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RESEARCH ARTICLE

Biological and chemical control and their combined use to control different stages of the *Rhizoctonia* disease complex on potato through the growing season

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Biological control; flutolanil; *Gliocladium catenulatum*; logit model; *Streptomyces griseoviridis*; *Trichoderma harzianum*.

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Abstract

Rhizoctonia solani causes stem canker and black scurf diseases on potato and negatively affects the yield in all potato-growing areas. While seed-borne infection can be efficiently controlled by dressing with fungicides, few means of effective control are available against soil-borne infection. In this study, commercially available antagonistic fungi and bacteria, and the combination of antagonistic *Trichoderma harzianum* and seed dressing with flutolanil, were tested for their efficacy in the control of soil-borne infection of *R. solani* in the field. Combined use of flutolanil and *T. harzianum* was found feasible because even the highest tested concentration of flutolanil [13.0 μg active ingredient (a.i.) mL^{-1}] had little effect on the growth of *T. harzianum in vitro*, whereas over 100-fold lower concentrations (0.1 μg a.i. mL^{-1}) were sufficient to strongly inhibit the growth of *R. solani* (EC_{50} 0.045 ± 0.0068 μg a.i. mL^{-1}). The variables under focus in plants inoculated with *R. solani* were the relative stem lesion index; sprout/stem number; stolon number, weight and incidence of symptoms on stolons; total yield and the yield of marketable sized tubers and incidence of black scurf on the marketable-sized tubers. Flutolanil and its combined application with *T. harzianum* reduced the damage to sprouts and severity of stem canker at the early stages of growth (up to 30 days postplanting). Towards the end of the growing season, *T. harzianum* was required to reduce disease severity. When applied in-furrow alone or in combination with flutolanil-dressed seed potatoes, *T. harzianum* increased the proportion of marketable-sized tubers in yield from 35% to 60% and decreased the incidence of black scurf on progeny tubers from 31% to 11%, which was not achieved using flutolanil alone. The number and weight of stolons and the yield of plants remained lower in the inoculated plants than un-inoculated control plants regardless of the method of control used. The other two antagonists tested, *Streptomyces griseoviridis* and *Gliocladium catenulatum*, showed no consistent control of *R. solani*. Taken together, the results suggest that combining the application of the antagonist *T. harzianum* with seed dressing with flutolanil may provide the best protection of the potato crop against damage caused by *R. solani* throughout the growing season.

Introduction

The soil-borne fungus, *Rhizoctonia solani* Kühn [teleomorph *Thanatephorus cucumeris* (Frank) Donk] causes a disease complex known as stem canker (lesions and necrosis on subterranean parts of the plant) and black scurf (sclerotia on the surface of tubers) on potato (*Solanum tuberosum* L.) (Carling & Leiner, 1986). In diseased plants, there is a shift

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in tuber size distribution towards increased proportion of small and/or large progeny tubers, and an increase in the number of malformed tubers, which reduces the marketable yield (Frank & Leach, 1980; Jager *et al.*, 1991; Jeger *et al.*, 1996). Yield reductions caused by *R. solani* occur wherever potatoes are grown (Banville *et al.*, 1996; Jeger *et al.*, 1996).

Inoculum of *R. solani* can be tuber-borne or soil-borne (Banville *et al.*, 1996). Infection initiated by the tuber-borne inoculum is efficiently controlled by dressing seed potatoes with fungicides (Hide & Cayley, 1982; Jeger *et al.*, 1996). However, infection based on soil-borne inoculum is poorly controlled by fungicides, especially when the levels of initial inoculum are high (Weinhold *et al.*, 1982; Jager & Velvis, 1985; Hide & Read, 1991; Brewer & Larkin, 2005; Tsror & Peretz-Alon, 2005). Application of fungicides into soil may have adverse environmental consequences, and the efficacy of control depends on soil type (Kataria & Sunder, 1988). Therefore, the available means for controlling *R. solani* on potato are efficient against tuber-borne inoculum, whereas better approaches for reducing soil-borne infection are required.

Biological control by antagonistic micro-organisms has been proposed as a component of integrated pest management, where a range of chemical, cultural and biological treatments are applied to combat soil-borne *R. solani* on potato (Jager *et al.*, 1991; Errampalli *et al.*, 2006). Various antagonistic fungi, including *Trichoderma* spp. (Beagle-Ristaino & Papavizas, 1985; Tsror *et al.*, 2001; Brewer & Larkin, 2005), *Verticillium biguttatum* Gams (Jager & Velvis, 1985; Van den Boogert & Luttikholt, 2004), *Laetisaria arvalis* Burdsall (Murdoch & Leach, 1993) and nonpathogenic *Rhizoctonia* spp. (Bandy & Tavantzis, 1990; Escande & Echandi, 1991; Tsror *et al.*, 2001), have been reported to control the seed- or soil-borne infection of *R. solani* on potato in the field. However, few studies have addressed the compatibility and level of control achieved by a combination of biological and chemical control of *R. solani*. Integrated control using an antagonist *V. biguttatum* and reduced rates of the fungicide pencycuron has been found to be equal to chemical control at full rate with respect to reducing the severity of black scurf and, hence, quality losses in yield (Jager *et al.*, 1991). It has also improved disease control compared with the use of the antagonist alone when infection pressure of *R. solani* is high (Jager & Velvis, 1986). Integrated control may also be more effective in reducing the amount of sclerotia developing on progeny tubers than the chemical control alone at a reduced rate (Van den Boogert *et al.*, 1990). However, in some studies, integrated control has not improved disease control compared with its components applied separately (Elad *et al.*, 1980; Wicks *et al.*, 1996; Schmiedeknecht *et al.*, 1998).

Our previous studies carried out in a greenhouse have shown that *T. harzianum* Rifai applied to soil decreases the severity of stem canker caused by soil-borne *R. solani* (Wilson *et al.*, 2008). Furthermore, the antagonist reduced the severity of black scurf on progeny tubers and decreased the proportion of small tubers, hence improving the quality of yield. The aim of this study was to determine whether control of stem canker and black scurf is attainable with *T. harzianum* also in the field, and whether combined application of this antagonist and a fungicide could improve the levels of control under the Nordic climatic conditions.

Materials and methods

Preparation of inoculum of *R. solani*

An isolate of *R. solani* (isolate R11) was obtained from a stem canker lesion of the potato cv. Posmo grown in Lammi, Finland. It belongs to the anastomosis group 3 that predominates on potato in Finland (Lehtonen *et al.*, 2008) and was used in the previous study for testing control of stem canker and black scurf under greenhouse conditions (Wilson *et al.*, 2008). Growth medium for the inoculum of *R. solani* was prepared as described (Wilson *et al.*, 2008). In brief, 250 g of quinoa seed (*Chenopodium quinoa* Willd) and 500 g of quartz sand (grade 0.5–1.2 mm; Optiroc Ltd, Finland) were moistened with 275 mL 2 of reverse osmosis water, and the preparation was sterilised in a 1-L bottle by autoclaving twice at 121°C for 1 h. Plugs (10 mm in diameter) cut from the edge of a 5-day-old colony of *R. solani* grown on potato dextrose agar (PDA) were added to the growth substrate in the bottle and incubated in the dark at 22°C for 3 weeks. The bottle was shaken daily to ensure an even colonisation of the substrate by the fungus. The inoculum was dried on a sterile plastic tray in a laminar flow cabinet for 48 h and stored at 4°C for 24–48 h before use.

Experimental set up

Field experiments were established in 2 years (2004 and 2005) in the experimental field of the University of Helsinki, Viikki, Helsinki, Finland (60°13′N, 25°1′E). The soil type was clay, with soil organic matter content of 6–12% and pH 5.8. Potatoes had not been previously grown in the fields and no *R. solani* pathogenic on potato was known to occur, which was further verified during the study (see Results). Different areas of the field were used for the experiments in each year to avoid sources of infection other than the added inoculum.

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Certified, healthy seed tubers of cv. Van Gogh and Rosamunda (free of black scurf) were obtained from the Finnish Seed Potato Centre Ltd (Tyrnävä, Finland). They were sprouted in daylight in the greenhouse at 20 ± 5°C for 2 weeks until the thermal heat sum (>5°C) reached approximately 220°C. The experimental unit or plot (1.2 m in length; referred to as treatment plot) consisted of four tubers planted by machine at 0.30-m intervals. The plots were located in 13-m-long rows. The space between the rows was approximately 0.75 m. All treatment plots were replicated three times and completely randomised within blocks. Each sampling date comprised a block. There were a total of nine and seven blocks (i.e. nine and seven sampling dates, respectively) in the experiments, totalling 378 (nine dates × 14 treatments × three replicates), and 168 (seven dates × eight treatments × three replicates) experimental plots in 2004 and 2005, respectively (Table 1).

In 2004, the seed potatoes were planted on 25 May and in 2005 on 19 May. Soil fertilisation was performed with an N : P : K = 8:5:19 manufacture (Perunan Y1; Kemira GrowHow, Finland) at a rate of 865 and 750 kg ha^{-1} in 2004 and 2005, respectively. Weeds were controlled with a combination of rimsulfuron [7.5 g active ingredient (a.i.) ha^{-1}; Titus WSB; Kemira GrowHow] and metribuzin (21.0 g a.i. ha^{-1}; Senkor WG; Berner, Finland) following the practices undertaken in commercial potato production. For control of potato, late blight Tanos (175 g a.i. both famoxate and cymoxanil ha^{-1}; Berner) and Epok 600 EC (80 and 160 g a.i. metalaxyl-M and fluazinam, respectively, ha^{-1}; Berner) were used in 2004 and 2005, respectively, when necessary.

Sampling was by hand 7, 14, 21, 28, 35, 42, 60, 90 and 120 days postplanting (dpp) in 2004, and 10, 20, 30, 40, 60, 90 and 120 dpp in 2005. Several sampling dates were included in the experiments to follow the development of disease and possible effects of the disease control treatments throughout the growing season. However, the assessment of yield (amount, proportion of marketable-sized tubers and incidence of black scurf) was only carried out at final harvest 120 dpp.

Inoculation

Experiment in 2004

Cultivar Van Gogh was used in the experiment. For the control of *R. solani*, treatments included (a) dressing tubers with *T. harzianum* by dipping the potatoes briefly in 1% aqueous solution of Trianum-G (isolate T-22,

Table 1 Experimental set-up in 2004 and 2005

Year	Treatment (abbreviation)	Distance of *Rhizoctonia solani* inoculum from seed potato (mm)	Number of sampling dates (blocks)	Number of replicates	Number of experimental plots
2004	*R. solani* (RHIZ)	0, 30, 60, 90	9	3	108
	RHIZ + *Trichoderma harzianum* dip (THDIP)	0, 30, 60, 90	9	3	108
	RHIZ + *S. griseoviridis* dip (MYCO)	30	9	3	27
	RHIZ + *G. catenulatum* dip (PRES)	30	9	3	27
	None (CON)	—	9	3	27
	T. harzianum dip (CON-THDIP)	—	9	3	27
	S. griseoviridis dip (CON-MYCO)	—	9	3	27
	G. catenulatum dip (CON-PRES)	—	9	3	27
	Total				378
2005	*R. solani* (RHIZ)	0	7	3	21
	RHIZ + *T. harzianum* dip (THDIP)	0	7	3	21
	RHIZ + *T. harzianum* 50 g m^{-1} (TH50)	0	7	3	21
	RHIZ + Moncut dip (MON)	0	7	3	21
	RHIZ + MON + TH50	0	7	3	21
	None (CON)	—	7	3	21
	T. harzianum dip (CON-THDIP)	—	7	3	21
	T. harzianum 50 g m^{-1} (CON-TH50)	—	7	3	21
	Total				168

CON, no inoculation and no disease control treatment; CON-MYCO, no inoculation with *R. solani* but dressing with Mycostop; CON-PRES, no inoculation with *R. solani* but dressing with Prestop (CON-PRES); CON-TH50, TH50 without inoculation with *R. solani*; CON-THDIP, no inoculation with *R. solani* but dressing with Trianum-G; MON, dressing tubers with 0.15% aqueous solution of flutolanil; MON-TH50, combination of dressing with flutolanil and TH50; MYCO, dressing tubers with 0.01% aqueous solution of *Streptomyces griseoviridis* (Mycostop); PRES, dressing tubers with 1% aqueous solution of *Gliocladium catenulatum* (Prestop); RHIZ, inoculation with *Rhizoctonia solani* but no disease control treatment; TH50, adding *T. harzianum* evenly into the furrow at a rate of 50 g m^{-1}; THDIP, dressing tubers with *T. harzianum* by dipping the potatoes briefly in 1% aqueous solution of Trianum-G.

1.5×10^8 spores g^{-1} preparation; Koppert BV, the Netherlands) (treatment referred to as THDIP), (b) dressing tubers with 0.01% aqueous solution of *Streptomyces griseoviridis* Anderson *et al.* [Mycostop, 10^8–10^9 colony-forming units (CFU) g^{-1} preparation; Kemira] (MYCO) or (c) dressing tubers with 1% aqueous solution of *Gliocladium catenulatum* Gilm & Abbott (Prestop, 10^7–10^9 CFU g^{-1} preparation; Verdera, Finland) (PRES) (Table 1). Treatments were performed immediately before planting.

Inoculum of *R. solani* (5 g) was added by hand on the seed tuber or as a ring at certain horizontal distances (30, 60 or 90 mm) from the tuber, as previously described (Wilson *et al.*, 2008). In the MYCO and PRES treatments, inoculum of *R. solani* was placed at 30 mm distance from the seed tuber, whereas in THDIP all aforementioned distances of inoculum were used (Table 1). Control treatments included (d) inoculation with *R. solani* but no disease control treatment (RHIZ); or no inoculation with *R. solani* but dressing with (e) Trianum-G (CON-THDIP), (f) Mycostop (CON-MYCO) or (g) Prestop (CON-PRES) or (h) no inoculation and no disease control treatment (CON) (Table 1). The treatment plots planted with cv. Van Gogh were separated from each other by planting two healthy seed tubers of cv. Rosamunda between them. Tubers of this cultivar are red-skinned in contrast to the white-skinned tubers of Van Gogh, which further facilitated separation of progeny tubers from the adjacent treatment plots. Two seed tubers of Rosamunda were also placed at the ends of each row.

Experiment in 2005

The inoculum of *R. solani* was added evenly in the furrows at a rate of 5 g m^{-1} at planting (treatment RHIZ). Treatments for the control of *R. solani* included (a) dressing tubers with 0.15% aqueous solution of flutolanil (Moncut 40 SC; 449 g a.i. L^{-1}; Berner) (MON); (b) THDIP (as in 2004); (c) adding the antagonist evenly into the furrow at a rate of 50 g m^{-1} (TH50) and (d) combination of dressing with flutolanil and TH50 (MON-TH50) (Table 1). Control treatments included CON and CON-THDIP, as in 2004, and TH50 without inoculation with *R. solani* (CON-TH50). The treatments plots were separated from each other as in 2004.

Disease and yield assessment

On each sampling date, seed tubers with unemerged or emerged sprouts, roots, stolons and stems, depending on the developmental stage, was collected and washed with running tap water. Samples were examined immediately after collection or were stored at 4°C in the dark for a maximum of 48 h before closer study. Symptoms such as lesions and death of unemerged sprouts, and lesions and discolouration of the subterranean parts of the stem were collectively called 'stem canker' for simplicity. The proportion of the sprouts or stem affected by the symptoms was scored according to Weinhold *et al.* (1982), and each potato stem (sprout) was placed in one of the six disease severity classes accordingly (0%, 1–5%, 6–25%, 26–50%, 51–75% or 76–100% of the subterranean part of the potato stem affected). For each plant, disease severity was then expressed as the Rhizoctonia stem lesion index (RSI) (Weinhold *et al.*, 1982) by multiplying the number of stems (sprouts) in each class by the midpoint of the class and dividing the sum of the values obtained by the total number of stems (sprouts) (Weinhold *et al.*, 1982). Thus, the possible maximum RSI-value for each plant was 88 (midpoint of the class 76–100%) (Weinhold *et al.*, 1982; Wilson *et al.*, 2007).

The incidence of infected stolons was determined for each plant at 60, 90 and 120 dpp. At the last sampling date 120 dpp, the weight and sizes of progeny tubers were assessed. Tubers were placed in two size classes: those 40–60 mm in diameter (marketable size for staple potato) and those outside this range. Black scurf was visually assessed on the marketable-sized tubers of each plant using the illustrated key (reference pictures) of Dijst (1985) based on the following scale: 0 = free of black scurf, 1 = very lightly, 2 = lightly, 3 = moderately or 4 = heavily infested with black scurf.

Sensitivity of *T. harzianum* and *R. solani* to flutolanil

The sensitivity to flutolanil of the isolates of *R. solani* (R11) and *T. harzianum* (T-22) was assessed as described for a collection of 119 *R. solani* isolates (including R11) in a previous study (Lehtonen *et al.*, 2008). In brief, autoclaved and cooled PDA was amended with different concentrations of flutolanil (0, 0.1, 0.5, 1.0, 3.0, 5.0, 10.0 or 13.0 μg a.i. mL^{-1}) and distributed onto Petri dishes (20 mL aliquots, diameter of the dish 90 mm). A plug of PDA with mycelia (diameter 7 mm) was cut from the leading edge of an actively growing colony of *R. solani* or *T. harzianum* and placed on the edge of the dish with fungicide-amended PDA. The dishes were sealed with Parafilm and incubated at 20°C in the dark. Each isolate and fungicide concentration and also the fungicide-free control dishes were replicated three times. Colony growth was measured along two straight lines drawn from the centre of the mycelial plug (angle between lines 50°) on days 3, 4, 7 and 10 after inoculation. The growth rate (mm day^{-1}) was used to estimate

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the effective concentration causing a 50% reduction in growth rate (EC50) compared with the fungicide-free control. The EC50-values were calculated from dose–response curves using nonlinear regression and the SPSS statistical software package (SPSS Inc., Chicago, IL, USA).

Statistical analyses

The effects of disease control treatments on the severity of stem canker and black scurf, and on stem and stolon numbers, stolon weight and tuber yield were compared by the analysis of variance (ANOVA) using the GLM procedure of the SAS statistical software package (SAS Institute Inc., Cary, NC, USA). In the 2004 experiment, a two-way ANOVA comprising the distance of *R. solani* inoculum from seed potato (0, 30, 60 or 90 mm) and disease control treatment (the presence or absence of *T. harzianum*) as factors was used. In the 2005 experiment, one-way ANOVA was used. The least significant differences were used to separate the means at $P = 0.05$.

The incidence of black scurf, the proportion of marketable tubers (40–60 mm in diameter) in the yield and the incidence of infected stolons were analysed by logit models. Logit models were preferred to ANOVA because the response variables were of a quantal nature (i.e. presence or absence, for which the results are reported as percentages or proportions). In logit models, the effects of explanatory variables are described using the concept of odds ratio, which is a relative measure of difference between the two probabilities compared: $[P_2/(1 - P_2)]/[P_1/(1 - P_1)]$ (Collett, 1991; Lindén *et al.*, 1996; Hiltunen *et al.*, 2005). Significance tests and confidence intervals for the single parameters were based on Wald statistics. The logit analysis was carried out by the procedure GENMOD of the SAS statistical software package. When necessary, overdispersion of the data was allowed by calculating a scale parameter that was used to adjust the standard errors and to correct the chi-squared statistics and *P* values.

Results

The growing season of 2004 was wetter than average. The mean rainfall in Helsinki in June, July and August was 110, 207 and 94 mm, respectively, which was 60, 147 and 19 mm higher than the mean of 30 years (Venäläinen *et al.*, 2005). However, the mean temperatures in June (13°C), July (17°C) and August (17°C) did not deviate from the mean of 30 years. In 2005, the temperatures and rainfall were similar to those recorded over the past 30 years, and the growing season was regarded as average.

In the following, the results of mainly those treatments and sampling dates that caused statistically significant differences in disease or yield parameters are described in detail. No stem canker, stolon symptoms or black scurf on progeny tubers were observed in uninoculated control plants in 2004 and 2005, indicating that no natural soil-borne inoculum of *R. solani* capable of causing symptoms was present in the experimental fields.

Severity of stem canker and influence of infection on stem number in 2004

In 2004, inoculum of *R. solani* (5 g) was placed at different distances from the seed tuber. At 7 dpp, no shoots had emerged and the RSI (Weinhold *et al.*, 1982) was determined based on the damage to the unemerged sprouts. RSI was highest in the treatments where the inoculum was placed on or close to the seed tuber (distances 0 or 30 mm) and decreased with increasing distance (60 and 90 mm) of inoculum from the seed tuber, as expected (Henis & Ben-Yephet, 1970; Tomimatsu & Griffin, 1982; Gilligan & Bailey, 1997; Wilson *et al.*, 2007) (Table 2a). At 28 dpp, many but not all shoots had emerged regardless of the treatment. Differences in RSI were less pronounced than at 7 dpp, but the severity of stem canker was still higher in plants with the closer distance from the inoculum (Table 2a). Overall, these results were explained by the time needed for the growth of *R. solani* towards the plants from the longer distance and which gradual increased the RSI over time.

Initially, at 7 dpp, sprout number was little affected by the distance of inoculum from the seed tuber (Table 2b). However, at 28 dpp, a lower total number of sprouts and stems were observed at shorter distances to the inoculum (Table 2b). At 60 dpp, the situation had reversed. The plants at closer inoculum distances had a higher number of sprouts and stems than those at a longer distance (e.g. 18.7 and 8.9 sprouts/stems per plant at an inoculum distance of 0 and 90 mm, respectively) (Table 2b), and the same trend was observed at 90 dpp. Furthermore, there were no longer significant differences in RSI, regardless of the distance of inoculum. These data were explained by branching of sprouts whose tip was killed by infection and compensatory growth of new sprouts (Baker, 1970; Errampalli *et al.*, 2006) in treatments where infection began early, that is, in treatments where the inoculum was placed close to the seed tuber, which increased the sprout/stem number per plant. However, the similar RSI-values could be explained because stems become more resistant to stem canker after emergence of potato plants (Van Emden, 1965). Hence, the new sprouts/stems of the plants infected early, and the stems of plants in treatments where *R. solani* was placed at a longer distance from the seed tuber and reached the stems late, managed to escape stem canker development more often than the others (Table 2a,b).

Table 2 The effect of biological disease control treatment and the horizontal distance of inoculum of *Rhizoctonia solani* from the seed tuber on (a) the Rhizoctonia stem lesion index (RSI)[a] and (b) the mean number of stems per plant in 2004

Treatment	7 dpp	28 dpp	60 dpp	90 dpp	120 dpp
(a)			Mean RSI		
Main effect[b]					
R. solani (RHIZ)	44.0	50.5	33.6	34.9	40.4
RHIZ + *Trichoderma harzianum* dip (THDIP)	46.9	53.0	32.1	38.7	44.7
SED (d.f. = 16)	7.1	3.0	3.9	4.7	3.8
Main effect[b]					
RHIZ 0 mm	70.3	54.8	27.5	29.7	35.3
RHIZ 30 mm	74.8	58.1	27.3	29.5	35.6
RHIZ 60 mm	36.0	48.7	37.7	39.7	47.0
RHIZ 90 mm	0.6	45.4	38.8	48.4	52.3
SED (d.f. = 16)	10.0	4.3	5.5	6.7	5.4
30 mm distance of inoculum					
RHIZ	72.5	53.4	26.1	32.0	37.0
THDIP	77.1	62.8	28.5	27.0	34.2
RHIZ + *S. griseoviridis* dip (MYCO)	69.2	57.4	29.2	29.7	30.4
RHIZ + *G. catenulatum* dip (PRES)	65.0	54.2	33.3	26.1	36.0
SED (d.f. = 8)	22.4	4.4	7.1	2.9	9.2
(b)			Mean stem number		
Main effect[b]					
RHIZ	5.6	9.0	12.4	8.0	3.9
RHIZ + THDIP	5.0	9.5	14.9	7.9	3.8
SED (d.f. = 16)	0.4	0.5	2.9	1.4	0.6
Main effect[b]					
RHIZ 0 mm	5.8	8.4	18.7	8.3	4.0
RHIZ 30 mm	4.6	8.6	14.7	9.5	3.7
RHIZ 60 mm	4.9	9.3	12.4	7.7	4.0
RHIZ 90 mm	5.8	10.8	8.9	6.3	3.8
SED (d.f. = 16)	0.6	0.8	4.0	2.0	0.9
30 mm distance of inoculum					
RHIZ	4.9	9.2	12.5	7.3	3.1
THDIP	4.3	8.0	16.8	11.7	4.3
RHIZ + MYCO	5.8	7.4	13.3	7.9	6.7
RHIZ + PRES	4.2	9.2	12.0	11.3	5.1
SED (d.f. = 8)	0.8	0.7	4.1	2.1	1.7

dpp, days postplanting; PRES, dressing tubers with 1% aqueous solution of *Gliocladium catenulatum* (Prestop); RHIZ, inoculation with *Rhizoctonia solani* but no disease control treatment; MYCO, dressing tubers with 0.01% aqueous solution of *Streptomyces griseoviridis* (Mycostop); SED, standard error of difference; THDIP, no inoculation with *R. solani* but dressing with Trianum-G.

[a]Scale 0–88, Weinhold *et al.* (1982).

[b]Interactions of disease control × distance were not significant at $P = 0.05$.

No antagonist had any significant control effect on stem canker regardless of the distance of inoculum or time post-inoculation in 2004 (Table 2a).

Combined chemical and biological control against stem canker in 2005

In 2005, inoculum of *R. solani* was applied at the same amount (5 g) but in a different manner than in 2004. It was placed evenly in the furrow to simplify the experimental set-up. Severity of stem canker was significantly lower in the MON and MON-TH50 treatments (RSI 3–22) than in RHIZ (RSI 42–73) at 10 and 30 dpp (Table 3a). At a later time (60 dpp), RSI was lower only in treatments including *T. harzianum* compared with RHIZ: TH50 had the lowest RSI-value, followed by MON-TH50 and THDIP (Table 3). At this time point, RSI of the MON treatment no longer showed any difference to RHIZ (Table 3). Similar trends were observed at 90 dpp, whereas at 120 dpp, no differences in RSI were observed between the treatments (RSI 24–41) (Table 3).

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Table 3 The effect of biological and chemical disease control treatments on (a) the Rhizoctonia stem lesion index (RSI)[a] and (b) the mean number of stems per plant in 2005

Treatment[b]	10 dpp	30 dpp	60 dpp	90 dpp	120 dpp
(a)	Mean RSI				
Rhizoctonia solani (RHIZ)	73.0	42.0	38.3	33.6	34.0
RHIZ + Moncut dip (MON)	8.3	21.6	32.3	35.0	38.5
RHIZ + *Trichoderma harzianum* dip (THDIP)	NA	40.6	25.2	31.9	41.2
RHIZ + *T. harzianum* 50 g m^{-1} (TH50)	49.0	41.1	17.2	20.8	24.0
RHIZ + MON + TH50	3.1	18.8	21.2	26.2	27.6
SED (d.f. = 10)	10.0 (d.f. = 7)	6.3	3.1	2.8	5.5
(b)	Mean stem number				
RHIZ	3.8	6.8	4.6	3.8	1.9
RHIZ + MON	3.7	10.3	12.3	7.5	1.6
RHIZ + THDIP	NA	7.8	6.3	6.0	1.9
RHIZ + TH50	4.6	6.5	11.7	7.7	1.2
RHIZ + MON + TH50	4.5	9.2	13.4	6.8	1.4
SED (d.f. = 10)	0.6 (d.f. = 7)	1.1	1.6	1.9	0.5

dpp, days postplanting; NA, data not available; SED, standard error of difference.
[a]Plants were inoculated with *R. solani* (RHIZ). Seed dressing with *Trichoderma harzianum* (THDIP), addition of *T. harzianum* to furrow (TH50), seed dressing with flutolanil (MON) or both (MON + TH50) were used for disease control.
[b]Scale 0–88, Weinhold *et al.* (1982).

The stem numbers first increased and subsequently decreased over time in response to the attack by *R. solani* when TH50 and/or MON were used for control (Table 3). However, without control (RHIZ) or using *T. harzianum* for seed dressing (THDIP), the stem numbers remained low over the whole growing season (Table 3), which was different from 2004 (Table 2). The data on development of sprout/stem numbers as a function of time in 2005 are also illustrated in Fig. 1. They indicate that inoculum pressure was very high, damage to unemerged sprouts was severe and no compensatory growth was possible and sprout/stem numbers remained constantly low without control (RHIZ) or with inefficient control (THDIP). However, the control treatments with TH50, MON and the combined used of MON and TH50 could delay the damage of sprouts albeit not prevent it, which was reflected as compensatory growth and increase of the sprout/stem numbers temporarily. Hence, observation of sprout/stem numbers provided additional information and indicated a difference in the severity of the disease in the two growing seasons. The difference could not be revealed based on the RSI values of plants inoculated with *R. solani* (RHIZ; no disease control) because they were similar in the 2 years. In 2004 and 2005, RSI for RHIZ was 51 and 42 at 28–30 dpp ($t = -1.802$, d.f. = 13, $P = 0.095$), and 34 and 38 at 60 dpp ($t = 1.094$, d.f. = 13, $P = 0.294$), respectively.

Compatibility of *T. harzianum* and flutolanil treatments was supported by results of *in vitro* tests. The growth rate of *T. harzianum* was little affected by any flutolanil concentration tested (Fig. 2) and its mean EC_{50} value was outside the range of the concentrations used (13.0 μg a.i. mL^{-1}). In contrast, the mean EC_{50}-value of flutolanil for the growth rate of *R. solani* was 0.045 ± 0.0068 (SE) μg a.i. mL^{-1}, which was consistent with results of a previous study (Lehtonen *et al.*, 2007) and indicated a markedly higher sensitivity to flutolanil of *R. solani* than *T. harzianum*.

Damage to stolons caused by *R. solani*

In both years, the mean fresh weight of stolons per plant was significantly lower in all the treatments inoculated with *R. solani* (1–3 g per plant) than in the uninoculated

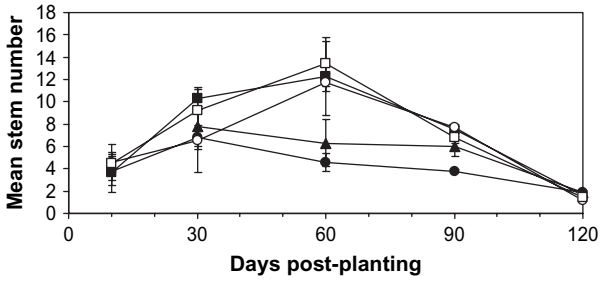

Figure 1 The effect of biological and chemical control treatments on the mean number of sprouts/stems per plant at different time points postplanting in 2005. All data are from plants inoculated with *Rhizoctonia solani*, without or with control. ●, *R. solani*, no control (RHIZ); ■, seed dressing with Moncut (MON); ▲, seed dressing with *Trichoderma harzianum* (THDIP); ○ application of *T. harzianum* to furrow at 50 g m^{-1} (TH50); □, combined chemical and biological control (MON + TH50). Each data point represents the mean of three replicates. Bars represent standard deviation.

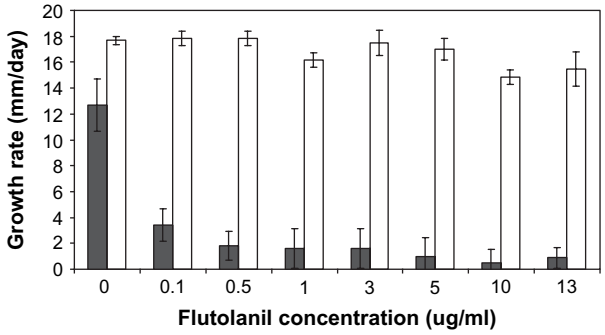

Figure 2 The growth rate (mm per day) of *Rhizoctonia solani* (isolate R11, shaded bars) and *Trichoderma harzianum* (isolate T-22, unshaded bars) at different concentrations (μg active ingredient mL^{-1}) of the fungicide flutolanil *in vitro*. Thin bars indicate standard deviation (n = 3).

control treatments (5–6 g per plant) and was little affected by the disease control treatments at any time of observation (for results of 2005, see Table 4a). Similarly, in 2005, the mean number of stolons per plant was lower in the treatments inoculated with *R. solani* (5–12 stolons per plant) than in the uninoculated control treatments (15–22 stolons per plant), regardless of the disease control treatments or time of observation (Table 4a).

All stolons in all plants inoculated with *R. solani* contained lesions in 2004, and no differences in the incidence of infected stolons were observed at 60, 90 or 120 dpp, regardless of the distance between the seed tuber and the applied inoculum and whether or not disease control was applied. In 2005, at 60 dpp, the incidence of stolon damage ranged from 32 to 78%, but differences were not significant between treatments (data not shown). However, observation of symptoms at 90 dpp indicated that the probability of stolons to be damaged by *R. solani* was significantly lower in the TH50 treatment (incidence of stolons with symptoms 36%) compared with MON-TH50 (49%), MON (56%), THDIP (65%) or no control (RHIZ; 64%). However, at 120 dpp, the incidence of stolon damage was significantly lower in the MON treatment (52%) than in MON-TH50 (70%), THDIP (70%), TH50 (80%) or RHIZ (81%) (Table 4b). Hence, protection of stolons against damage was mostly not statistically significant with the control methods used; however, results suggested some positive effects because the incidence of stolons with symptoms was never higher in plants treated with MON and/or TH50 than the plants (RHIZ) in which no control against *R. solani* was used.

Yield and size distribution of progeny tubers

In both years, the mean yield per plant was significantly lower in all treatments inoculated with *R. solani* (200–600 g per plant) compared with the treatments without of *R. solani* (1000–1400 g per plant) (Tables 5 and 6a). No disease control treatment had any detectable effect on the total yield.

In 2004, inoculation of plants with *R. solani* (RHIZ) resulted in significantly lower proportions (42–48%) of marketable-sized tubers (diameter 40–60 mm) in the yield compared with uninoculated plants (CON, CON-THDIP, CON-MYCO and CON-PRES) (72–75%), regardless of the distance (0–90 mm) at which inoculum was placed from the seed tuber (Table 5). The disease control treatments tested (THDIP, MYCO and PRES) did not result in any statistically significant increase in the total yield or the proportion of marketable-sized yield in plants inoculated with *R. solani*; however, the values for THDIP were the highest for the total and marketable yields when inoculum was placed at a 30-mm distance from the seed tuber (Table 5). The biological control agents *per se* (CON-THDIP, CON-MYCO and CON-PRES) had no negative effect on the yield compared with the uninoculated and untreated plants (CON) (Table 5).

In 2005, the proportion of marketable-sized tubers could be increased by the disease control. TH50 increased the proportion of marketable yield to 60% compared with 35% in RHIZ (Table 6a). Similarly, the combined use of chemical and biological control (MON-TH50) resulted in a significantly higher proportion of marketable-sized progeny tubers than RHIZ (Table 6b). With the two other disease control treatments (THDIP and MON), the proportion of marketable yield tended to be higher than with RHIZ (odds ratio >1), but the difference was not statistically significant (Table 6b). *Trichoderma harzianum* had no negative impact on yield because plants treated with *T. harzianum* alone (CON-TH50 and CON-THDIP) produced similar high yields as the untreated and *R. solani*-free plants (CON) (Table 6a,b).

Incidence of black scurf

In 2004, the incidence of marketable sized progeny tubers with black scurf was higher when the inoculum of *R. solani* was placed on the seed tuber (incidence 60%), as compared to placement at distances of 60 mm (incidence 44%; OR 0.48, P = 0.024) or 90 mm (incidence 42%; OR 0.55, P = 0.046) from the seed tuber. No biological control agent could significantly prevent formation of black scurf. While the incidence of black scurf was 46% in RHIZ (no control), it was 49%, 40% and 32% in the treatments THDIP, PRES and MYCO, respectively, at 30 mm distance of inoculum from the seed tuber (the corresponding OR 1.16, P = 0.6245; OR 0.79, P = 0.4797 and OR 0.54, P = 0.0798, respectively).

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Table 4 The effect of biological and chemical disease control treatments on (a) the mean stolon weight and number per plant and (b) the incidence of stolons with symptoms at 90 and 120 days after planting (dpp) in 2005. The incidences of stolons with symptoms were compared using logit analysis

Treatment	Mean stolon weight (g)		Mean stolon number	
	90 dpp*	120 dpp	90 dpp	120 dpp
(a)				
Rhizoctonia solani (RHIZ)	1.7	1.6	6.2	9.5
RHIZ + Moncut dip (MON)	1.5	2.7	8.3	11.9
RHIZ + *Trichoderma harzianum* dip (THDIP)	1.3	1.6	8.3	10.1
RHIZ + *T. harzianum* 50 g m^{-1} (TH50)	2.0	2.4	8.8	9.9
RHIZ + MON + TH50	1.8	1.8	5.7	8.4
Control (CON)	5.8	5.3	15.4	19.3
CON + THDIP	5.8	5.6	17.6	17.1
CON + TH50	4.8	4.6	22.0	19.4
SED (d.f. = 19)	1.0	1.3	2.1	2.2

Treatment	Incidence of infected stolons (%)	Odds ratio[a]	95% confidence interval[b]	*P* value of Wald test
(b)				
90 dpp				
Comparison RHIZ versus				
RHIZ	63.9	1	ND	ND
RHIZ + THDIP	65.1	1.05	0.37–2.99	0.9279
RHIZ + TH50	36.1	0.22*	0.08–0.62	0.0041
RHIZ + MON	55.9	0.68	0.24–1.91	0.4704
RHIZ + MON + TH50	49.4	0.55	0.17–1.80	0.3262
Comparison RHIZ + TH50 versus				
RHIZ + THDIP	65.1	4.76*	1.83–12.40	0.0014
RHIZ + MON	55.9	3.10*	1.21–7.96	0.0185
120 dpp				
Comparison RHIZ versus				
RHIZ	81.1	1	ND	ND
RHIZ + THDIP	70.1	0.96	0.54–1.69	0.8767
RHIZ + TH50	79.7	1.48	0.80–2.72	0.2082
RHIZ + MON	51.8	0.44*	0.26–0.76	0.0029
RHIZ + MON + TH50	69.6	0.93	0.51–1.68	0.8042
Comparison MON versus				
RHIZ + THDIP	70.1	2.16*	1.28–3.65	0.0040
RHIZ + TH50	79.7	3.35*	1.90–5.88	<.0001
RHIZ + MON + TH50	69.6	2.10*	1.21–3.64	0.0084

CON, no inoculation and no disease control treatment; MON, dressing tubers with 0.15% aqueous solution of flutolanil; ND, not determined; RHIZ, inoculation with *Rhizoctonia solani* but no disease control treatment; adding *T. harzianum* evenly into the furrow at a rate of 50 g m^{-1}; THDIP, dressing tubers with *T. harzianum* by dipping the potatoes briefly in 1% aqueous solution of Trianum-G.

[a]Odds ratio, 1 is equivalent to no difference in the comparison; values <1 indicate increased probability in the first term; values >1 indicate increased probability in the second term.

[b]If unity (=1) is not within the confidence interval, the comparison shows a significant difference ($P < 0.05$)*.

In 2005, incidences of progeny tubers with black scurf were, in general, similar to those in 2004. The highest incidences in 2005 were observed in THDIP (50%), MON (44%) and RHIZ (31%) which were not statistically different from each other (Table 7a). Low incidences of 11% and 18% were observed in the MON-TH50 and TH50 treatments, respectively. In pairwise comparisons using logit analysis, it was found that the incidence of tubers with black scurf was significantly lower in MON-TH50 than in RHIZ, MON or THDIP (Table 7b). Furthermore, the incidence of black scurf in TH50 was lower than in THDIP and MON (Table 7b).

In both years, the severity of black scurf was only 1.0–1.5 on the scale of Dijst (1985) in all plants inoculated with *R. solani*, and the control treatments had no noticeable impact on it (data not shown).

Discussion

Concomitant control of stem canker, black scurf and other negative effects on potato yield, that is, an effective control through the whole growing season, is expected from the measures applied to protect the crop against *R. solani* (Elad *et al.*, 1980; Brewer & Larkin, 2005). In addition,

Table 5 The effect of biological disease control treatment and the horizontal distance of inoculum of *Rhizoctonia solani* from the seed tuber on the mean yield (g per plant), and the proportion of marketable sized progeny tubers (diameter 40–60 mm) at harvest (120 days postplanting) in 2004

Treatment	Mean yield (g)	Proportion of marketable-sized tubers (%)
Main effect		
R. solani (RHIZ)	580.0	50.8
RHIZ + *Trichoderma harzianum* dip (THDIP)	619.6	53.6
SED (d.f. = 19)	78.3	ND
Main effect		
RHIZ 0 mm	336.0	48.4
RHIZ 30 mm	379.3	52.5
RHIZ 60 mm	351.0	41.5
RHIZ 90 mm	538.7	43.5
Uninoculated control (CON) with and without *T. harzianum* (THDIP)	1353.3	72.7
SED (d.f. = 19)	94.2	ND
30 mm distance of inoculum		
RHIZ	349.5	47.6
RHIZ + THDIP	409.0	57.9
RHIZ + *S. griseoviridis* dip (MYCO)	287.2	33.9
RHIZ + *G. catenulatum* dip (PRES)	193.6	43.1
CON	1378.2	74.4
CON + THDIP	1328.3	71.9
CON + MYCO	1253.3	73.2
CON + PRES	1099.9	75.4
SED (d.f. = 15)	60.2	ND

CON, no inoculation and no disease control treatment; MYCO, dressing tubers with 0.01% aqueous solution of *Streptomyces griseoviridis* (Mycostop); ND, not determined; PRES, dressing tubers with 1% aqueous solution of *Gliocladium catenulatum* (Prestop); RHIZ, inoculation with *Rhizoctonia solani* but no disease control treatment; SED, standard error of difference; THDIP, dressing tubers with *T. harzianum* by dipping the potatoes briefly in 1% aqueous solution of Trianum-G.

Table 6 The effect of biological and chemical disease control treatments on (a) the mean yield (g per plant) and the proportion of marketable-sized progeny tubers (diameter 40–60 mm) at harvest (120 days postplanting) in 2005. (b) The proportions of marketable tubers were compared using logit analysis

(a) Treatment[a]	Mean yield (g)	Proportion of marketable sized tubers (%)
Rhizoctonia solani (RHIZ)	280.4	35.4
RHIZ + Moncut dip (MON)	392.2	48.1
RHIZ + *Trichoderma harzianum* dip (THDIP)	225.6	48.8
RHIZ + *T. harzianum* 50 g m^{-1} (TH50)	495.8	59.7
RHIZ + MON + TH50	388.1	52.1
Control (CON)	990.1	55.1
CON + THDIP	1093.6	59.5
CON + TH50	1184.3	52.4
SED (d.f. = 19)	97.3	ND

(b) Comparison of RHIZ versus	Odds ratio[b]	95% confidence interval[c]	*P* value of Wald test
RHIZ + THDIP	1.74	0.81–3.73	0.1538
RHIZ + TH50	2.71*	1.39–5.27	0.0034
RHIZ + MON	1.69	0.83–3.43	0.1452
RHIZ + MON + TH50	1.98*	1.04–3.78	0.0373
CON	2.24*	1.28–3.92	0.0045
CON + THDIP	2.69*	1.50–4.80	0.0008
CON + TH50	2.02*	1.15–3.53	0.0140

CON, no inoculation and no disease control treatment; MON, dressing tubers with 0.15% aqueous solution of flutolanil; ND, not determined; RHIZ, inoculation with *Rhizoctonia solani* but no disease control treatment; sed, standard error of difference; TH50, adding *T. harzianum* evenly into the furrow at a rate of 50 g m^{-1}; THDIP, dressing tubers with *T. harzianum* by dipping the potatoes briefly in 1% aqueous solution of Trianum-G.

[a]Plants were inoculated with *R. solani* (RHIZ). Seed dressing with *T. harzianum* (THDIP), addition of *T. harzianum* to furrow (TH50), seed dressing with flutolanil (MON) or both (MON + TH50) were used for disease control. For comparison, plants were not inoculated (CON) and some them were treated with THDIP and TH50 as above.

[b]Odds ratio: 1 is equivalent to no difference in the comparison; values <1 indicate increased probability in the first term; values >1 indicate increased probability in the second term.

[c]If unity (= 1) is not within the confidence interval, the comparison shows a significant difference ($P < 0.05$)*.

the control should be effective against seed- and soil-borne infection, but effective means to control the latter are scarce. When effective, biological control agents may have a potential to partially replace or extend the control spectrum of chemicals (Van den Boogert & Luttikholt, 2004). In this study, we tested whether the control of soil-borne inoculum of *R. solani* is attainable with three biological control agents in the field but found only one (*T. harzianum*) to provide significant control of the disease. Furthermore, the results indicated that a combination of chemical control with flutolanil and biological control with *T. harzianum* could be more securely effective against the different components of the *Rhizoctonia* disease complex than either method alone. The reason is that *T. harzianum* seems to exhibit control effects throughout the growing season. However, the combined use of flutolanil and *T. harzianum* tended to increase the disease control effects also at the early growth stages, which suggested that the dose of flutolanil used was not harmful to *T. harzianum*. Because little data were available on flutolanil sensitivity in *T. harzianum*, it was tested in an *in vitro* assay. Compared with *R. solani*, *T. harzianum* tolerated over 100-fold higher doses of flutolanil with little effect on growth, which

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Table 7 (a) The effect of biological and chemical disease control treatments on the incidence of progeny tubers with black scurf at harvest (120 days postplanting) in the marketable-sized progeny tubers in 2005. (b) Logit analysis was used to compare the incidences

(a) Treatment	Incidence of black scurf (%)
Rhizoctonia solani (RHIZ)	31.0
RHIZ + *Trichoderma harzianum* dip (THDIP)	50.0
RHIZ + *T. harzianum* 50g m^{-1} (TH50)	17.5
RHIZ + Moncut dip (MON)	44.0
RHIZ + MON + TH50	10.5

(b) Comparison	Odds ratio[a]	95% confidence interval[b]	*P*-value of Wald test
RHIZ versus			
RHIZ + MON + TH50	0.26*	0.07–0.96	0.0433
RHIZ + THDIP versus			
RHIZ + TH50	0.21*	0.06–0.70	0.0111
RHIZ + MON + TH50	0.12*	0.03–0.46	0.0020
RHIZ + MON versus			
RHIZ + TH50	0.27*	0.09–0.84	0.0238
RHIZ + MON + TH50	0.15*	0.04–0.55	0.0043

TH50, adding *T. harzianum* evenly into the furrow at a rate of 50 g m^{-1}; THDIP, dressing tubers with *T. harzianum* by dipping the potatoes briefly in 1% aqueous solution of Trianum-G; MON, dressing tubers with 0.15% aqueous solution of flutolanil; RHIZ, inoculation with *Rhizoctonia solani* but no disease control treatment.

[a]Odds ratio: 1 is equivalent to no difference in the comparison; values <1 indicate increased probability in the first term; values >1 indicate increased probability in the second term.

[b] If unity (=1) is not within the confidence interval, the comparison shows a significant difference ($P < 0.05$)*.

supports the combined use of flutolanil and *T. harzianum* against *R. solani*.

Seed dressing with flutolanil and its combined use with *T. harzianum* was effective in reducing the severity of stem canker, the disease syndrome caused by *R. solani* early in the growing season (Hide & Cayley, 1982; Read *et al.*, 1989; Scholte, 1989). At this growth stage, the pathogen can damage and kill sprouts, cause additional branching and increase in the shoot number but also delay emergence (Baker, 1970; Errampalli *et al.*, 2006). However, after the emergence of shoots, development of new stem canker symptoms ceases (Van Emden, 1965). These dynamics were also observed in the current study, as explained in results and illustrated in Fig. 1. It was found that observation of the sprout and stem numbers at several time points postplanting provided valuable information about progression of the disease, for which RSI alone was not sufficient.

Seed dressing with flutolanil had little effect on the control of the phases of the disease, which affected the quality of yield, including the proportion of marketable-sized tubers and black scurf-free tubers among them. For control of these symptoms that develop late in the growing season (and further away from the seed tuber), application of *T. harzianum* was required. These findings are supported with data from previous studies showing that *T. harzianum* is able to grow and colonise new stems, stolons and roots of the potato plant throughout the growing season, and thus maintain or increase its controlling efficacy (Harman, 2000; Howell, 2003). In contrast, the chemical fungicide used for seed dressing is effective only close to the seed tuber and probably diluted and washed away during the growing season. The current study is one of the few (Jager *et al.*, 1991), where the proportion of marketable-sized tubers could be increased by application a biological control agent in field conditions. The size distribution achieved following treatment with *T. harzianum* was similar to those of uninoculated control plants. Previously, *T. harzianum* has been found to reduce the proportion of small progeny tubers of plants inoculated with *R. solani* in a greenhouse experiment (Wilson *et al.*, 2008). However, the treatments could not prevent the significant yield losses caused by *R. solani*. Therefore, while the quality of yield was improved following application of *T. harzianum*, the total marketable yield remained lower than in the non-inoculated plants.

The data of this study suggest that the probability of stolon infection was reduced by adding *T. harzianum* in-furrow or dressing seed potatoes with flutolanil. These positive effects on the health of stolons were merely suggestive because they were not consistently observed. An important aspect of stolon infection and damage is that it may interfere with tuber development. In previous studies, stolon damage was found to correlate with an altered size distribution of progeny tubers, by increasing the proportion of small and large tubers (Cother, 1983; Scholte, 1989). However, in the present study, while flutolanil seemed to alleviate stolon infection, it could not improve tuber size distribution, in contrast to what was observed following treatment with *T. harzianum*. Furthermore, *T. harzianum* decreased the incidence of black scurf on progeny tubers, which was not observed with application of flutolanil alone. These results are consistent with the ability of *T. harzianum* to colonise stolons and roots, and hence maintain its effectiveness throughout the growing season (Harman, 2000; Howell, 2003).

Differences observed in the progression of disease and efficiency of disease control in 2004 and 2005 suggested that the variable weather conditions and differences in applying the inoculum played a role. In general, drought may be controlled by irrigation in field experiments, but the main difference between the two growing seasons of this study was the abundant rain falling in 2004 that could not be controlled. There is some information

available concerning the effect of moisture on the growth of some organisms used as antagonists (Wong & Griffin, 1974; Eastburn & Butler, 1991; Hussain *et al.*, 2005), but there is little information about the effect of moisture on biocontrol activity of the agents tested in this study (Burpee, 1990). Taken together, it is possible that, in 2004, soil moisture was excessive for the antagonists tested. However, a larger amount of inoculum was placed close to the seed tuber in 2004 than 2005. These factors may explain the low efficiency of control against *R. solani* in 2004. Temperatures did not significantly differ between the two growing seasons and were in the range suitable for the antagonists and *R. solani*. According to the manufacturers of the biocontrol agents tested, the suitable temperatures for the growth of *T. harzianum* is 10–34°C, for *S. griseoviridis* 15–25°C and for *G. catenulatum* 10–30°C. This is the first time that *S. griseoviridis* and *G. catenulatum* have been tested for the control of *R. solani* on potato. They are known to effectively control soil-borne fungal pathogens, such as *Fusarium* sp. and *Pythium* sp. on greenhouse-grown cucumber (Punja & Yip, 2003; Rose *et al.*, 2003) but had little effect on *R. solani* in this study.

Taken together, the results of this study suggest that *T. harzianum* can control *R. solani* in the field. Previously, similar results have been obtained in Israel (Elad *et al.*, 1980; Tsror *et al.*, 2001)m where the climate is much warmer and therefore the growth conditions are different from those of this study carried out in Northern Europe. The inoculum source (seed or soil) must be taken into account when planning control strategies against *R. solani* on potato (Tsror & Peretz-Alon, 2005). This study concentrated on soil-borne inoculum of *R. solani*, against which chemical control is considered less effective than against the seed-borne inoculum. Using healthy seed and adding the inoculum to soil free of natural soil-borne inoculum of *R. solani* allowed equalising the infection pressure in treatment plots, whereas most of the previous studies carried out in the field have relied on natural and possibly spatially variable infestation of the soil. This study is also one of the very few to report control of *R. solani* using seed dressing with a fungicide combined with an application of a biological control agent. In the majority of studies, fungicides have been applied directly into soil and their effect compared with those of the biological control agents (Elad *et al.*, 1980; Beagle-Ristaino & Papavizas, 1985; Jager & Velvis, 1986; Jager *et al.*, 1991; Virgen-Calleros *et al.*, 2000; Van den Boogert & Luttikholt, 2004). The data obtained in this study suggest that the combined application of an effective antagonist and seed dressing with a fungicide might provide the most effective means of controlling the different stages of disease and types of damage to the yield caused by *R. solani* over the whole growing season.

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