomass in the presence of wild type *Trichoderma viride* and *T. harzianum* strains and their mutants resistant to iprodione. Dry matter of control plants in experiments with: *T. viride* – 5.20 g, *T. harzianum* – 6.33 g

caused an increase of dry matter ranging from 50 to 65%, as compared to non treated control. Application of all mutants of *T. viride* resulted in a noticeable increase of dry biomass in comparison to wild type strain Td50 and control object. The highest increase in dry matter occurred in the case of inoculation of soil substrate with isolates Td50-22, Td50-B35 and Td50-57 (by 41, 44, and 27%, respectively). Among mutants of *T. harzianum* the highest increase of the dry biomass yield – in the range from 78 to 106% – was observed after applications of isolates F84-244, F85-247 and F85-257 to the soil substrate (Fig. 1). The observed increase in growth of cucumbers could be associated with higher degree of colonization of root systems by these *Trichoderma* species. The isolates Td50-57 and Td50-B35 significantly better colonized root systems of cucumbers than wild type strain Td50 ($p < 0.05$, Student's t-test; Table 2).

A similar effect was observed in the case of Td50-140-4 and Td50-B34 mutants. From 1 g of roots 922–1159 cfu were isolated. These amounts were higher by 10 to 37% in comparison with the wild type strain. Among tested strains of *T. viride*, the smallest number of cfu was isolated from the roots of cucumbers growing in the soil substrate treated with the Td50-35 isolate (Table 2). The numbers were lower by 36% in comparison with the wild type strain – Td50. A decreased effect on the growth of cucumber plants occurred. However, the number of *T. harzianum* cfu isolated from roots was noticeably smaller in comparison with the strains of *T. viride* and varied from 13 to 253 cfu per 1 g (Table 2). Both wild type strains of *T. harzianum* colonized roots to a similar extent. Among the tested strains of *T. harzianum*, the smallest number of cfu was isolated from roots of cucumbers grow-

Table 2

Colonization of cucumber roots by wild type *Trichoderma viride* Td50, *T. harzianum* F84 and F85 and their iprodione-resistant mutants in comparison to control

Trichoderma viride		*Trichoderma harzianum*	
strain	cfu per 1 g of root	strain	cfu per 1 g of root
Control	7 f	Control	3 g
Td50	843 c	F84	113 de
Td50-22	535 d	F84-240	163 cd
Td50-35	301 e	F84-241	13 g
Td50-40	479 d	F84-242	110 de
Td50-57	924 b	F84-243	47 fg
Td50-140-4	1 159 a	F84-244	177 bc
Td50-B34	922 b	F85	93 ef
Td50-B35	1 159 a	F85-247	140 bc
		F85-257	227 ab
		F85-260	180 bc
		F85-261	253 a

Values followed by the same letter are not significantly different according to Student's t-test ($p = 0.05$).

ing in the soil substrate treated with the F84-241 isolate: the root system was colonized to the same degree as the root system of control plants. However, in these conditions the roots were colonized by naturally occurring *Trichoderma* species, which were present in the soil substrate. The F85-261 isolate of *T. harzianum* was found the best root colonizer. The number of cfu per 1 g of roots was 172% higher in comparison with the wild type (Table 2). We isolated significantly more cfu from the roots treated with *T. viride* strains than with *T. harzianum*. Finally, the application of *T. harzianum* resulted in a more robust growth of cucumber plants.

In the second pot experiment, we tested the possibility of combining the treatment with fungicides together with selected iprodione-resistant mutants for control of soil-borne pathogens. For this purpose, we used following three isolates: *T. viride* Td50-B35, *T. viride* Td50-57 and *T. harzianum* F85-257. They were most resistant to both fungicides iprodione and propamocarb and represented the best colonizers of cucumber roots as was described above. Using different methods to control the tested soil-borne pathogens *F. culmorum* and *S. sclerotiorum* we discovered that the application of fungicides significantly decreased the number of pathogen cfu (Fig. 2). The reduction of the number of cfu collected from the roots ranged from 34 to 52% in comparison with the control object for *F. culmorum* and *S. sclerotiorum*, respectively. The reduction of the number of cfu after application of iprodione or propamocarb did not result in stimulation of plant biomass production (Fig. 3). When *T. harzianum* F85-257 was used alone a significant decrease of cfu of *S. sclerotiorum* F110 was observed. In this case, the number of pathogen cfu

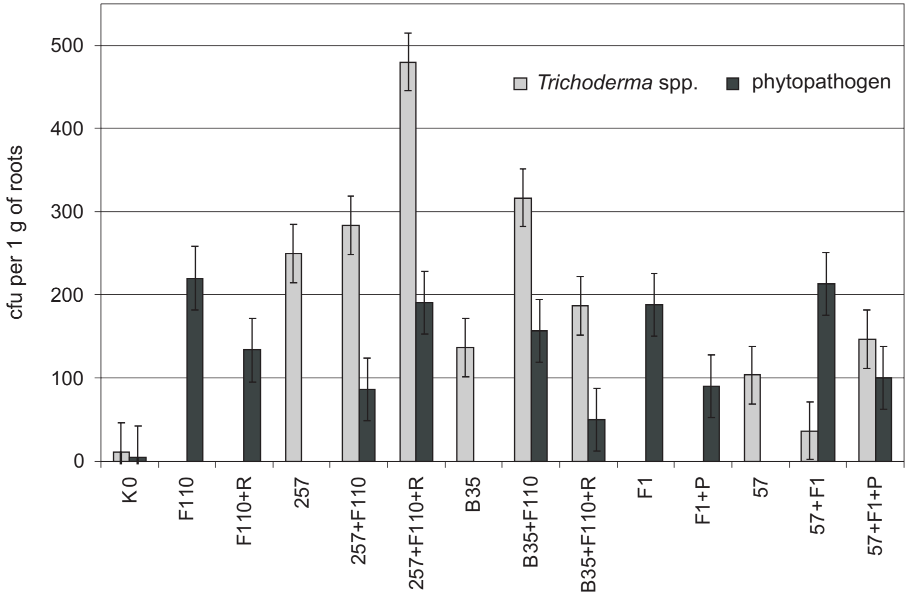

Fig. 2. Root colonization of cucumber by isolates of *Trichoderma* and phytopathogenic fungi in soil substrate after use of different methods of protection. K0 – control, F1 – *Fusarium culmorum,* F110 – *Sclerotinia sclerotiorum,* R – iprodione, P – propamocarb hydrochloride, 257 – *Trichoderma harzianum* F85-257, B35 – *T. viride* Td50-B35, 57 – *T. viride* Td50-57

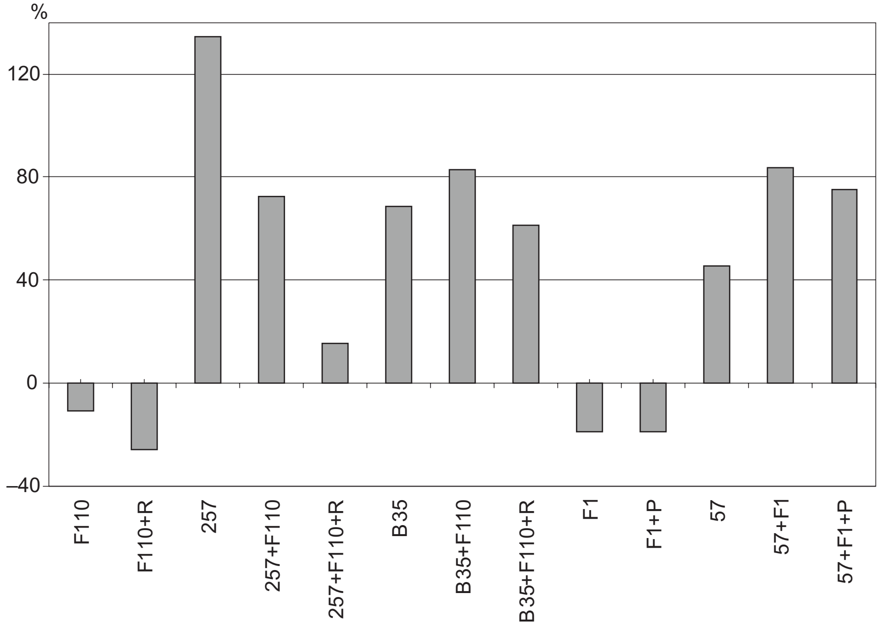

Fig. 3. Relative yield of dry biomass of cucumber grown in soil substrate after use of different methods of protection. Dry matter of control plants – 8.42 g. Symbols – see Figure 2

was 61% lower than in the control object. Furthermore, a significant decline (by 47% of cfu number) of *F. culmorum* F1 was observed after application of the Td50-57 isolate together with propamocarb to the soil substrate. Additionally, the application of *T. viride* Td50-B35 strain together with iprodione to the soil substrate resulted in a synergistic effect of both agents and the number of cfu of *S. sclerotiorum* F110 decreased by 77%. We also noticed a higher degree of root colonization by tested mutants after their co-application with the fungicides. A significant increase of root colonization was found in the case of *T. viride* Td50-257 ($p < < 0.05$, Student's t-test; Fig. 2). Moreover, Td50-B35 and F85-257 mutants occupied the roots to a higher extent after the introduction of biomass of *S. sclerotiorum* F110 to the soil substrate, whereas, after the addition of biomass of *F. culmorum* F1, the number of *T. viride* mutant Td50-B35 cfu isolated from the root pieces significantly decreased. The observed reduction of cfu of tested pathogens was not connected with the improvement of plant growth.

The application of tested *Trichoderma* strains as protective agents before introduction of phytopathogenic fungi caused a noticeable stimulation of plant biomass production and the effect ranged from 61 to 84% in comparison with control plants grown in untreated soil substrate (Fig. 3), whereas the combined application of fungicides with *T. viride* Td50-B35 and Td50-57 did not result in a significantly higher growth of cucumbers in comparison with application of these mutants alone. The F85-257 mutant caused stimulation of plant biomass production in higher degree, when was applied alone into uninfected soil substrate than in the presence of *S. sclerotiorum* F110. Moreover, we did not observe growth stimulation after combined application of *T. harzianum* F85-257 with propamocarb.

Discussion

Trichoderma fungi are well known biocontrol agents of soil-borne plant pathogenic fungi. The increased growth response of several plants, following application of *Trichoderma* spp. to soil under both greenhouse and field conditions, has also been well documented (Baker 1989, Kleifeld and Chet 1992, Inbar et al. 1994, Ousley et al. 1994, Bailey and Lumsden 1998). Moreover, the combined application of chemical and biological agents for controlling plant pathogens appears to be a solution which can reduce pesticide input into environment as well as provide a better phytopathogen control (Cook 1988, Papavizas and Lewis 1988, Elad and Shtienberg 1994, De et al. 1996, O'Neill et al. 1996). The tested isolates of *T. viride* and *T. harzianum* showed high levels of resistance towards iprodione and propamocarb in comparison with wild type strains. The level of resistance was not correlated with their ability to colonize roots or to stimulate growth of cucumbers during a five-week vegetation period. The observed variability in the ability to colonize roots among tested iprodione-resistant mutants suggested that there was a significant metabolic pathway change due to mutation. Griffiths et al. (2003) showed that iprodione had a selective effect on lipid metabolism, especially on choline (ethanolamine) phosphotransferase of *Botrytis cinerea.* In addition, Dry et al. (2004) confirmed the presence of mutations leading to premature termination of the translated histidine kinase gene in the case carboximide-resistant *Alternaria alternata* isolates. Ochiai et al. (2001) presented evidence that mutation sites were located on an upstream element of histidine kinase in the case of osmotic-sensitive mutant of *Neurospora crassa,* which exhibited strong resistance to dicarboximides. Such alternation of metabolism resulted in significant changes in permeability of biological membranes and probably also took place in the case of our iprodione-resistant isolates of *Trichoderma.* Alternation of biological membrane characteristics changed the secretion of metabolites including antibiotic and enzymatic proteins, which play a role in root colonization and growth stimulation (Altomare et al. 1999, Cuevas et al. 1994, Inbar et al. 1994, Kücük and Kivanc 2003, Viterbo et al. 2005). Our results indicate that the introduction of *Trichoderma* species to the soil substrate generally caused an increase of cucumber growth in comparison to control plants. The stimulation of cucumber growth was associated with higher degree of root colonization when compared with isolates originating from the same species. The differences in the capacity to colonize roots and stimulate plant growth observed in our studies suggest that our iprodione-resistant isolates probably demonstrated alternation of metabolite secretion, which play a role in root colonization and in plant growth promotion. Increased colonization of the soil substratum and the root systems enhances the biocontrol properties of saprotrophic fungi and their impact on plant growth stimulation.

It has been suggested that a rapid production of spores, which are then carried down the root by water, fuels the root colonization process and this is a key feature in the establishment of the biocontrol agent on the root (Whipps 2001). However, the results of our experiments showed that isolates of *T. harzianum,* which were obtained in lower numbers from rhizoplane of cucumber than *T. viride,* were in contrast to the suggestion of Whipps (2001). This fact may indicate that our iprodione-resistant isolate of *T. harzianum* employs a different mode of action than our isolates of *T. viride*. Numerous modes of action of various isolates of *Trichoderma* were described in literature (Altomare et al. 1999, Benitez et al. 2004, Cuevas et al. 1994, Inbar et al. 1994, Kücük and Kivanc 2003, Viterbo et al. 2005). In addition, it is possible that in our five-week-long pot experiment the process of spore transportation with water did not play an important role. In addition, we could conclude that metabolic activity of *T. harzianum* F85-257 responsible for plant growth promotion was affected by iprodione in the soil substrate.

The results of our study showed that after application of *Trichoderma* spp. with fungicide to the soil substrate the number of cfu of *S. sclerotiorum* F110 and of *F. culmorum* F1 decreased by 15–78% and 47%, respectively. Additionally, we found that the numbers of cfu of F85-257 and Td50-B35 were about two times higher than the numbers of *S. sclerotiorum* F110 cfu. This effect of stronger suppression of *Fusarium* could be explained by better colonization of root system by *Trichoderma* in the presence of fungicide, despite of the fact that propamocarb was non active against *Fusarium*. We assume that better control of *Fusarium* by *Trichoderma* in the presence of propamocarb resulted from suppression of *Oomycetes* in soil. The biomass of killed fungi serves as additional easily accessible source of carbon and energy for biocontrol agents. It also suggests that *Oomycetes* were a significant part of fungal biomass in soil under the conditions in question.

Our earlier study showed already the improved efficiency of *T. viride* Td50-B35 application after previous soil disinfections with reduced doses of fungicides in production of cabbage, celery, tomato and strawberry transplants under field conditions (Pietr et al. 2002, Ślusarski and Pietr 2003). Moreover, Ślusarski (2005) applying *T. viride* Td50-B35 into previously chemically disinfected rockwool, observed also reduction of severity of *F. oxysporum* f. sp. *radicis-lycopersici* to tomatoes.

Our data suggest that decreased numbers of the pathogens cfu on cucumber roots could be related to a higher degree of competitiveness of fungicide-resistant isolates in the presence of fungicides, due to weakening of pathogens and other saprotrophic fungi in this specific ecological niche. Fungicide-resistant isolates of *Trichoderma* can be combined either simultaneously or subsequently with appropriate fungicide in order to obtain new formulations for use in more efficient control of plant diseases and plant growth stimulation.

In conclusion, the efficacy of plant protection with combined application of fungicide-resistant strains and fungicides requires appropriate selection of isolates. Iprodione-resistant isolates originating from the same species may have differentiated alternation of metabolic pathways. Selection of fungicide-resistant isolates able to express essential features in arable soils and horticulture substrate in the presence of fungicides can contribute to reducing pesticide use in agriculture.

Streszczenie

KOLONIZACJA KORZENI I STYMULACJA WZROSTU OGÓRKA PRZEZ IZOLATY *TRICHODERMA* SPP. ODPORNE NA IPRODION, ZASTOSOWANE ODDZIELNIE I W KOMBINACJI Z FUNGICYDAMI

Możliwości praktycznego zastosowania biologicznych środków ochrony roślin na bazie grzybów rodzaju *Trichoderma* są w znacznym stopniu ograniczone ze względu na stosowanie fungicydów. Sposobem eliminacji tego problemu może być zastosowanie odpornych na fungicydy izolatów. Celem badań było określenie zdolności szczepów macierzystych *T. harzianum* i *T. viride* oraz ich mutantów odpornych na iprodion do kolonizacji korzeni, a także ich wpływu na stymulację biomasy ogórków. Badano również wpływ wybranych izolatów *Trichoderma* w połączeniu z fungicydami (iprodionem i propamokarbem) na ograniczenie liczebności *Sclerotinia sclerotiorum* i *Fusarium culmorum*.

Badane szczepy charakteryzowały się znaczną odpornością na fungicydy. Dawki iprodionu hamujące wzrost radialny mutantów w 50% zawierały się w przedziale od 26 do 821 μg/ml, a dawki propamokarbu – w przedziale od 389 do 1824 μg/ml. Wartości ID_{50} wyliczone dla mutantów były 90-krotnie w przypadku iprodionu i 40-krotnie w przypadku propamokarbu większe niż dla szczepów macierzystych.

Po wprowadzeniu szczepów *Trichoderma* do substratu glebowego wystąpił wyraźny wzrost biomasy ogórków (od 41 do 106% w porównaniu z roślinami kontrolnymi) oraz lepsza kolonizacja systemu korzeniowego przez testowane szczepy *Trichoderma* spp. (od 227 do 1159 jtk na 1 g). Ponadto łączne zastosowanie mutantów *T. viride* z fungicydami dało efekt synergistyczny. Izolat Td50-B35, po zastosowaniu z iprodionem, spowodował istotne ograniczenie liczebności jtk *S. sclerotiorum*, a Td50-57 z propamokarbem – ograniczenie liczebności jtk *F. culmorum*. Obserwowany efekt synergistyczny można prawdopodobnie powiązać z eliminacją pod wpływem zastosowanych fungicydów grzybów – zarówno patogenicznych, jak i saprotroficznych, których biomasa mogła być wykorzystana przez testowane izolaty *Trichoderma* jako dodatkowe źródło pokarmu, ułatwiając im lepszą kolonizację ryzosfery.

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