

Effect of seed dressing treatment of *Streptomyces griseoviridis* on barley and spring wheat in field experiments

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The effect of seed dressing with the antagonist *Streptomyces griseoviridis* on root rots and yields of wheat and barley was studied in field experiments. In long-term field experiments, where different levels of soil-borne inoculum of root rots were maintained with different crop sequences, seed treatment with the antagonist increased yields slightly on average over all experimental years. However, variations between years, crops and crop sequences were considerable. The highest yield increases were in excess of 600 kg/ha, whilst treatment occasionally resulted in slight yield losses. In experiments in which seed naturally infested with *Fusarium* spp. was used, seed treatment with *S. griseoviridis* increased yields of wheat but not those of barley. Seed dressing with an organomercurial fungicide resulted in higher yield increases than the biopreparate.

Key words: biological control, *Bipolaris sorokiniana*, *Fusarium* spp., *Gaeumannomyces graminis*, Mycostop, crop rotation,

Foot and root rot diseases are a world wide problem in intensive cereal crop production. In Finland common root rot (*Fusarium avenaceum* (Corda ex Fr.) Sacc., *F. culmorum* (W. G. Sm.) Sacc., *Bipolaris sorokiniana* (Sacc.) Shoem.) and take-all (*Gaeumannomyces graminis* (Sacc.) v. Arx & Olivier) are widespread throughout the cereal growing area (Mäkelä and Parikka 1980).

Yield losses caused by common root rot may exceed up to 10% (Uoti 1976, Kurppa 1985). The damage is most severe in cereal monoculture. Dry growing seasons favour infection by *Fusarium* species (Wiese 1987). Take-all is extremely damaging in cereal monoculture on light soils

during wet seasons, when 90% crop losses have been reported (Yarham 1981).

Common root rot fungi are seed- and soil-borne. Seed-borne inoculum can be controlled by chemical seed dressing (Uoti 1979, Kurppa 1985, Wiese 1987). There is, however, no effective chemical control against soil-borne inoculum of common root rot and truly soil-borne take-all. Soil-borne root rots are mainly controlled by diverse crop rotations and other cultural practices (Yarham 1981, Wiese 1987).

Much effort has gone into investigation of the biocontrol of soil-borne root-rots of cereals. Numerous soil-inhabiting micro-organisms, e.g.

Trichoderma and *Gliocladium* fungi and *Streptomyces* bacteria, have been shown to prevent growth of root rot pathogens on agar and to suppress disease severity in bioassays and field trials (Domsch and Gams 1968, Uoti 1976, Harman and Taylor 1990). Root inhabiting fluorescent *Pseudomonas* bacteria have demonstrated great potential in the control of take-all (Bowen and Rovira 1976, Weller and Cook 1983, Ryder et al. 1990).

Seed dressing with spores or mycelia of the antagonist has proved to be a reliable way of controlling a number of diseases (Mangenot and Diem 1979, Papavizas and Lewis 1980). The antagonist applied to seeds protects them from infection and may also colonize the rhizosphere. The activity of the antagonist can be intensified by different additives, e.g. nutrients (Harman and Taylor 1990).

Streptomyces griseoviridis Anderson et al. has been successfully used for controlling seed-borne diseases of cruciferous plants (Tahvonen and Avikainen 1987) and numerous soil-borne diseases (Tahvonen 1982, 1988). Preliminary studies of Tahvonen and Avikainen (1990) indicated that the antagonist also has potential for controlling common root, *Fusarium* spp. and *B. sorokiniana*, of cereals. This study investigates the efficacy of *S. griseoviridis* seed dressing on barley and spring wheat against foot rot diseases in field conditions and its effect on spring wheat and barley yields.

Material and methods

Crop-rotation experiments

The performance of the antagonist *Streptomyces griseoviridis* in the field was studied in two crop-rotation experiments, with rotations maintaining different levels of soil-borne inoculum of root-rot pathogens, *Fusarium* spp., *Gaeumannomyces graminis* and *B. sorokiniana*.

The five year crop rotation experiment conducted at Helsinki (Viikki) in 1982–86 consist-

ed of four crop sequence types containing 100%, 75%, 50% or 25% wheat (cv. Ruso) or barley (cv. Kustaa). Both barley and wheat were grown in monoculture (100%), which was interrupted every three years with fallow (75%), barley and wheat were exchanged every other year for oats, turnip rape and field bean (50%) and barley and wheat were grown in four year rotation with oats, turnip rape and field bean (25%) (Hannukkala 1985). At Jokioinen, a four-year barley (cv. Pokko) and wheat (cv. Luja) monoculture together with barley and wheat grown in one, two and three consecutive years after grass ley maintained three, two and one years, respectively were included in the crop rotation experiment in 1985–87.

Both experiments consisted of two sub-trials, one with barley and the other with wheat as the test crop. The experimental design was a split-plot model with four replications. Crop sequences were placed in the main plots and the seed treatments in subplots. Plot size at Helsinki was 8 m² and at Jokioinen 10 m².

Seed dressing experiments

The effect of *S. griseoviridis* on seed-borne root-rot diseases was studied in 1984–86. A set of field experiments using artificially infected seed was carried out at three locations, Jokioinen, Kotkaniemi and Mietoinen, in 1984–85. Two barley seed lots (cv. Pokko) were inoculated by soaking in aqueous suspension of two pathogens, *Fusarium culmorum* and *B. sorokiniana* as described by Tahvonen and Avikainen (1990). Seed lots heavily contaminated with common root rot pathogens were screened in pot experiments in 1986 (Tahvonen and Avikainen 1990). Four seed lots of barley (cvs. Arra, Etu, Pokko and Potra) and three seed lots of wheat (cvs. Luja and Tapio) were selected for the field test. A split-plot experimental design with four replications, where the main plots were infected seed lots and the sub-plots were seed dressings, was used in both sets of experiments. The plot size was 10 m².

Biocontrol agent and seed treatments

The *S. griseoviridis* used as a biocontrol agent in all studies was originally isolated from Finnish peat (Tahvonen 1982). In 1982–84 the seed was treated with the spore suspension of the antagonist in sterile water as described by Tahvonen (1982). In 1985–87 the seed was treated with a powdery product, "Mycostop" Kemira Oy, Finland, containing mycelium and spores of *S. griseoviridis* at a minimum of 10^8 cfu per g (Tahvonen and Avikainen 1987).

Mycostop was applied to seeds by shaking them with the powdery product in the same manner as standard fungicides. The standard dose was 3 g Mycostop/kg seed. An additional dose of 10 g Mycostop/kg seed was included in the crop rotation experiment at Jokioinen and the experiment with naturally infested seed. An untreated control was included in all experiments. Standard organomercury treatment (Täyssato, Kemira Oy or Ceresan, Berner Oy (metoxyethylmercurychloride a.i.) 2g/kg seed) was used in all experiments except the crop-rotation experiment at Helsinki.

Other trial methods and analyses

The soil type in all experiments was heavy clay with the exception of those at Kotkaniemi, where it was fine sand. Soil acidity ranged from pH 5.5 to 6.5. Trials received 80 kg N/ha in the form of compound fertilizer. The type of fertilizer chosen depended on the nutritional status of each individual experimental field. Commercial herbicides were used for weed control, depending on the dominant weed population in the field.

Growing seasons during the study were extremely variable. Sowing dates varied from the last week of April to the third week of May and harvesting dates from the fourth week of August to the third week of September. The beginning of the growing season in 1982 and 1985 was exceptionally cool and moist. In 1983, 1984 and 1986 the spring was warm and, especially in

1984, very dry. The 1987 Growing season was one of the coldest this century.

The stem bases of the plants were rated for disease at growth stage 20–30 (Zadoks et al. 1974) from a sample of 25–50 plants/plot. At Helsinki an additional sample was taken at growth stage 75 and both stem bases and roots were rated for the disease. The disease data were studied by the loglinear modelling available in the SAS CATMOD procedure. For modelling the original disease ratings were grouped into three symptom classes (healthy, moderate and severe). Disease ratings are not presented in the results, because there were no statistically significant differences between treatments.

The yield data were analysed by modifications of the analysis of variance according to each experimental design using the SAS GLM procedure. Before the analyses of variance the data were studied by the SAS UNIVARIATE procedure to confirm that the data fulfilled the assumptions of analysis of variance. No transformations were needed for the data. The data were further studied by the Tukey HSD test. Statistical significances are expressed by asterixes (***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; NS, $P > 0.05$) and/or using different letters to show individual means belonging to the different group at the 0.05% significance level.

Results and discussion

Seed treatment with *S. griseoviridis* suspension increased yields considerably in the crop rotation experiment at Viikki in 1982. The maximum yield increase in barley was about 640 kg/ha and in wheat 310 kg/ha. The high yield increases encouraged us to continue testing and to start new field studies. The following years however, showed that neither the suspension nor the powdery product of the antagonist could provide any consistent positive effect on yield. On average over years and crop sequences, the antagonist increased barley yields slightly but caused minor losses to wheat yields (Table 1).

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Table 1. Effect of *Streptomyces* seed treatment on wheat and barley yields in a five year crop rotation experiment at Viikki in 1982–86.

Crop sequence	Yield increase/decrease kg/ha					mean	Mean yield of untreated control
	1982	1983	1984	1985	1986		
Wheat							
Monoculture	311	-146	58	-110	85	40	3650
75% wheat	117	-223	-130	-85	-56	-75	4108
50% wheat	-88	-298	15	5	-146	-102	4155
25% wheat	-383	-110	-70	9	-13	-113	4017
Mean yield of untreated seed	4831	5021	5066	2417	2949		
Barley							
Monoculture	289	37	174	-61	-264	35	4358
75% barley	638	-102	83	-64	-219	68	4384
50% barley	328	234	11	124	12	142	4435
25% barley	481	-180	-6	-174	-92	6	4745
Mean yield of untreated seed	4889	5703	5145	3397	3090		
F-values:							
			Wheat		Barley		
	Crop sequence (Cs)		14.73***		16.41***		
	Year (Y)		1354.61***		848.25***		
	Treatment (T)		10.97**		10.24**		
	Y x Cs		10.58**		6.17**		
	Y x T		1.66 NS		7.21**		
	Cs x T		1.12 NS		1.85 NS		

In the crop rotation experiment at Jokioinen, none of the seed treatments had a statistically significant effect on barley yields. Ley as the precrop decreased yields in 1986 and 1987 (Table 2). There were however, no statistically significant differences in visible stem base symptoms between seed treatments or between crop sequences.

In wheat all seed treatments increased yields as compared with the untreated control. Mercury treatment always gave higher yield increases than *Streptomyces*-treatment. Ley as a precrop decreased wheat yields each experimental year. The highest yield increases due to seed treatments were obtained after ley as the precrop (Table 3). No statistically significant differences in visible stem base symptoms were detected between seed treatments or crop sequences.

The yield decrease after ley was probably caused by a biotic factor, which could be controlled to some extent by seed treatments. Ley probably increased the inoculum of some 'minor' pathogens (Salt 1979) e.g. snow mould, *Microdochium nivale* (Ces. ex Berl. & Vogl.) Samuels & Hallet, an agent that typically kills grasses in winter, may cause mild symptoms in both barley and wheat (Wiese 1987).

The variations in the effects of seed treatments between years and experiments indicate that establishment of the antagonist is highly dependent on environmental factors, e.g. soil moisture and temperature, as was also stressed by Bowen and Rovira (1976), Papavizas and Lewis (1980) and Harman and Taylor (1990). The yield increases obtained with seed treatment despite insignificant differences in disease ratings may

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Table 2. Effect of *Streptomyces* and mercury seed treatment on barley yields in a four year crop rotation experiment at Jokioinen in 1985–87.

Year	Precrop	Untreated control kg/ha	Increase/decrease kg/ha		Mercury	Mean kg/ha	F value
			<i>Streptomyces</i> 3 g/kg	10 g/kg			
1985	ley	4427	35	19	256	4505 A	precrop 2.53 seed tr. 0.69 prec. x seed. 0.80
	barley	4643	67	39	19	4674 A	
	mean	4535 a	51 a	29 a	138 a	4589 A	
1986	ley + ley	3430	40	63	50	3468 A	precrop 9.36* seed tr. 1.27 prec. x seed. 0.27
	barley + ley	3177	126	126	313	3318 A	
	barley + barley	3958	129	56	186	4051 B	
	mean	3522 a	98 a	82 a	183 a	3612	
1987	ley + ley + ley	3048	244	-168	174	3111 AB	precrop 3.92* seed tr. 0.18 prec. x seed. 1.92
	barley + ley + ley	3306	-521	-50	-293	3091 AB	
	barley + barley + ley	2398	238	-78	248	2500 A	
	barley + barley + barley	3266	100	163	30	3340 B	
	mean	3005 a	15 a	-33 a	40 a	3010	

Table 3. Effect of *Streptomyces* seed and mercury treatment on spring wheat yield in a four-year crop rotation experiment at Jokioinen in 1985–87.

Year	Precrop	Untreated control kg/ha	Increase/decrease kg/ha		Mercury	Mean yield kg/ha	F value
			<i>Streptomyces</i> 3 g/kg	10 g/kg			
1985	ley	3466	528	342	860	3899 A	precrop 17.26** seed tr. 30.99 *** prec. x seed. 1.54
	wheat	3986	433	437	632	4362 B	
	mean	3726 a	480 b	390 b	746 c		
1986	ley + ley	2714	80	219	423	2894 A	precrop 14.82*** seed tr. 18.97*** prec. x seed. 0.50
	wheat + ley	2682	66	149	439	2845 A	
	wheat + wheat	3150	35	-4	352	3245 B	
	mean	2848 a	61 a	121 a	405 b	2995	
1987	ley + ley + ley	1670	416	475	546	2029 A	precrop 14.36*** seed tr. 0.70 prec. x seed. 1.71
	wheat + ley + ley	2365	119	50	-39	2398 AB	
	wheat + wheat + ley	2503	106	-115	171	2543 B	
	wheat + wheat + wheat	3218	-163	-1	-278	3108 C	
	mean	2439 a	119 a	102 a	100 a	2519	

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Table 4. Effect of *Streptomyces* and mercury treatment of uninoculated seeds(I) or of barley seeds inoculated with *Fusarium culmorum* (II) or *Bipolaris sorokiniana* (III) on yield at three different experimental places.

Location	Seed treatment	Yield and yield increase/decrease kg/ha			
		I	II	II	Mean
1984					
Jokioinen	Untreated	5611	5689	5639	5646
	Streptomyces	-192	-213	-159	-188
	Mercury	-17	-17	-106	-50
Kotkaniemi	Untreated	2528	2378	2911	2606
	Streptomyces	+230	+314	+198	+247
	Mercury	+234	+295	+31	+187
Mietoinen	Untreated	4660	4710	4710	4693
	Streptomyces	-120	+130	+10	+7
	Mercury	+90	+30	+130	+83
1985					
Jokioinen	Untreated	6232	6519	6209	6320
	Streptomyces	-52	-310	+301	-20
	Mercury	+95	+195	+606	+299
Kotkaniemi	Untreated	6492	6193	6100	6262
	Streptomyces	-383	+177	-33	-80
	Mercury	+3	+185	+422	203
Mietoinen	Untreated	4560	4330	4460	4450
	Streptomyces	-100	+200	+140	+80
	Mercury	+150	+230	+160	+180
Mean	Untreated	5013	4970	5005	4996
	Streptomyces	-71	+3	+145	+25
	Mercury	+93	+153	+207	+151

F values: No significant differences between treatments

be due to the ability of the antagonist to stimulate plant growth. Numerous soil and rhizosphere bacteria are known to stimulate crop growth (Gerhardson et al. 1985). Certain *Actinomycetes* belonging to the genus *Streptomyces* have been reported to promote wheat growth (El-Shan-shoury 1989).

In experiments with artificially infested seed, inoculation with *F. culmorum* or *B. sorokiniana* caused little increase in disease incidence or decrease in yields as had been the case in preliminary pot experiments (Tahvonen and Avikainen 1990). Seed dressings with mercury or *Streptomyces* had no statistically significant effects on yields, though both treatments caused slight yield increases on average in all experiments (Table 4).

In 1986, when naturally infested seed was used in the experiments, treatment with Mycostop increased wheat yields by 130 kg/ha and with the organomercurial compound by 580 kg/ha. Seed treatments had no statistically significant effects on barley yields (Table 5). *Fusarium* is known to be more destructive to wheat than barley, and healthy barley plants in the stand have a high capacity to compensate for the negative effects of diseased plants by more vigorous growth (Wiese 1987). These results indicate that seed treatment with either fungicide or biopesticide is more important in wheat than in barley for the control of common rot caused by *Fusarium* spp. The studies of Kurppa (1985) have shown that fungicides can significantly reduce

Table 5. Effect of seed treatment with *Streptomyces* and mercury on yield of four barley seed lots and six wheat seed lots in 1986 at Jokioinen.

Treatment	Wheat		Barley	
	Yield kg/ha	Range	Yield kg/ha	Range
Untreated	4177 a	3705 - 4446	4347 a	2903 - 5291
Mycostop 3g/kg	+134 b	+12 - +267	+10 a	-355 - +234
Mycostop 10g/kg	+129 b	+32 - +224	+47 a	-116 - +193
Mercury	+577 c	+383 - +925	-44 a	-247 - +149

Different letters show individual means belonging to the different group at the 0.05 % significance level.

seed surface infection by *B. sorokiniana* but that they do not provide reliable control when seed is heavily infested.

Some antagonists have potential in the biological control of root rots of cereals. *S. griseoviridis* was originally isolated from peat, and the biopreparate Mycostop was developed to control diseases of greenhouse crops grown in a controlled environment (Tahvonen and Avikainen 1987, Lahdenperä 1987, 1992). *S. griseoviridis* has shown potential to control eyespot disease of cereals (Clarkson and Lucas 1993) and ear blight of wheat caused by *Fusarium* spp. (Lahdenperä et al. 1992). To control truly soil-borne diseases in heavy clay soils in a cool cli-

mate it is essential to look for microbes adapted to the soil environment where they will actually be used.

In conclusion, wheat yields can be increased by seed dressings more efficiently than can barley yields. Both fungicides and biopesticides can also affect soil-borne infection when the infection pressure is low. The *S. griseoviridis* prepreparate gave some protection against common root rot and was able to increase yields. However, chemical control with an organomercurial compound resulted in yield increases two to three times higher than those with the biopreparate tested.

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SELOSTUS

Streptomyces griseoviridis siemenpeittauksen vaikutus ohraan ja kevätvehnään kenttäkokeissa

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Streptomyces griseoviridis -sädesientä ja siitä tehtyä jauhemaista Mycostop-valmistetta testattiin vehnän ja ohran siemen- ja maalevintäisten tautien torjumiseksi. Siemenet kasteltiin mikrobin itiösuspensiolla tai ravistettiin mikrobijauheen kanssa peittauslaitteessa. Peittauksen tehoa selvitettiin luontaisesti ja keinotekoisesti saastutetuilla siemenillä ja erilaisissa esikasvikokeissa. Monipuolisessa kiertoviljelykokeessa oli esikasveina eri pituisia aikoja kaura, rypsi ja härkäpapu. Toisessa esikasvikokeessa oli esikasvina nurmi eri pituisia aikoja. Kokeet tehtiin vuosina 1982–1987 Viikissä, Kotkaniemessä, Mietoissa ja Jokioissa.

Monipuolisessa vuoroviljelykokeessa *Streptomyces*-peittaus lisäsi ohrasatoja, mutta vehnällä ei keskimäärin saatu sadonlisäyksiä. Vuosien väliset vaihtelut olivat suuria. Vuoroviljelykasvit lisäsivät vehnän satoa, mutta ohralle yksipuolinen viljely ei sanottavasti alentanut satoa suhteessa vuoroviljelyyn.

Nurmi ohran ja vehnän esikasvina alensi satoja, voimakkaammin vehnällä kuin ohralle. Siemenen peittaus lisäsi satoja varsinkin vehnällä, kun esikasvina oli ollut nurmi. Kemiallinen elohopeapeittausaine antoi suuremman sadonlisän kuin *Streptomyces*-peittaus. Parhaimmat sadonlisät olivat 860 kg/ha.

Siemenlevintäiset *Fusarium*- ja *Bipolaris*-sienet eivät vaikuttaneet ohran satoihin, jolloin peittauslakaan ei saatu sadonlisäyksiä. Vehnällä siemenen peittaus lisäsi satoja, kun siemen oli luontaisesti *Fusarium*-sienten saastuttamaa. Kemiallinen elohopeapeittaus antoi paremman tuloksen kuin biologinen peittaus.

Tehdyt kokeet osoittivat, että biologisella *Streptomyces*-peittauksella voidaan saada sadonlisäyksiä, mutta kemiallisella peittauksella tulos on pelto-oloissa aina parempi. Tämän takia pelto-oloja varten tarvitaan tehokkaampia antagonisteja, jos viljan viljelyssä halutaan käyttää biologista torjuntaa.