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Performance of the *Pseudomonas chlororaphis* biocontrol agent MA 342 against cereal seed-borne diseases in field experiments

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Abstract

The bacterial biocontrol agent *Pseudomonas chlororaphis*, strain MA 342, was tested for activity against a number of cereal seed-borne diseases in a total of 105 field experiments carried out at different locations in Sweden during the years 1991-1996. Bacterial liquid culture was directly applied to pathogen-infested seeds of barley, oats, wheat and rye without additives. The seeds were then dried and sown in field together with fungicide-treated and untreated seeds used as controls. The bacterization controlled seed-borne diseases caused by *Drechslera (Pyrenophora) graminea, D. teres, D. avenae, Ustilago avenae*, *U. hordei*, and *Tilletia caries*, as effectively as guazatine + imazalil, and these effects were consistent over the years and over varying climatic zones. Diseases caused by pathogens like *U. nuda*, soil-borne *T. caries* and *T. contraversa* were not controlled and the bacterization gave less than full effect against diseases caused by *Microdochium (Fusarium) nivale*, and *Bipolaris sorokiniana (Cochliobolus sativus)*. Bacterized seeds could be stored dry for at least two years without losing the disease suppressing effect of the bacterial treatment, when tested in the field.

Introduction

The use of biocontrol agents, or biopesticides, for plant disease control may not be more effective than other control methods, but is commonly encouraged for environmental and other reasons (Cook, 1993; Campbell, 1994). Several bacterial and fungal strains showing disease-suppressing abilities have also been extensively studied to this end (e. g. Hornby, 1990). Developmental work for large scale use has been successfully accomplished for certain of these agents (Harman, 1991; Ryder and Jones, 1990; Lumsden et al., 1995), or is anticipated for others (Köhl et al., 1995; Knudsen et al., 1995). A repeatedly reported problem, however, is the often encountered inconsistency in field trial effects (Weller, 1988; Gerhardson and Larsson, 1991; Hornby et al., 1993; Tahvonen et al., 1995). Another common drawback in developing large scale utilizable biopesticides has been the small range of diseases often possible to control by any single biological agent (Cook, 1993; Campbell, 1994). To overcome these disadvantages, several ways to improve performance of known antagonistic organisms have been envisaged (Dowling et al., 1992; Chet et al., 1993; Moënne-Loccoz et al., 1996). Another strategy has been a continuous screening for new and better agents from natural sources (Leyns et al., 1990; de Bruyne et al., 1991; Knudsen et al., 1997).

In a search for a new and commercially utilizable antagonist in an inter-Scandinavian research project (Knudsen et al., 1997), cereal seed-borne diseases were chosen as suitable targets for screening and testing. These diseases are agriculturally important in Sweden and are commonly controlled chemically by fungicides (Olofsson and Johnsson, 1985), and convenient diagnostic and testing methods are easily available. In this search we isolated a number of bacterial strains from Swedish soils that gave a strong disease-controlling effect when applied to infested cereal seeds. One of these strains, MA 342, gave consistent effects in all the preliminary field experiments carried out, strong inhibition of several diseases, and the seeds treated with this strain could be stored for months without losing the disease controlling effect

Table 1. Listing of pathogens, crops, and cultivars tested, and locality of the field experiments carried out. The last column shows the Table number where results from the different experimental series are given. All barley seed lots are spring barley

Pathogen	Crop tested	Cultivar	Locality of experiments[a]	Results shown in Table No.
Drechslera graminea	6-row barley	Agneta	M, E, B, C, P, AC	2, 3, 4
D. teres	2-row barley	Golf	M, E, R, T, P, C, AC	2, 3, 4, 14
D. avenae	Oats Vital	M, C	2, 3, 4, 14	
Microdochium nivale	Spring wheat	Drabant	M, C	7
M. nivale	2-row barley	Pernilla	C	6
M. nivale	Winter wheat	Kosack	M, N, R, T, E, C, B	8, 9
M. nivale	Winter rye	Danko/Amilo	C, W	8
Bipolaris sorokiniana	2-row barley	Blenheim	B	5
Tilletia caries, seed-borne	Winter wheat	Kosack	M, N, R, T, E, C, B	9
T. caries, seed-borne	Spring wheat	Dragon	N, R	10
T. caries, soil-borne	Spring wheat	Drabant	C	In text
T. contraversa, soil-borne	Winter wheat	Kosack	I	In text
Ustilago nuda	6/2-row barley	Agneta/Pernilla	M, E, C, AC	2, 6
U. avenae	Oats	Svea/Vital	M, N, E, R, C	11, 12, 14
U. hordei	2-row barley	Golf	M, C	13
Stagonospora nodorum[b]	Winter wheat	Forno [c]	Greenhouse	15
Stored MA 342 seed	Barley/oats	Golf/Vital	C	14

[a] Letter denotation of Swedish counties where MA 342 was tested in the field. See map of Sweden in Figure 1.
[b] Only greenhouse test.
[c] Seed lot from Switzerland.

(Hökeberg et al., 1997). Since these characteristics point to feasibility for large scale, practical use of MA 342, a more extensive field testing program of its performance under various plant production conditions was carried out.

We here report on the results obtained in the testing of this strain as a biological control agent in 105 field experiments under various climatic conditions, against several seed-borne and a few soil-borne diseases, and during six growing seasons.

Materials and methods

Pathogens and cereal seeds tested

The pathogens, the crop species and the cultivars tested are listed in Table 1. The naturally infested seed lots used in most field experiments were in each case, as far as possible, highly infested with a single pathogen. Thus, for all tests performed with the pathogens *Drechslera graminea* (Rabenh. ex Schlecht.) Shoemaker, *Drechslera teres* (Sacc.) Shoemaker, *Drechslera avenae* (Eidam) Scharif, *Microdochium nivale* (Fr.) Samuels & I. C. Hallett, *Bipolaris sorokiniana* (Sacc.) Shoemaker, *Ustilago nuda* (Jens.) Rostr. and *Stagonospora nodorum* (Berk.) Castellani & E.G. Germano, we were able to use naturally and highly infested seed lots. For tests with *Ustilago avenae* (Pers.) Rostr. control, we used either artificially or naturally infested seed lots. All the seed lots used in the experiments with seed-borne infestation of *Tilletia caries* (DC.) Tul. and *Ustilago hordei* (Pers.) Lagerh. were artificially infested with 2–4 g crushed bunt spikes per kg seed. The crushed bunt spikes and the seed were mixed in a cement mixer during 3 min. In the experiments with soil-borne infestation of *T. caries*, we placed inoculum (8 l per sq.m) in the sow row above and close to the kernels at sowing. The inoculum was produced by mixing 5 gram of crushed bunt spores with 500 ml of a mixture of equal volumes of sand and humus (Enhetsjord K Normal). The experiments for testing *Tilletia contraversa* Kühn control were placed on naturally infested soil. To boost this soil infestation, about 0.1 g per sq.m crushed spikes infected by *T. contraversa* were spread over the soil surface just after sowing the wheat seed.

Bacterial inoculum production and seed treatment procedures

All inocula were produced from the *Pseudomonas chlororaphis* strain MA 342, as described in Hökeberg et al. (1997). Bacterial cultures grown on a shaker in TSB 50 (Tryptic Soy Broth (Difco Ltd.), 50% strength) for two days (resulting in around 4 x 10^9 colony forming units (cfu) per ml), were mixed with the seeds to be treated in a plastic bag and shaken vigorously for 4-5 min. In most experiments 300 ml bacterial broth was applied per kg seed. This treatment resulted in a bacterial number of approximately 10^7cfu per seed. An exception to this treatment was in the experiments carried out in 1993, when 200 ml bacterial broth per kg seed was regularly used. The seeds were then spread out on a paper for drying over night or longer. Two controls, untreated seeds and fungicide-treated seeds, were included in most of the experiments. For the fungicide controls we used either Panoctine Plus 400 (guazatine 150 g per l, imazalil 10 g per l), Panoctine 400 (guazatine 150 g per l) or Sibutol LS (bitertanol 280 g per l, fuberidazol 18 g per l) depending on the disease to be controlled. The fungicides were applied in standard doses, 4 ml per kg seed for the Panoctine products and 2 ml per kg or 2.68 (*T. contraversa* experiment) ml per kg seed for Sibutol LS. They were applied in ordinary seed treatment equipment (Ziro, Gamac Zweden, Hallsberg, Sweden).

In one experiment seed lots of barley and oats were treated with MA 342 and then stored for up to two years at room temperature.

Field experiment localities and plant growing conditions

The field experiments were situated in the various climatic zones cropped to cereals in Sweden (Figure 1). In Table 1 the localities of the various experiments carried out are denoted by letters. These letters represent counties/localities as shown in the map in Figure 1. The experiments were, where convenient, placed on official experimental stations, i. e. Alnarp (M), Lanna (R), Stenstugu (I), Ultuna (C) and Röbäcksdalen (AC), and others were placed on experimental localities regularly used by the plant breeding company Svalöf Weibull AB, i.e. Landskrona (M), Svalöv (M), Bjertorp (R), Nygård (P) and Kölbäck (E). A few experiments were further placed on private or county experimental farms at Märsta (B), Haga (C),

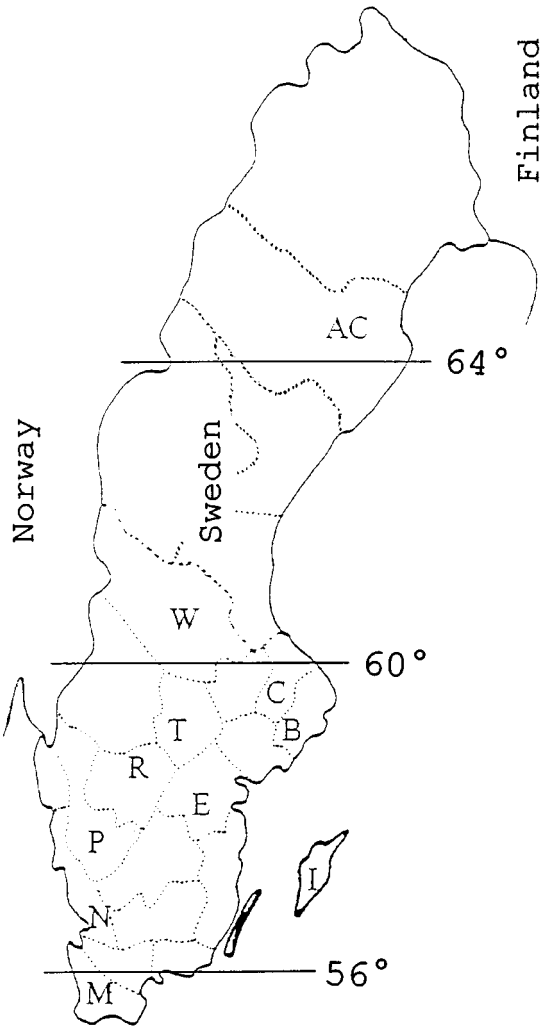

Figure 1. Map showing county letter code designating the different Swedish counties where field experiments with MA 342 were carried out. The daily mean temperature exceeds 5 °C (vegetation period limit) more than 200 days per year for county M and N, 180–200 days for B, C, E, I, P and R, 160–180 days for T and W, and 140-160 days for county AC. The precipitation during this period is 600–650 mm for county N, 450–500 mm for M, P, R and T, 400–450 mm for E and W, 350–400 mm for B, C and I, and 300–350 mm for county AC (Eriksson, 1992).

Böslid (N), Skara (R), Örebro (T) and Hedemora (W). Altogether 105 field experiments were conducted.

The sowing times for the experiments with *T. caries* in spring wheat were always as early as possible in the spring in order to get high bunt infection (Johnsson, 1991). In all the other experiments the sowing times used were normal for each locality.

The soil types at the different experimental localities varied. The predominating soil types were clay

Table 2. Results obtained in field experimental series testing effects of MA 342 against i) *Drechslera graminea* and *Ustilago nuda* in spring barley (cv. Agneta) 1991-1996, ii) *Drechslera teres* in spring barley (cv. Golf) 1991-1996, iii) *Drechslera avenae* in oats (cv. Vital) 1991-1995

Treatment	Yield, kg ha^{-1}	No. of plants m^{-2}	No. of infected plants m^{-2}	Hecto-litre weight, kg	1000-kernel weight, g	No. of *U. nuda* infected spikes m^{-2}
i) *D. graminea* and *U. nuda*						
Control	3110 B[a]	378 AB	51.5 A	62.7 A	32.9 AB	5.1 A
Pan. Plus [b]	3670 A	383 A	4.0 B	62.8 A	32.4 B	5.0 A
MA 342 [c]	3660 A	365 B	6.9 B	62.9 A	33.5 A	4.7 A
No. of analysed plots	76	69	77	6	6	37
ii) *D. teres*						
Control	5050 B[a]	354 B	63.4 A	67.0 B	46.8 A	
Pan. Plus [b]	5300 A	365 A	1.1 B	67.5 A	47.5 A	
MA 342 [c]	5320 A	348 B	3.6 B	67.6 A	47.6 A	
No. of analysed plots	101	112	118	22	22	
iii) *D. avenae*						
Control	4940 A[a]	412 B	33.7 A	56.0 A	31.2 B	
Pan. Plus [b]	4970 A	442 A	12.6 B	54.9 B	31.3 B	
MA 342 [c]	4900 A	425 AB	8.2 B	54.4 B	32.1 A	
No. of analysed plots	40	40	44	5	5	

[a] Means followed by the same letter in each column are not significantly different at P = 0.05 according to Duncan's multiple range test.
[b] Pan. Plus = Panoctine Plus 400, 4 ml kg^{-1} seed.
[c] 200 or 300 ml kg^{-1} seed.

Table 3. Results obtained in single years in field experiments testing effects of MA 342 against i) *Drechslera graminea* in barley (cv. Agneta) 1991–1996, ii) *Drechslera teres* in barley (cv. Golf) 1991-1996, iii) *Drechslera avenae* in oats (cv. Vital) 1991–1995

Treatment	No. of infected plants m^{-2} during the years						
	1991	1992	1993	1994	1995	1996	Mean
i) *D. graminea*							
Control	30.8 A[a]	101.1 A	60.4 A	19.3 A	35.8 A	21.9 A	51.5 A
Pan. Plus [b]	1.5 B	5.2 B	3.8 B	3.9 B	3.8 B	3.3 B	4.0 B
MA 342 [c]	1.0 B	6.9 B	12.0 B	6.8 B	6.5 B	3.6 B	6.9 B
No. of analysed plots	4	20	12	16	17	8	77
ii) *D. teres*							
Control	46.4 A[a]	47.9 A	89.3 A	68.1 A	85.6 A	54.0 A	63.4 A
Pan. Plus [b]	0.3 B	0.7 B	1.1 B	0.9 B	3.7 B	0.2 B	1.1 B
MA 342 [c]	0.5 B	1.0 B	4.9 B	6.8 B	10.3 B	0.6 B	3.6 B
No. of analysed plots	16	20	15	16	19	32	118
D. avenae							
Control	74.0 A[a]	22.1 A	72.5 A	18.8 A	12.6 A		33.7 A
Pan. Plus [b]	32.0 B	12.6 B	15.6 B	6.6 B	6.1 B		12.6 B
MA 342 [c]	17.3 C	2.8 C	20.0 B	4.0 B	7.0 B		8.2 B
No. of analysed plots	4	16	8	8	8		44

[a,b and c] See in Table 2.

Table 4. Results obtained in field experimental series testing effects of MA 342 against i) *Drechslera graminea* in spring barley (cv. Agneta) 1991-1996, ii) *Drechslera teres* in spring barley (cv. Golf) 1991–1996, iii) *Drechslera avenae* in oats (cv. Vital) 1991–1995 at different experimental localities (see Figure 1)

Treatment	No. of infected plants m^{-2} in the counties of					
	M	R	E	C	AC	Mean
i) *D. graminea*						
Control	45.1 A[a]	111.5 A	44.4 A	60.6 A	38.1 A	51.5 A
Pan. Plus [b]	3.8 B	11.5 B	3.0 B	3.1 B	5.8 B	4.0 B
MA 342 [c]	6.4 B	7.7 B	5.0 B	9.1 B	7.7 B	6.9 B
No. of analysed plots	20	4	23	20	10	77
ii) *D. teres*						
Control	55.0 A[a]	79.4 A	76.5 A	55.0 A	15.0 A	63.4 A
Pan. Plus [b]	0.8 B	1.9 B	1.1 B	0.8 B	0.5 B	1.1 B
MA 342 [c]	4.5 B	4.6 B	3.5 B	2.7 B	0.8 B	3.6 B
No. of analysed plots	32	31	23	20	4	118
iii) *D. avenae*						
Control	31.4 A[a]			35.6 A		33.7 A
Pan. Plus [b]	12.9 B			12.5 B		12.6 B
MA 342 [c]	6.9 B			9.3 B		8.2 B
No. of analysed plots	20			24		44

[a],[b] and [c] See in Table 2.

Table 5. Results obtained in a field experimental series, testing effects of MA 342 against *Bipolaris sorokiniana* in barley (cv. Blenheim) during the years 1991, 1993 and 1994

Treatment	Yield, kg ha^{-1}	No. of plants m^{-2}	Brown coleoptiles,%	Hectolitre weight, kg	1000-kernel weight, g
Control	5760 A[a]	276 B	79.4 A	68.3 A	49.8 A
Pan. Plus [b]	5890 A	306 A	30.8 C	68.2 A	48.6 A
MA 342 [c]	5870 A	318 A	50.7 B	68.2 A	49.0 A
No. of analysed plots	12	12	13	2	2

[a],[b] and [c] See in Table 2.

Table 6. Results obtained in 1992 in one field experiment testing effects of MA 342 against *Microdochium nivale* and *Ustilago nuda* in barley (cv. Pernilla)

Treatment		Yield, kg ha^{-1}	No. of plants m^{-2}	Infected coleoptile,%	No. of *U. nuda* infected spikes m^{-2}
Control		5700 A[a]	455 A	86.0 A	15.0 A
Panoctine Plus 400,	4 ml kg^{-1}	5860 A	428 A	51.0 B	20.0 A
MA 342,	300 ml kg^{-1}	5640 A	414 A	50.5 B	31.0 A
No. of analysed plots		4	4	4	4

[a] See in Table 2.

Table 7. Results obtained in field experiments during 1992 and 1994 testing effects of MA 342 against *Microdochium nivale* in spring wheat (cv. Drabant)

Treatment		Yield, kg ha^{-1}	No. of plants m^{-2}	Stand in spring, 0-100	Brown coleoptile,%
Control		4600 A[a]	224 C	80 C	84 A
Panoctine 400,	4 ml kg^{-1}	4520 AB	378 A	97 A	40 B
MA 342,	300 ml kg^{-1}	4310 B	312 B	91 B	51 B
No. of analysed plots		8	12	8	8

[a] See in Table 2.

Table 8. Results obtained in field experimental series testing effects of MA 342 against *Microdochium nivale* in winter wheat (cv. Kosack, 1990/91 and 1994/95) and winter rye (cv. Danko/Amilo, 1990/91 and 1995/96)

Treatment		Winter wheat Yield, kg ha^{-1}	Stands in spring, 0–100	Winter rye Yield, kg ha^{-1}	Stand in spring, 0–100
Control		4320 B[a]	74 B	4210 B	76 B
Panoctine 400,	4 ml kg^{-1}	4620 A	95 A	5280 A	99 A
MA 342,	300 ml kg^{-1}	4310 B	86 AB	4800 AB	85 AB
No. of analysed plots		4	12	4	2

[a] See in Table 2.

soils, with different amount of clay, but sandy and organic soils were also represented. Cultivation practices such as fertilization, weed control etc. were according to official recommended practice in the area concerned.

Disease, emergence, stand and yield registration

In most of the field experiments with spring cereals, the number of germinated plants were recorded by counting the seedlings emerged in about 0.5 sq.m per plot. In most of the field experiments with winter cereals, the stand in the spring was graded on a scale from 0 to 100, where 0 means no stand and 100 means full/optimal stand. In experiments concerning *D. teres* and *D. avenae*, the number of infected plants were recorded when the plants had 3–4 leaves. In experiments with *D. graminea*, the number of infected plants were recorded at heading. In the experiments with *Drechslera* pathogens, the recorded area with infected plants per plot was 1.0 sq.m. In the *Bipolaris* experiments, the percentage of plants with brown coleoptiles was recorded when the plants had 2–3 leaves. In the experiments with different kinds of bunt or smut, the number of infected spikes were recorded 2–3 weeks before maturity. The area counted varied depending on the smut infection level.

In most of the experiments where the yield was recorded, the 1000-kernel weight and hectolitre weight were measured.

In the greenhouse experiment with *S. nodorum*-infested wheat, the frequency of infected leaves and coleoptiles was recorded when the plants had 3–4 leaves. The experimental design for the greenhouse experiment is described by Hökeberg et al. (1997).

Experimental design and statistical analysis

All field experiments were performed in a randomized block design. Usually, we used four replications, but in a few cases only three replications were used. The plot size varied from about 8 sq.m in the bunt experiments to about 25 sq.m in those experiments where the yield was measured. The statistical analyses are based on the single plot value for the yield, the number of germinated plants, the stands in spring and autumn and the frequency of infected plants, coleoptiles or spikes. For the variables 1000-kernel weight and hectolitre weight, the analyses are based on a pooled value from all the plots values for each treatment in every separate experiment. Differences between means are tested with Duncan's multiple range test, P = 0.05. Means followed by the same letter are not significantly

Table 9. Results obtained in a field experimental series testing effects of MA 342 against seed-borne *Tilletia caries* and *Microdochium nivale* in winter wheat (cv. Kosack) during the years 1992, 1993, 1995 and 1996

Treatment	No. of infected spikes m^{-2} during the years					Stand, 0–100	
	1992	1993	1995	1996	Mean	autumn	spring
Control	69.0 A[a]	133.8 A	56.2 A	105.1 A	75.2 A	88.3 B	73.5 B
Panoctine 400, 4 ml kg^{-1}	6.5 B	30.0 B	5.8 B	9.6 B	9.2 B	93.3 A	84.6 A
MA 342 [b]	0.0 B	20.0 B	2.1 B	4.6 B	4.3 B	93.3 A	77.6 B
No. of analysed plots	4	4	22	7	37	18	26

[a] See in Table 2.
[b] 200 or 300 ml kg^{-1} seed.

Table 10. Results obtained in two field experiments testing effects of MA 342 against seed-borne *Tilletia caries* in spring wheat (cv. Dragon) during 1995

Treatment		No. of infected spikes m^{-2}	Stand, 0–100
Control		10.0 A[a]	100 A
Panoctine 400,	4 ml kg^{-1}	1.1 B	100 A
MA 342,	300 ml kg^{-1}	0.2 B	100 A
No. of analysed plots		8	8

[a] See in Table 2.

different from each other. All analyses with percent figures are done after arcsine transformation.

Results

The results from the disease readings (frequency of infected plants, spikes/panicles or coleoptiles), registration of emergence, stand, yield, 1000-kernel weight and hectolitre weight for the different experiments carried out are given in Tables 2 to 15. The diseases tested and locality of testing are listed also in Table 1.

MA 342 applied as a biopesticide has shown as good disease controlling effect against *D. teres* in barley and *D. avenae* in oats (Tables 2, 3, 4 and 14), against *D. graminea* in barley (Tables 2, 3 and 4), against *U. hordei* in barley (Table 13) and against seed-borne *T. caries* in wheat (Tables 9 and 10) as the fungicides tested. The effect of MA 342 against *U. avenae* in oats was significantly better than of the tested fungicide and the non-treated control (Tables 11, 12 and 14). In the years 1991 and 1992 (Table 3) the effect of MA 342 against *D. avenae* was significantly better than the fungicide effect; and in the years 1993–1996 the effects were at the same level as the fungicidal effect.

The effect of MA 342 against *D. teres* was still very good after storing MA 342 treated seeds for one and even for two years, and against *D. avenae* and *U. avenae*, after storing MA 342 treated seeds for one year (Table 14). The stability of MA 342 over the years is shown in Tables 3, 9 and 11 and at different localities in Table 4.

The disease controlling effect of MA 342 was weaker than the fungicidal effect against *M. nivale* in barley, wheat and rye (Tables 6 to 9) and against *B. sorokiniana* (Table 5) in barley, MA 342 treatment was, however, in most cases significantly better than controls. MA 342 had no effect against *U. nuda* in barley (Table 2 and 6), against soil-borne *T. caries* or against *T. contraversa* in wheat (data not shown). In the *T. caries* and *T. contraversa* experiments, the effect of MA 342 was compared to the effect of Sibutol LS. This fungicide significantly reduced the number of infected spikes, as compared to the non-treated control, while MA 342 did not reduce disease incidence from these soil-borne inocula. In a greenhouse experiment, MA 342 showed good effect against *S. nodorum* (Table 15).

Discussion

The results presented provide evidence that seed bacterization with the strain MA 342, as carried out here, gives excellent control of several cereal, seed-borne diseases in the field and under normal cropping conditions. They further show that the effects are consistent

Table 11. Results obtained in single years 1991–1996 in field experiments testing effects of MA 342 against *Ustilago avenae* in oats (cv. Vital and Svea)

Treatment	No. of infected panicles m^{-2} during the years						
	1991	1992	1993	1994	1995	1996	Mean
Control	7.0 A[a]	46.5 A	45.0 A	8.2 A	15.7 A	5.1 A	19.3 A
Pan. Plus [b]	3.4 B	37.5 A	40.0 A	8.3 A	16.4 A	4.2 A	16.6 A
MA 342 [c]	0.8 B	6.3 B	5.0 B	1.7 B	5.6 B	0.2 B	2.9 B
No. of analysed plots	4	4	4	3	4	8	27

[a],[b] and [c] See in Table 2.

Table 12. Results obtained in field experiments testing effects of MA 342 against *Ustilago avenae* in oats (cv. Vital and Svea). Results from experiments carried out during 1991–1996

Treatment	Yield, kg ha^{-1}	No. of plants m^{-2}	No. of infected panicles m^{-2}
Control	3630 A[a]	327 A	25.7 A
MA 342 [b]	3840 A	323 A	3.6 B
No. of analysed plots	7	28	50

[a] See in Table 2.
[b] 200 or 300 ml kg^{-1} seed.

not only over time, but also under different climatic conditions, from the south of Sweden to the northern parts (Figure 1). For diseases caused by the seed-borne pathogens *D. teres* (Tables 2 to 4, 14), *D. graminea* (Tables 2 to 4), *T. caries* (Tables 9 to 10), *U. hordei* (Table 13) and *S. nodorum* (Table 15), the bacterial disease suppressing effect was as reliable and effective as conventional fungicide (Panoctine products) treatment, and it was even better than the fungicidal effect against *D. avenae* (Tables 2 to 4) and *U. avenae* (Tables 11 to 12, 14). MA 342 treatment was, on the other hand, not effective against soil-borne infections from *T. caries* and *T. contraversa* or against seed-borne infections from *U. nuda* (Tables 2, 6), which show selectivity in effects. Also, from a practical point of view, less than fully satisfactory control was obtained of diseases caused by seed-borne *B. sorokiniana* (Table 5), and *M. nivale* (Tables 6 to 9), further pointing to selectivity depending on disease and/or infection route. MA 342 bacterization, thus, gives effective and reliable control of a selected number of seed-borne diseases, and although field effects of biocontrol agents are well documented (Knudsen, 1994; da Luz, 1994; Hornby et al., 1993; Tahvonen et al., 1995), we know of no earlier reports where a consistent effect on a broad range of cereal diseases has been shown in long series of field experiments.

The mode of action of MA 342 is not elucidated in detail. A plausible interpretation of the field effects shown here is that MA 342 suppresses the pathogens by production of antifungal metabolites with a broad activity spectrum. Its capacity to control many seed-borne diseases, or their inocula, but inability to affect soil-borne inocula could, further, reflect a strong spermosphere, but a poor coleoptile and/or rhizosphere colonizing ability. This possibility has been indicated also in other investigations in our laboratory (D.J. van der Gaag, unpubl.), where the spatial-temporal colonization pattern of MA 342 is studied, using cell-tagging with gfp (green fluorescent protein) (Tombolini et al., 1997). A combination of a localized, metabolite-mediated mode of action and a strong spermosphere colonizing ability, could, thus, account for the varying biocontrol effects obtained depending on type and placement of disease inocula. This interpretation is also in agreement with the findings that those seed-borne pathogens that are effectively controlled by MA 342, are usually rather superficially localized in or on the seeds (Neergaard, 1977). Some seed-borne pathogens not controlled, like *U. nuda*, which is localized in the seed embryo (Neergaard, 1977), could then be too deeply hidden in the seed to be affected by MA 342, or its metabolites.

The consistency over years/seasons and varying climates found, further, points to a disease control mode of action where the MA 342 strain does not directly antagonize the pathogens on or outside the plant surface. Rather, it seemingly is more dependent on

Table 13. Results obtained in field experiments testing effects of MA 342 against *Ustilago hordei* in barley (cv. Golf) during the years 1991 (C-county) and 1996 (M- and C-counties, see Figure 1)

Treatment	No. of infected spikes m^{-2}				No. of plants m^{-2}
	1991 C-county	1996 M-county	1996 C-county	Mean	
Control	4.8 A[a]	2.5 A	0.7 A	2.7 A	320 A
Pan. Plus [b]	1.5 B	2.7 A	0.2 B	1.4 A	330 A
MA 342 [c]	0.3 B	1.0 A	0.0 C	0.4 B	321 A
No. of analysed plots	4	4	4	12	12

[a] and [b] See in Table 2.
[c] 300 ml kg^{-1} seed.

Table 14. Results obtained in field experiments testing effects of storing seeds treated with MA 342 against *Drechslera teres* in barley (cv. Golf), *Drechslera avenae* in oats (cv. Vital) and *Ustilago avenae* in oats (cv. Vital) in the year 1994 (seeds were stored at room temperature in the laboratory)

Treatment	Barley	Oats	
	No. of *D. teres* infected plants m^{-2}	No. of *D. avenae* infected plants m^{-2}	No. of *U. avenae* infected panicles m^{-2}
Control	34.0 A [a]	12.4 A	8.2 A
Pan. Plus [b]	0.0 B	4.2 B	8.3 A
MA 342, treated in spring 1992 [c]	4.0 B	n. t.	n.t.
MA 342, treated in spring 1993 [d]	2.8 B	6.7 B	2.6 B
MA 342, treated in spring 1994 [c]	3.8 B	3.9 B	1.7 B
No. of analysed plots	4	7	3

[a] and [b] See in Table 2.
[c] 300 ml kg^{-1} seed.
[d] 200 ml kg^{-1} seed.

Table 15. Results obtained in one greenhouse experiment testing effects of MA 342 against *Stagonospora nodorum* in winter wheat (cv. Forno) during 1996

Treatment		Infected plants,%
Control		22.5 A[a]
Panoctine Plus 400,	4 ml kg^{-1}	0.5 B
MA 342,	300 ml kg^{-1}	1.5 B
No. of replications		4

[a] See in Table 2.

the host plant, or its seed and seedling, than on the surrounding environment, where the varying seasonal and climatic conditions otherwise would have had a stronger influence on the bacterial performance.

From an agricultural point of view, the results obtained on consistency in biocontrol effect of MA 342 over the years and under different climatic conditions, emphasize the possibilities of large scale practical utilization of this strain. This is further emphasized by the proven possibilities of storing MA 342-treated seeds under dry conditions without losing the bacterial disease-controlling effect (Table 14), and by the rather broad range of diseases that may be controlled. MA 342 may from this point of view constitute a sound alternative to chemical fungicides for cereal seed-borne disease control. To be commercially competitive however, it also has to fulfil other requirements.

Harman (1991) suggested three general approaches for achieving the level of reliability and efficacy needed for biocontrol agents to be as competitive as chemical fungicides: (i) genetically superior strains (obtained through selection or genetic manipulation), (ii) cost-effective, large scale production methods (fermentation) for producing biocontrol agents of high quality and long shelf life, and (iii) development of delivery systems that give the biocontrol agent a competitive advantage.

The results so far obtained with the strain MA 342 clearly fulfil the first of these criteria, while the other two are still to be better elucidated. The findings that liquid cultures of MA 342, grown in laboratory scale, could be stored for at least one month without losing their activity (Hökeberg et al., 1997), like the possibil-

ity to store treated seeds without losing the biocontrol effect, indicate that the shelf life of MA 342 is not a main obstacle. Considering the possibilities of easily producing cells of MA 342 in the laboratory, cost-effective and reliable large scale fermentation also seems reachable. An acceptable and competitive delivery system is, on the other hand, still to be worked out. From practical and economical points of view the simple laboratory application used in these studies is not acceptable for large scale use. The high amount of liquid cells suspensions used for seeds application (200-300 ml suspension per kg seed), requires drying of the seed to a water content where safe storage is possible. Since drying is energy demanding, costly and also has to be performed at a relatively low temperature in order not to harm the bacterial cells, it is not applicable for large scale seed treatment. It is, further, difficult to apply higher liquid amounts than about 10 ml per kg seed in most modern seed dressing machines.

Some of the diseases here tested affect yield very moderately at the infection pressures at hand in the experiments, and for these diseases it is usually more important to hinder a long term build-up of pathogen inocula than to directly affect the yield loss by control measures. The main issue in this study was also the potential of MA 342 as a disease control agent, rather than tests of dose response effects or effects on yield. It can, however, be seen from the results presented in Tables 7 and 8 that yields obtained from MA 342 treated seeds in the wheat experiments were not fully comparable to those obtained from fungicide treated seeds. We assume that the bacterial dose, or rests from the bacterial broth, was unnecessary high in these experiments. Ongoing greenhouse and field experiments with varying bacterial doses, formulations and seed application methods (to be published elsewhere), have given evidence that these factors may affect plant germination capacity as well as storability/shelf life and biocontrol efficacy. From this we conceive that e.g. wheat yield effects of MA 342 can be overcome by optimizing bacterial dose, bacterial formulation and application procedure.

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