European Journal of Plant Pathology **104:** 243–251, 1998.

Screening for fungal antagonists of seed-borne *Fusarium culmorum* on wheat using *in vivo* tests

Esa Teperi[1], Milja Keskinen[2], Elise Ketoja[3] and Risto Tahvonen[4]
[1] *Agricultural Research Centre, Laukaa Research and Elite Plant Station, FIN-41330 Vihtavuori, Finland (Fax: +358-14-633835);* [2] *Agricultural Research Centre, Plant Protection, FIN-31600 Jokioinen, Finland;* [3] *Agricultural Research Centre, Data and Information Services, FIN-31600 Jokioinen, Finland;* [4] *Agricultural Research Centre, Horticulture, FIN-21500 Piikkiö, Finland*

Accepted 30 November 1997

Key words: biological control, *Gliocladium*, field tests

Abstract

A screening system is described for identifying fungal antagonists from soil which are effective in controlling seed-borne *Fusarium culmorum* on wheat (*T. aestivum* L.). The procedure comprised three consecutive *in vivo* tests done under controlled conditions using three different plant growing media. Altogether 1701 fungal isolates from Finnish field soils were screened for biocontrol activity. 210 fungal isolates that had advanced to various stages in the screening system were also included in a field experiment. The isolates that were the most effective in controlling seedling blight under natural conditions belonged to the genus *Gliocladium*. The screening tests separated *Gliocladium* spp. rather clearly from the rest of test fungi and thus the overall agreement among the results was good. The assays run under controlled conditions did not efficiently predict which of the *Gliocladium* isolates would be best in protecting wheat seedlings under field conditions.

Introduction

Seedling blight of wheat (*Triticum aestivum* L.), caused by *Fusarium* spp., arises primarily from seed-borne inoculum (Wiese, 1987). In Northern Europe the level of contamination in seed lots varies considerably from year to year and sometimes a large proportion can be infected (Daamen et al., 1991; Skou, 1991; Uoti and Ylimäki, 1974).

Fusarium culmorum (W.G. Sm.) Sacc. is the most prevalent (Uoti and Ylimäki, 1974) and probably the most economically damaging (Uoti, 1976) of the *Fusarium* species in Finnish wheat fields. Microbial antagonists of this pathogen, when applied to infected seeds, have appeared capable of preventing disease development (Knudsen et al., 1995; Sivan and Chet, 1986). Seed treatments comprising bacterial (Kempf and Wolf, 1989) and fungal (Sivan and Chet, 1986) isolates have also protected wheat seedlings from soil-borne *F. culmorum* in tests done in the greenhouse.

The objectives of this study were to identify fungal antagonists of *F. culmorum* from Finnish soils and to evaluate the screening methods used. Only *in vivo* tests were used during screening: the pathogen and the candidate antagonists were allowed to interact on germinating seeds under controlled conditions after which the degree of antagonism was determined by inspecting the disease symptoms on young seedlings. A field experiment was set up to provide information on the potential of the screening tests for predicting biocontrol activity under natural conditions. A preliminary report of this research was presented (Tahvonen and Teperi, 1995).

Materials and methods

Isolation of the test fungi

Soil samples were collected from 143 fields from different areas of Finland in the autumns of 1989 and

1990. About 1 ha plots from fields of diverse soil type and cropping histories were selected for sampling. About twenty subsamples from the depth of 0–15 cm were mixed properly and 1 kg of the mixture was stored in plastic bags at +4 °C for up to 4 weeks prior to isolation of fungi. Fungal isolates were obtained either from roots of bait plants grown in the soil samples (root isolates, RI), or directly plated soil (soil isolates, SI).

Bait plants were used to acquire fungi able to colonise the surfaces of young roots at low temperatures, which was regarded as an important property of a biocontrol agent used as a seed treatment. Three different plant species, wheat, barley (*Hordeum vulgare* L.) and turnip rape (*Brassica rapa* subsp. *oleifera* DC.) were used, because the isolated fungi were intended to be used for screening against seed and soil-borne pathogens of various cultivated plants. Plastic pots (50 ml) were filled with soil from a sample, seeds of bait plants were placed on the soil and were covered with sand. The pots were watered and incubated in a growth chamber at 15 °C with a 14 h daylength. After two weeks the roots of the seedlings were washed under running tap water and sections of them were plated on either Littman oxgall agar (LOA, Difco), peptone -PCNB -agar (Nash and Snyder, 1962) or potato dextrose agar (PDA, Biokar) supplemented with streptomycin sulf. (Sigma, 300 μg/ml), and incubated for 2–4 days at room temperature. Individual fungi with different morphologies were then subcultured on PDA plates.

Direct plating was initiated by thoroughly mixing a 10 g subsample of soil in sterile water thickened with 0.5% (w/v) agar. 1.0 and 0.1% (w/v) suspensions of soil were prepared and shaken for 30 min in Erlenmeyer flasks. 1 ml samples of both suspensions were mixed separately with 20 ml of LOA (55 °C) in a sterile Petri dish which was then incubated at room temperature in the dark. Three subsamples were processed from each soil sample. After 3–7 days the plates were inspected and pure cultures of fungi were isolated on PDA.

After the cultures on PDA plates were fully developed, their purity was checked and most of the fungi were identified according to Domsch et al. (1980). Most *Botrytis, Rhizopus, Mucor, Penicillium, Fusarium avenaceum, F. culmorum, F. graminearum* and *Monographella nivalis* isolates (710 isolates altogether) were discarded without any testing because of their abundance or their assumed pathogenity/inefficiency as antagonists. Agar plugs from the remaining cultures (about 4000 isolates) were stored at −80 °C until needed for the first screening test.

Screening fungal isolates

The screening program consisted of three separate *in vivo* tests carried out under controlled conditions; each with a different plant growing medium. Sand was used in the first test, peat in the second and field soil in the third. After each screening stage the ineffective isolates were excluded from further testing.

Spring wheat cv. Luja (certified seed, Jokioinen Seed Center) was the test plant in all the screening tests. The fungal isolates tested against *F. culmorum* were cultured on PDA plates (diameter 85 mm) for 3–4 weeks at room temperature. The basic water suspensions used in screening tests were prepared similarly for all the isolates: the spores and mycelia from the surface of one plate were scraped into 25 ml of distilled water. After homogenization, the suspension or dilutions were used to treat the wheat seeds. One pathogenic *F. culmorum* isolate, originating from Jokioinen, was used for pathogen inoculations in all the tests. Inoculum was prepared as for test fungi, but the suspension was stored at −20 °C before use. Three rounds of the three stage screening program were carried out, one each winter 1991, 1992 and 1993. 1701 isolates (1313 RI and 388 SI) were tested in total.

Testing in sand

In the first screening test pots (50 ml) were used. In each pot three wheat seeds were placed on 45 ml of non-sterile sand (grain size 0.2–0.7 mm, moistened to saturation). In test treatments, three amounts of each test isolate (1 ml of the basic water suspension and dilutions of this suspension, 10^{-1} and 10^{-3}) and two concentrations of *F. culmorum* (10^6 and 10^4 conidia / pot) were used. To test one isolate, each of these six concentration combinations was drenched on to the seeds in each of five pots. 10–20 isolates were tested simultaneously and on each sowing date separate control pots were prepared. Ten of these were treated with 10^6 conidia of *F. culmorum* per pot, ten with 10^4 conidia per pot and ten with water only. After inoculations the pots were filled with additional sand and put into plastic containers which were then covered with transparent polyethylene sheets.

The pots were incubated in a growth chamber (12 ± 2 °C, 14 h photoperiod, 25 μE s^{-1} m^{-2}) for 16–18 days. If the untreated seeds produced healthy

sprouts, and in *Fusarium* control pots severely diseased plants were found, observations were made on the test treatments. The isolates that did not damage the wheat, but clearly prevented pathogenesis by *F. culmorum*, were retained for further testing in peat.

Testing in peat

Steam sterilized *Sphagnum* peat supplemented with dolomite limestone (8 kg/m^3) and with commercial NPK fertilizer for peat (Kemira Agro Oy, containing 130 g N, 130 g P, 240 g K /m^3 and small amounts of micro nutrients) was used as the growing medium. Four treatments were used to test each isolate: healthy control, *Fusarium* control, *Fusarium* + the basic water suspension of the test isolate and *Fusarium* + 10^{-2} dilution of the basic water suspension. The wheat seeds were first dipped in a suspension of *F. culmorum* (10^6cfu/ml) (or in water for the healthy control) and dried overnight on laboratory tables. The two suspensions of the test isolate were then prepared and applied by shaking them with the seeds in plastic bottles (2 min). The amount used was the maximum that the seed could absorb. The seeds for the two control treatments were treated similarly with water. The seeds were then dried on laboratory tables for 2–4 h and sown.

One plastic box (29×50×10 cm) filled with peat was used for testing one isolate. For each four treatments 60 seeds were sown in two 29 cm rows and one border row with uninoculated seed was sown at each end of the box. The boxes were incubated in a greenhouse (15 °C, 12 h of extra light from multimetal lamps, 240 μE s^{-1} m^{-2}) and they were hand-watered when needed. 18 days after sowing the healthy plants were counted (sprouts without symptoms and ungerminated seeds that had remained hard). The test of each isolate was considered valid if the number of healthy plants did not exceed eight in the *Fusarium* control, and was not less than 48 in the healthy control. An isolate was generally rejected for the field soil test, unless the application of either of the two suspensions prepared from it produced 41 or more healthy seedlings. In practice, the selection was based almost entirely on the basic suspension treatment. Only the results from it are used in comparing screening results with the observations from the field experiment.

Testing in soil

In this screening test, wheat was planted in 1.5 l plastic pots containing non-pasteurized silty clay (pH 6.4) from Jokioinen. Each autumn prior to running the tests, soil was taken from the depth of 0–25 cm and stored at 15 °C for up to six months. Before use, the soil was first air-dried at room temperature, then graded and moistened with water. Only one concentration (the basic water suspension) of each test isolate was used for seed treatment. Otherwise the *Fusarium* and antagonist inoculations were as for the peat tests. There were three replications in the experiments and 36 seeds were sown in each pot. 10–15 isolates were tested in one experiment. The pots were completely randomized on greenhouse tables and the growing conditions were as for the peat tests. Four weeks after sowing the number of healthy sprouts was recorded. The isolates that gave the best results were considered to be worth more detailled examination and further testing under field conditions.

Field experiment to evaluate the effectiveness of the screening procedure

To evaluate the three-stage screening system described above, an experiment was sown in the field in spring 1993. The isolates that had been tested up to different stages of the screening system during the preceding winter were used for selecting isolates to be tested in this experiment (487 isolates in all, 228 RI and 259 SI). Two restrictions on these isolates were set up before sampling: 1) Only fungi belonging to genera represented by at least one isolate in the soil tests performed during the three years of screening were included. 2) Isolates which had been pathogenic to wheat or had not had any effect on foot and root rot in the sand tests, were excluded (one *Coniothyrium*, three *Cylindrocarpon*, 31 *Fusarium*, two *Gliocladium*, 11 *Mortierella*, 17 *Penicillium*, three *Scopulariopsis*, two *Trichoderma* and two *Ulocladium* isolates). The purpose of these restrictions was to increase the possibility of finding effective biocontrol agents among the isolates rejected during greenhouse screening and to make the test on the selection procedure more critical. 330 isolates (164 RI and 166 SI) remained for sampling.

All the 58 isolates that had been used in the soil tests were included in the field experiment. 15 of them had been selected for further examination according to the results from this third screening test. Before sampling, the fungi that had been rejected in the peat tests (174 isolates) were grouped into those isolated from bait plant roots (92 RI) and those isolated directly from soil (82 SI). From both were drawn samples of equal size (46 isolates). The same method was applied to the

Table 1. The 210 fungal isolates included in the field experiment grouped by genera, by stage of screening to which they proceeded and by the method that was used in their isolation from soil (SI = isolates from soil platings, RI = isolates from bait plant roots)

	Isolates rejected in sand tests		Isolates rejected in peat tests		Isolates rejected in soil tests		Isolates approved in soil tests	
genus	SI	RI	SI	RI	SI	RI	SI	RI
Alternaria spp.	1	1	1	–	–	–	–	–
Coniothyrium spp.	2	–	–	–	–	1	–	–
Cylindrocarpon spp.	1	3	–	7	–	–	–	–
Fusarium spp.	7	17	8	13	–	2	–	–
Gliocladium spp.	5	1	1	3	22	6	13	2
Mortierella spp.	6	4	6	7	2	1	–	–
Penicillium spp.	6	2	12	11	1	2	–	–
Scopulariopsis spp.	1	2	10	1	–	1	–	–
Trichoderma spp.	1	–	4	4	2	2	–	–
Ulocladium spp.	–	–	4	–	1	–	–	–
Total	30	30	46	46	28	15	13	2

fungi rejected in sand tests (98 isolates in all, 55 RI and 43 SI) but the sample sizes were smaller (2 x 30 isolates). This was because it was suspected that during screening most incorrect selection decisions were made on the basis of the results from peat tests. The properties of pasteurized *Sphagnum* peat as a growing medium could be claimed to be so unique that the use of it prevented efficient selection of isolates with good field performance. The genera included in the field experiment and the sample sizes are given in Table 1.

Four fungal isolates were included in the experiment as controls. The effect of treating cereal seed with them had been tested in field experiments in 1992 (E. Teperi, unpubl.). Two of these J1446 (*Gliocladium catenulatum* Gilman & Abbott) and J76 (*Myrothecium* sp.) controlled different fusaria as effectively as fungicide dressings while two others J98 (*Gliocladium roseum* Bain.) and J810 (*Idriella bolleyi* (Sprague) von Arx) induced considerably lower levels of protection. There were two more treatments in the experiment: non-treated control and fungicide control (Baytan I dressing, Bayer, 300 mg of triadimenol and 50 mg of imazalil /1 kg of seed).

The infected seed (spring wheat cv. Luja) used in the field experiment was produced in the previous summer. At the beginning of flowering stage the wheat plot was irrigated for one day and in the evening a water suspension of *F. culmorum* was sprayed on the canopy with a knapsack mist blower. A similar inoculation was repeated two weeks later. The following winter, samples of the seed obtained were plated onto PDA. Hundred per cent of the seeds appeared to be infected. Dressing the field inoculated seed with Baytan I improved sprouting from 43 to 63% in greenhouse tests.

The seed for the field experiment was treated with the 214 isolates two days before sowing. 0.18 ml of the basic water suspension of each was applied per 1 g of seed using the method described above for the peat tests. The seed lots used for untreated and fungicide controls were wetted. After drying the seed overnight at room temperature, the fungicide was applied. Next day the field at Jokioinen (silty clay, pH 6.0) was fertilized with a commercial N-P-K (18-5-10) fertilizer (80 kg N/ha) and the experiment was sown (Wintersteiger Seedmatic 6) three weeks later than normal practice. The field plot was irrigated with 20 mm of water two days after sowing. One month later the seedlings were dug out of the soil and the number of healthy ones, without any foot rot symptoms, was assessed for each row. The seedlings were washed clean when needed for the observation.

A cubic lattice design (Cochran and Cox, 1957) was used. It allowed use of small incomplete blocks and took into account environmental variation attributable to soil factors. One 1.5 m long row sown with 5.4 g of seed formed an experimental unit. In each block (1.5 $\times$ 0.9 m) there were six rows and 36 such incomplete blocks were arranged into one complete replicate of the 216 treatments. There were six replicates of all treatments (1296 plots) in the experiment.

The analysis of the results from the field experiment was performed with a spreadsheet following the methods of Homeyer et al. (1947). The treatment means were adjusted to remove the influence of differences among the blocks, and both the efficiency of the cubic lattice design relative to a randomized complete block design, and the average standard error of differences between all adjusted treatment means, were computed. The relationships between the treatment means from the screening tests and the field experiment were tested using Pearson product-moment correlation. The absolute number of healthy seedlings was used as the measure for biocontrol activity. The percentages were not used as the target pathogen caused pre-emergence death of seedlings.

Table 2. The progress of fungal isolates belonging to various genera into the different stages of screening during the three years of testing (1991-93)

	Number of isolates				
genus	Rejected in sand tests	Rejected in peat tests	Rejected in field soil tests	Approved in field soil tests	Tested
Acremonium spp.	7	6			13
Alternaria spp.	12	6	2		20
Aspergillus spp.	1	1			2
Arthrinium spp.	7	5			12
Bipolaris spp.	9	4			13
Botrytis sp.	1				1
Chaetomium spp.	1	2			3
Cladosporium spp.	24	4			28
Coniothyrium spp.	5	8	2		15
Cylindrocarpon spp.	25	16	2		43
Dendryphion sp.	1				1
Doratomyces sp.	1				1
Epicoccum spp.	2	5			7
Fusarium spp.	386	113	13	3	515
Geotrichum spp.	–	2			2
Gliocladium spp.	30	14	43	28	115
Gymnoascus spp.	28	12			40
Humicola sp.	1				1
Idriella bolleyi	–	1	–	1	2
Mortierella spp.	174	52	4	1	231
Mucor spp.	12	9			21
Myrothecium sp.	–	–	2	1	3
Paecilomyces spp.	2	1			3
Penicillium spp.	95	77	10		182
Phialophora spp.	1	1			2
Phoma spp.	2	4			6
Preussia sp.	–	1			1
Pythium spp.	5				5
Rhizoctonia sp.	–	1			1
Sclerotium spp.	2	1			3
Scopulariopsis spp.	23	21	6		50
Stemphylium spp.	2	2			4
Thermomyces sp.	1				1
Trichocladium spp.	1	1			2
Trichoderma spp.	87	52	27	3	169
Trichothecium sp.	2				2
Ulocladium spp.	4	4	1		9
Verticillium spp.	21	12			33
Yeasts	2				2
Unidentified	71	43	3		117
Total	1062	487	115	37	1701

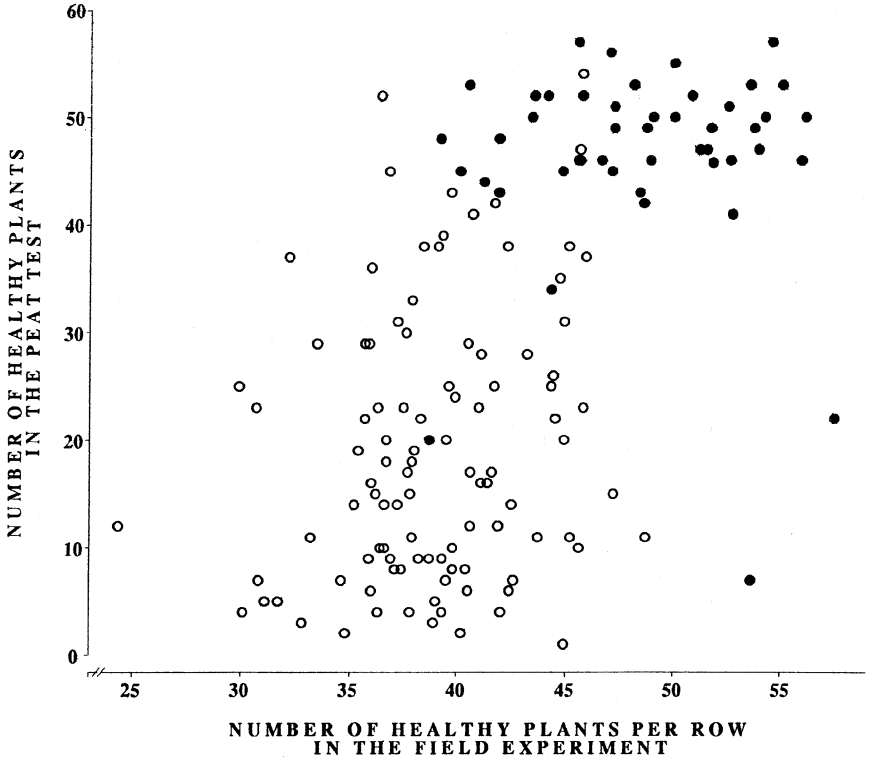

Figure 1. The disease observations made from the field experiment and from the peat test for the 150 isolates that were tested in both. ● = *Gliocladium* -isolates ○ = isolates belonging to other genera.

Results

Gliocladium spp. clearly differed from the rest of the fungi tested during the three years of screening. Less than 7% of all the isolates tested, but 76% of the isolates selected in all three tests belonged to this genus (Table 2). The fungi that most efficiently prevented disease development in the field experiment belonged also to the genus *Gliocladium* (Figures 1, 2). Of the most effective isolates only few were rejected according to the two first screening tests (Figure 2). The peat test was a little better than the sand test in being useful for predicting the performance of the fungi under field conditions. The average number of healthy seedlings per row in the field experiment for the 58 isolates that had passed the peat tests was 46.4. For the 58 isolates that were best in the sand tests, the average was 43.1. The use of different concentrations of the test fungi in the two first screening tests was not successful (data not presented). The stronger the inoculation, the closer was the relationship to the results from the field experiment. Likewise, the more *F. culmorum* applied to the seed, the better was the agreement.

The soil test was the best of the three screening tests to predict the biocontrol activity under field conditions. The 15 isolates that had passed this test yielded 49.6 healthy seedlings per row on average in the field experiment. A worse result would have been obtained if an isolate group of this size had been chosen according to the peat tests. The corresponding mean would have been 47.5.

Because *Gliocladium* spp. were superior to other fungal isolates, both in the greenhouse tests and in the field experiment, there was clear linear correlation between the results (Table 3). The two first screening tests selected *Gliocladium* spp. out of all tested fungi rather efficiently but inside this genus they did not predict the field performance at all (Figures 1 and 2, Table 3). In this regard the soil test, for which mainly *Gliocladium* isolates were left, was better (Table 3). However, the relationship between the results from the soil test and the field experiment was far too weak to justify its use after the two former screening tests (Figure 2).

In the field experiment no differences were established between biocontrol activity of the soil isolates

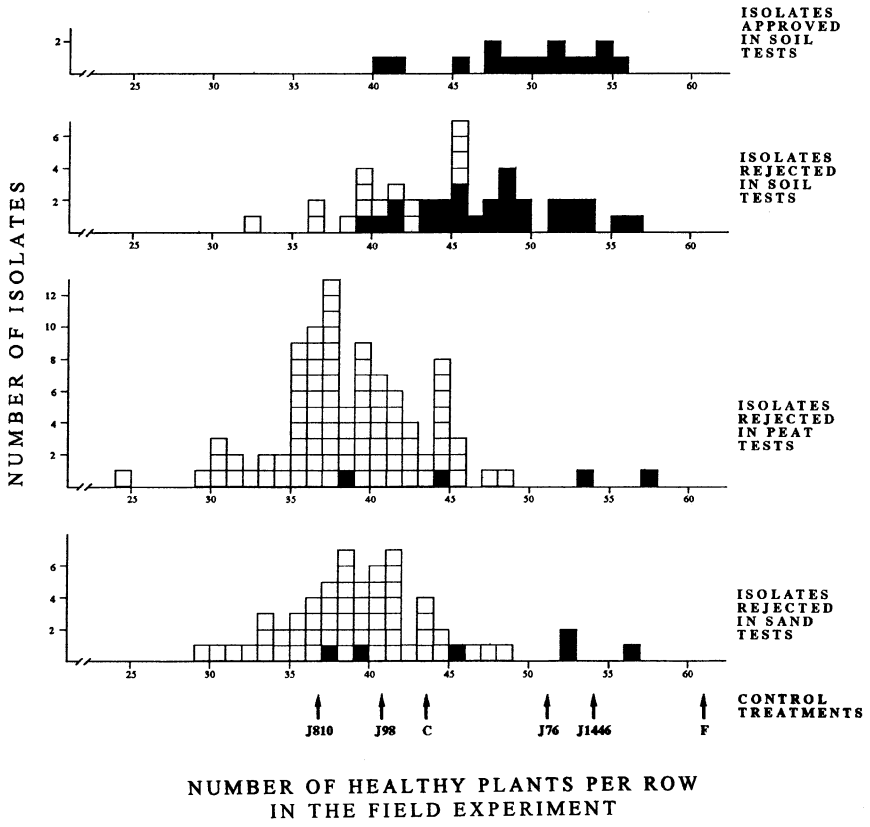

Figure 2. Distributions of the adjusted average numbers of healthy plants in the field experiment for isolates that had advanced to the different stages in the screening process. ■ = *Gliocladium* -isolates □ = isolates belonging to other genera. **C** = untreated control; **F** = fungicide control; J76, J98, J810 and J1446 = control isolates.

and the root isolates belonging to any single genus (data not presented). The fungicide control gave higher number of healthy sprouts than any of the biocontrol treatments (Figure 2).

The incomplete block design increased the precision of the field experiment. The relative efficiency over the randomized complete block design was 141%. This means that by using randomized blocks and 8 replications per treatment somewhat less precise comparisons between treatments would have been possible than was the case with 6 replications. The average standard error of differences between treatment means was 4.56 healthy sprouts per plot.

Discussion

It was not unexpected that *Gliocladium* spp. were effective in controlling seed-borne *F. culmorum* as they are known to be potential biocontrol agents of various plant pathogens and produce antifungal metabolites (Papavizas, 1985). Antagonism through antibiosis is thought to be a particularly promising strategy for biological protection of seeds (Baker, 1990). *Gliocladium roseum* Bain. can effectively control root rots and seedling diseases caused by *Fusarium* spp. (Castejón-Muñoz and Oyarzun, 1995; Knudsen et al., 1995).

In addition to *Gliocladium* spp., effective fungal antagonists of fusaria causing root diseases have been found from the genera *Trichoderma* (Harman et al., 1989; Kommedahl and Brock, 1954; Lynch et al., 1987; Sivan and Chet, 1986], *Idriella* (Knudsen et al., 1995, Spiegel and Schönbeck, 1991), *Fusarium* (Oyarzun et al., 1994), *Aspergillus, Nigrospora, Penicillium* (Kommedahl and Brock, 1954) and *Chaetomium* (Chang and Kommedahl, 1968). In our field experiment 94 isolates represented these genera but none compared with the best *Gliocladium* isolates for biocontrol activity. The methods used may in part explain the one-sidedness of the group of fungi identified as

Table 3. The relationships between biocontrol efficacy of fungal isolates in the field experiment and in two of the screening tests done under controlled conditions, expressed as linear correlation between number of healthy plants in them. Values of coefficient of correlation are presented separately for all the fungi, for *Gliocladium* isolates alone, and for all isolates except *Gliocladium* spp. For the sand test, the correlation could not be calculated because the least effective isolates were excluded from the field experiment

	The relationship between the field experiment and			
	Peat test		Soil test	
	coefficient of correlation	n[1)]	coefficient of correlation	n
all the fungi	0.58	150	0.53	58
Gliocladium spp.	−0.016	47	0.32	43
all the fungi except *Gliocladium* spp.	0.20	103	0.006	15

1) sample size, number of isolates tested both in the field experiment and in the screening tests.

promising biocontrol agents. Both the procedures used for isolating the test organisms from soil yielded fungi which grew fast at relatively low temperatures. Also, preparation of the inocula of the test fungi intentionally favoured the fungi which sporulated strongly on agar cultures. It is obvious however, that the genus *Gliocladium*, in particular, contains disproportionate numbers of species/isolates capable of growing actively and competing with other micro-organisms on germinating seeds

There are few reports on the applicability and efficiency of *in vivo* screening under controlled conditions to select biocontrol agents for use outdoors. Duczek (1994) studied screening of microbes against soil-borne *Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur causing common root rot on cereals. He used wheat and barley as test plants and observed the disease symptoms at the seedling stage in the greenhouse assays and after flowering in the field tests. He found no statistically significant relationship between the disease ratings and opted to do all screening in the field from thereon. Kommedahl and Windels (1978) tested bacteria and fungi for use as biocontrol agents against soil-borne pathogens of pea (*Pisum sativum* L.). In field tests they confirmed the results obtained under controlled conditions only for a year when the disease occurred early in the season.

In the present study the target pathogen was seed-borne and the wheat seed used was also heavily inoculated with it both for the greenhouse tests and the field experiment. The superiority of the fungicide control treatment in the field experiment demonstrates the severity of the pathogen inoculation. As a matter of fact, the seedlings were more diseased than ever in practice. In several field experiments run in 1991–95, where the use of seed naturally infected by different fusaria led to clear seedling blight, the best biocontrol fungi controlled the disease at least as effectively as triadimenol + imazalil (E. Teperi, unpubl). Because of the heavy pathogen inoculation, clear symptoms could be detected on young seedlings in all the tests. Also, the possible prevention of the disease development by different seed treatments had to occur in an very early stage to be observed. Consequently, satisfactory agreement between the results from controlled and uncontrolled conditions was achieved. Likewise, the rapidness of the biocontrol activity required explains why the ability of the tested isolates to colonise root surfaces turned out not to be a significant property. As the proportion of *Gliocladium* spp. was clearly larger for the soil isolates than for the root isolates, the additional effort to obtain root isolates appeared to be wasted.

The concept behind the screening procedure was to ensure the reliability of observations by moving towards a more natural environment at each consecutive testing stage. To some extent this was achieved as the peat test was better than the sand test to predict the field performance of the candidate fungi and the soil test was the best one for the *Gliocladium* spp. However, the two first screening tests were quite effective in separating *Gliocladium* isolates from the rest. When running the screening routine, considerable work was saved by relying on results from these two first tests and not evaluating all the isolates in field soil. As the soil test can't be used for further selection, screen-

ing should be continued by testing all the *Gliocladium* isolates in the field.

The test conditions clearly had an impact on the ability of *Gliocladium* isolates to demonstrate their potential biocontrol activity. Each of the screening tests reported in this paper rejected some *Gliocladium* isolates that performed well in the field experiment. On the other hand, some of them effectively prevented the development of foot and root rot in the greenhouse, but did not do so under natural conditions. It is therefore quite possible that if the field experiment presented in this paper had been done in another field or in another year, different *Gliocladium* isolates would have given the best results. Thus, the future field screenings will have to be extensive enough to identify those *Gliocladium* isolates which most consistently reduce seedling blight in various environments and at diverse locations. The incomplete block designs will be helpful in this work.

Acknowledgements

We are grateful to Asko Hannukkala for his help in the identification of fungi. We also thank Hanna Kortemaa for her critical comments on the manuscript and Dr. Jonathan Robinson for correcting the language. This work was financially supported by Finnish Ministry of Agriculture and Forestry, NJF -project 75.

References

Baker R (1990) An overview of current and future strategies and models for biological control. In: Hornby D and Cook RJ (eds) Biological Control of Soil-borne Plant Pathogens. (pp.375–388) CAB International, Wallingford

Castejón-Muñoz M and Oyarzun PJ (1995) Soil receptivity to *Fusarium solani* f.sp. *pisi* and biological control of root rot of pea. Eur J Plant Pathol 101: 35–49

Chang I-pin and Kommedahl T (1968) Biological control of seedling blight of corn by coating kernels with antagonistic microorganisms. Phytopathology 58: 1395–1401

Cochran WG and Cox GM (1957) Experimental designs. John Wiley & Sons, New York

Daamen RA, Langerak CJ and Stol W (1991) Surveys of cereal diseases and pests in the Netherlands. 3. *Monographella nivalis* and *Fusarium* spp. in winter wheat fields and seed lots. Neth J Pl Path 97: 105–114

Domsch KH, Gams W and Anderson T-H (1980) Compendium of soil fungi. Academic Press, London

Duczek LJ (1994) Relationship between a greenhouse and field assay for biological control of common root rot of spring wheat and barley. Can Plant Dis Surv 74: 135–140

Harman GE, Taylor AG and Stasz TE (1989) Combining effective strains of *Trichoderma harzianum* and solid matrix priming to improve biological seed treatments. Plant Disease 73: 631–637

Homeyer PG, Clem MA and Federer WT (1947) Punched card and calculating machine methods for analyzing lattice experiments including lattice squares and the cubic lattice. Iowa State College of Agriculture and Mechanic Arts; Research Bulletin 347

Kempf H-J and Wolf G (1989) *Erwinia herbicola* as a biocontrol agent of *Fusarium culmorum* and *Puccinia recondita* f.sp. *tritici* on wheat. Phytopathology 79: 990–994

Knudsen IMB, Hockenhull J and Jensen DF (1995) Biocontrol of seedling diseases of barley and wheat caused by *Fusarium culmorum* and *Bipolaris sorokiniana*: effects of selected fungal antagonists on growth and yield components. Plant Pathology 44: 467–477

Kommedahl T and Brock TD (1954) Studies on the relationship of soil mycoflora to disease incidence. Phytopathology 44: 57–61

Kommedahl T and Windels CE (1978) Evaluation of biological seed treatment for controlling root diseases of pea. Phytopathology 68: 1087–1095

Lynch JM, Ebben MH, Whipps JM, Claydon N, Magan N, Hand P. Budge SP, Moyneux SA, Ridout CR, Coley-Smith JR and Hanson JR (1987) Biological control of soil-borne diseases. In: Annual Report Glasshouse Crops Research Institute, 1985. Littlehampton, UK. (pp. 140–146)

Nash SM and Snyder WC (1962) Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52: 567–572

Oyarzun PJ, Postma J, Luttikholt AJG and Hoogland AE (1994) Biological control of foot and root rot in pea caused by *Fusarium solani* with nonpathogenic *Fusarium oxysporum* isolates. Can. J. Bot. 72: 843–852

Papavizas GC (1985) *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Annual Review of Phytopathology 23: 23–54

Sivan A and Chet I (1986) Biological control of *Fusarium* spp. in cotton, wheat and muskmelon by *Trichoderma*. J. Phytopathology 116: 39–47

Skou JP (1991) Spireskadende svampe og deres betydning i vårbyg i Danmark. Tidsskr. for Landøkonomi 3: 123–141

Spiegel D and Schönbeck F (1991) Occurrence and significance of *Microdochium bolleyi* in barley roots (Abstr). In: Breemster ABR, Bollen GJ, Gerlach M, Ruissen MA, Schippers B and Tempel A (eds) Proceedings of the First Conference of the European Foundation of Plant Pathology (p. 215) Elsevier, Amsterdam

Tahvonen R and Teperi E (1995) Biological control of *Fusarium culmorum* of wheat with fungus antagonists isolated from Finnish soils. In: Manka M, (ed) Environmental biotic factors in integrated plant disease control, 3rd conference of EFPP (pp. 541–544) The Polish Phytopathological Society, Poznan

Uoti J (1976) The effect of five *Fusarium* species on the growth and development of spring wheat and barley. Ann. Agric. Fenn. 15: 254–262

Uoti J and Ylimäki A (1974) The occurence of *Fusarium* species in cereal grain in Finland. Ann. Agric. Fenn. 13: 5–17

Wiese MV (1987) Compendium of wheat diseases. 2nd ed. American Phytopathological Society, APS Press, St. Paul, Minnesota.