Improving the efficacy of biocontrol agents against soilborne pathogens

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Control of soilborne pathogens of tomato using a commercial formulation of *Streptomyces griseoviridis* and solarization

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Abstract

Mycostop® is a commercial formulation of strain K61 of *Streptomyces griseoviridis*. This strain was isolated from *Sphagnum* peat and can control or suppress some root rot and wilt diseases by colonizing the rhizosphere prior to pathogens. The present work was carried out to test the ability of the commercial formulation, combined or not with soil solarization, to control different diseases of greenhouse-grown tomato. Data obtained from four trials carried out over two years (2001 and 2002) demonstrated that *S. griseoviridis* could play a role in the integrated control of tomato soilborne diseases. This study is among the first to test *S. griseoviridis*'s effectiveness against corky root rot caused by *Pyrenochaeta lycopersici* when it is applied throughout the irrigation system (10 l of water per m^2). The biofungicide was very effective against *Fusarium oxysporum* f.sp. *lycopersici* and *Verticillium dahliae* in 2002 in artificially infested soils; however, in 2001 there was no statistically significant reduction of the vascular wilts compared to the control. Soil spraying was more effective than soil irrigation to control tomato wilts. The bacterial antagonist was not effective against Fusarium crown and root rot caused by *F. oxysporum* f.sp. *radicis-lycopersici*, when applied

alone, but was less effective when applied with *S. griseoviridis*. Soil solarization provided good control of *V. dahliae* and *F. oxysporum* f.sp. *lycopersici*, but also was slightly less effective when combined with *S. griseoviridis* A significant increase in fruit mass and a higher yield m^{-2} was recorded when solarization and the biofungicide were applied together in 2001. This indicated there may be a potential additive effect of the commercial biofungicide and solarization in increasing tomato yield; however, it was not consistent and generally not significantly different from the inoculated control. Metham sodium provided the most effective control of corky root and greatest yield increase of all the treatments evaluated.

Keywords: biological control, *Fusarium oxysporum* f.sp. *lycopersici*, *Fusarium oxysporum* f.sp. *radicis-lycopersici*, integrated control, *Pyrenochaeta lycopersici*, solarization, *Streptomyces griseoviridis*, tomato, *Verticillium dahliae.*

1. Introduction

In the last thirty years, glasshouse cultivation of tomato (*Lycopersicon esculentum* Mill.) has greatly increased in mild and warm climate regions (Albajes et al., 1999). The intensification of glasshouse tomato production has created optimal conditions for many pathogens (Jones et al., 1991). Collar rot, caused by *Rhizoctonia solani* Kühn and verticillium wilt, caused by *Verticillium* spp. (*V. dahliae* in Italy), occur in all tomato growing regions. *Pyrenochaeta lycopersici* Schneider & Gerlach, *Fusarium oxysporum* Schlechtend.: Fr. f.sp. *lycopersici* (Sacc.) Snyder & Hans. and *F. oxysporum* f.sp. *radicis-lycopersici* Jarvis & Shoemaker are also widespread pathogens whose severity varies with regional cultural practices.

Soilborne pathogens were effectively controlled by methyl bromide and tomato crops account for about 30% of the methyl bromide used in the world (UNEP, 1995) and 43% of its use in Italy (Gullino et al., 2003). Concern for the potential depletion of ozone by methyl bromide led to its inclusion among the substances controlled by the Montreal protocol which stated that use by

industrialized countries should have been eliminated by the end of 2004 (Bell et al., 1996; Gullino et al., 2003). Alternatives of biological control and soil solarization, alone or in combination, are promising methyl bromide replacement methods for controlling soilborne diseases of tomato.

Microbial antagonists have been widely studied as biological controls the last few years. *Penicillium oxalicum* reduced the incidence of *F. oxysporum* f. sp. *lycopersici* (Cal et al., 1997). *Trichoderma harzianum* and *T. koningii* controlled Fusarium root and crown rot (Bourbos et al., 1997). Non-pathogenic strains of *F. oxysporum*, obtained from suppressive soil, controlled Fusarium wilts (Alabouvette, 1988; Minuto et al., 1995a; Minuto et al., 1997). Many of these biological control agents, however, are still being tested and are not commercially available.

Actinomycetes have been recognized as sources of several secondary metabolites, antibiotics, and lytic enzymes that affect fungal growth (Goodfellow and Williams, 1983). A strain of *Streptomyces* sp., isolated from the rhizosphere of field-grown tomato, has been reported to suppress damping-off of tomato transplants caused by *R. solani* in a peat-based, soilless potting mix under greenhouse conditions (Sabaratnam and Traquair, 2002). Isolates of *Streptomyces* spp. were assessed in Finland for 20 years for their ability to control fungal diseases (Tahvonen, 1982) and a selected *S. griseoviridis* has been developed as a biofungicide by Verdera OY and tested on a wide range of organisms on glasshouse and field crops (Tahvonen and Avikainen, 1987; Lahdenperä, 1987). Mycostop® is a biofungicide based on the K61 strain of *S. griseoviridis* isolated from *Sphagnum* peat (White et al., 1990). Streptomycetes are active in the rhizosphere and the modes of action of species tested include antibiosis, lysis of fungal cell walls, competition and hyperparasitism (Mohammadi and Lahdenperä, 1992; Tapio and Pohto-Lahdenperä, 1991). The commercial product based on strain K61 can control or suppress some root rot and wilt diseases, caused by *Pythium* spp., *Fusarium* spp., *Rhizoctonia* spp. and *Phytophthora* spp., if it colonizes the rhizosphere prior to the pathogens. Mycostop® is registered in many European countries for use on different vegetables, herbs, and ornamentals, such as basil, cucumber, eggplant, melon, pepper, tomato, pumpkin, gerbera, cyclamen, and carnation (in Italy: Registration 10506 of the Italian Ministry of Health).

Solarization, carried out by covering the soil with plastic film during the hot season, has been widely exploited in a number of warm countries as well as in climatically marginal ones (Katan and DeVay, 1991). In Northern Italy, its efficacy is improved when applied with transparent mulch (30-40 μm thick) in the greenhouse (Garibaldi and Gullino, 1991). Many growers are skeptical about its effectiveness since it requires soil free of cultivation for at least four weeks. A practical possibility, often adopted to increase soil solarization efficiency and, especially, to enlarge the spectrum of efficacy of biocontrol agents, is the combination of both methods (Katan and DeVay, 1991). Previous studies clearly demonstrate the feasibility of a combination of soil solarization and biocontrol agents that target reducing the mulching period (Minuto et al., 1995b).

The present work was carried out under semi-commercial conditions to evaluate the effectiveness of *Streptomyces griseoviridis* isolate K61for control of different soilborne pathogens of greenhouse-grown tomato. Its combination with soil solarization also was investigated in order to evaluate potential beneficial effects of *S. griseoviridis* applied after mulching and to explore the possibility of reducing its duration. All of these activities were used to better define the formulation label in terms of application methods, according to European legislation (Directive 91/414 CE) related to pesticide registration. In this regard, different dosages and application methods were assessed for their disease control effectiveness. A general objective was to study the relationship between plant productivity and disease incidence as they were affected by the different treatments applied. To increase the incidence of some diseases during specific experiments, some target pathogens were inoculated into the soils.

2. Materials and methods

2.1. Layout of tomato trials

During 2001-2002, four experimental trials were carried out with different pathogens in four different glasshouses of the Experimental Station (CeRSAA) of the Chamber of Commerce of

Savona located in Albenga (Italian Riviera). Experiments used tomato cv. Cuore di Bue planted in soil systems (sandy loam: sand 75%, silt 20%, clay 5%; pH, 8.1; organic matter content, 2.5%; cation exchange capacity, 8.5 meq $100g^{-1}$ soil) to assess the efficacy of biological and physical methods against different soilborne pathogens. A randomized complete block design was used with four replicates per trial. The cultivation density was 2 plants m^{-2}. During the first and second trial, 16 plants per replicate were used; during the third and fourth trial, the number of plants per replicate was increased to 40. The efficacy against tomato soilborne pathogens of a commercial formulation of *Streptomyces griseoviridis* alone, or combined with solarization, was compared to the efficacy of chemical pesticides used for soil disinfestations in intensive horticulture (metham sodium) or known for its efficacy against vascular diseases (benomyl). The layout of the four trials is summarized in Table 1.

2.2 Soil infestation with pathogen inocula

In order to evaluate the effectiveness of Mycostop® under semi-commercial conditions, the experimental activities were carried out in greenhouses where, without any soil disinfestation, tomato had been grown at least the two previous growing seasons and showed some symptoms of vascular wilt (*F. oxysporum* f.sp. *lycopersici* and *V. dahliae*) and basal root rot (*R. solani*, *F. oxysporum* f.sp. *radicis-lycopersici*). Nevertheless, to achieve a uniform soil infestation and higher disease pressure before solarization, fumigation or Mycostop® application, artificial soil inoculations of some pathogens were executed. Two strains of each pathogen (*F. oxysporum* f.sp. *radicis-lycopersici*, *F. oxysporum* f.sp. *lycopersici*, *V. dahliae*, or *P. lycopersici*), freshly isolated from tomato plants, were grown on autoclaved wheat kernels. Inocula were incorporated into the soil by rototilling to a depth of 15 cm 7-10 d prior to soil treatments at the dosages reported in Table 1. Soil was kept moist for one week by periodic sprinkler irrigation (5-10 mm).

2.3. Soil treatments

153 In trial 1, the efficacy of the commercial biocontrol agent *S. griseoviridis* (Mycostop®; Verdera
154 OY, Helsinki, Finland) applied at 10^7 colony forming units (cfu) m^{-2} was compared with the
155 efficacy of solarization and a combination of the two methods. *S. griseoviridis* was delivered as
156 suspension of the powder formulation (0.1 g m^{-2} with 0.5 l of water per m^2) immediately after
157 transplanting and repeated three times every four to five weeks.
158 Solarization was accomplished by covering the soil for 26 d with standard low-density polyethylene
159 (LDPE, Eiffel, Fontanellato, Italy, 40 μm thick). Soil was irrigated (30-35 mm of water m^{-2}) the day
160 before mulching. In the combination antagonist and solarization, the biofungicide was applied to the
161 soil immediately at the end of solarization. In trial 2 the same treatments were compared but *S.*
162 *griseoviridis* was applied at 10^7 cfu. and 5 x 10^6 cfu. m^{-2} (0.05 g of commercial formulation in 0.25
163 l of water m^{-2}).
164 Soil solarization was not evaluated in 2002, and three different methods of application of *S.*
165 *griseoviridis* were compared with fumigation by metham sodium (trial 3) or benomyl (trial 4).
166 Mycostop® was applied at 10^7 cfu m^{-2} (0.1 g m^{-2}) or at 5x10^6 cfu m^{-2} (0.05 g m^{-2}) by irrigation or at
167 10^7 cfu m^{-2} (0.1 g m^{-2}) by spraying. Irrigation was accomplished by drenching the soil with 5 l m^{-2}
168 of water immediately before applying the biofungicide in 2 l m^{-2} with another 3 l m^{-2} immediately
169 afterwards. When spraying, the commercial formulation was delivered with 0.5 l m^{-2} of water.
170 Metham sodium (Vapam, SIPCAM, 32.7% a.i. corresponding to 380 g a.i./ l formulation) was
171 applied as a water suspension (76 g a.i. m^{-2}) using 15 l m^{-2} of water. Benomyl (Benlate, DuPont,
172 50% a.i.) was distributed as a water suspension (2 g a.i. m^{-2}) using 10 l m^{-2} of water.
173 In every trial a not treated control was introduced.
174
175 *2.4. Tomato transplant and cultural practices*
176 Tomato plants, 40-50 d old, belonging to the cultivar Cuore di Bue, were transplanted 7 d after soil
177 treatment. Plants were placed in two rows per bed approximately 15-20 cm from the bed edge and
178 40-50 cm apart. Plants were fertilized in the drip irrigation system five times at 10 day intervals

with a solution of $N:P_2O_5:K_2O$ (20:10:10) at 100kg/ha each time. Three insecticide sprays to reduce the presence of virus (TSWV, TYLC) vectors were applied at 7-10 day intervals after transplanting.

2.5. Data collection and analysis

Infection by *F. oxysporum* f.sp. *radicis-lycopersici* (FORL), *P. lycopersici* and the two vascular pathogens, *F. oxysporum* f.sp. *lycopersici* (FOL) and *V. dahliae*, were assessed at the beginning of each trial and several times later to correctly identify the pathogens by plating vascular tissues from diseased plants on potato dextrose agar (PDA, Merck) or Komada's semi-selective medium (Komada, 1975). Natural infections of *Rhizoctonia solani*, which frequently occurred during the cropping period, were also evaluated. Disease development was evaluated every 10-14 d by counting and eliminating symptomatic and collapsed plants (disease pressure). The final data is reported for all but trial 1and also indicates the number of healthy plants. The yield was evaluated on healthy plants by weighing and counting the number of fruits per plant. Data are expressed as number and mass of marketable fruits per plant. All data collected were statistically analyzed according to Duncan's Multiple Range Test (P=0.05).

3. Results

3.1 Trial 1

The inoculated control plants (Table 2) at the end of the trial had a high level of infection by the three pathogens inoculated (*F. oxysporum* f.sp. *radicis-lycopersici*, *F. oxysporum* f.sp. *lycopersici* and *V. dahliae*). In addition, a very high percentage (31.3%) of the control plants were naturally infected by corky root rot (*P. lycopersici*). The application of *S. griseoviridis* at 10^7 cfu m^{-2} partially controlled the natural infection of corky root rot (18.8%), but was ineffective against the wilt pathogens (12.5%) and Fusarium crown and root rot (20.8%). Nevertheless, two months after planting, the antagonist had partially reduced the incidence of the inoculated pathogens (Table 2).

Solarization, applied alone, significantly reduced vascular wilts (2.1%), and also Fusarium crown and root rot (2.1%). The plots treated with solarization and *S. griseoviridis* were severely infected by *F. oxysporum* f.sp. *radicis-lycopersici* (18.8%), but the wilt pathogens (2.1%) and corky root (18.8%) were controlled satisfactorily (Table 2).

No significant difference was observed in the number of fruits produced per healthy plant and in the mass of fruits per healthy plant (Table 3). The average mass of tomato fruits was significantly higher with solarization and *S. griseoviridis* (138.8 g), than the control (110.0 g).

3.2 Trial 2

The second trial was carried out at the same time as the first one, from July to December 2001. In this trial, the plots were inoculated with *F. oxysporum* f.sp. *radicis-lycopersici*, which infected 21.2% of the control plants (Table 4). Corky root natural infestation was very low. Only a few plants were attacked by *R. solani* at the beginning of the cropping season and no differences were observed between treated and untreated plots.

Neither the physical or biological control method, nor their combination, significantly reduced the incidence of Fusarium crown and root rot. This confirms the highly aggressive nature of the causal agent and its difficulty to control. Neither formulation of *S. griseoviridis*, alone, controlled the wilt pathogens, while solarization alone and the combination of antagonist and physical treatment were able to control them.

Solarization, alone or combined with *S. griseoviridis* controlled the wilt pathogens, while neither formulation of *S. griseoviridis* controlled the wilt pathogens.

A lower mass and number of fruits per plant were observed in the solarized plots (Table 5). Moreover, the yield per m^2 data showed that the application of *S. griseoviridis* combined with solarization increased yield (428 g) statistically more than the control (270 g).

3.3 Trial 3

231 This trial was carried out in a soil artificially infested with *P. lycopersici* where severe infection by
232 corky root rot had been observed in the previous tomato crop. During the experiment, a low
233 incidence of *F. oxysporum* f.sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *lycopersici* was
234 observed (Table 6). The incidence of *F. oxysporum* f.sp. *radicis lycopersici* was not statistically
235 different between the treatments and the inoculated control. Attacks of *F. oxysporum* f.sp.
236 *lycopersici* registered in the control were low (1.3%) but they were statistically higher in the plots
237 treated with metham sodium.
238 At the end of the cropping cycle, the average percentage of the root surface of surviving plants
239 attacked by *P. lycopersici* was calculated to determine the severity of infection (Table 6). In the
240 inoculated control, 10.3% of the root surface showed symptoms of corky root compared with only
241 5.9% in the plots treated with metham sodium. All of the treatments containing *S. griseoviridis*
242 provided medium levels of efficacy regardless of the mode of application or the dosage applied.
243 Disease severity in the plots treated with the bacterial antagonist was not different from the plots
244 treated with metham sodium or the control.
245 In contrast, the incidence of *P. lycopersici* was very high in the control (55.3%) and lowest in the
246 plots fumigated with metham sodium (7.4%). Application of the biofungicide through the irrigation
247 system at 0.05 and 0.1 g per m^2, significantly reduced the incidence of infections compared with the
248 untreated control. The application of *S. griseoviridis* by spraying at 0.1 g per m^2 was not effective.
249 None of the treatments significantly affected either the quality or the yield, represented by the
250 average mass of single fruit or their quality (Table 7). The total mass and number of fruits produced
251 per plant were significantly higher (yield) in the plots fumigated with metham sodium. *S.*
252 *griseoviridis* given by irrigation at the rate of 10^7 cfu m^{-2} nearly doubled the yield (mass/plant)
253 compared to the untreated inoculated control, even though the yield increase was not statistically
254 significant.
255
256 *3.4 Trial 4*

Natural infection by *R. solani* in 2002, caused slight damage to the crop with no significant differences among the treatments (Table 8). No infection by *V. dahliae* and only a low infection of *F. oxysporum* f.sp. *radicis-lycopersici* were recorded in 2002and there were no significant effects of any treatment on the incidence of disease. All of the treatments generally limited the incidence of Fusarium wilt but only *S. griseoviridis* applied by spraying at 10^7 cfu m^{-2} provided a protection significantly different from the control. Heavy yield losses caused by a strong reduction in fruit set due to adverse temperature conditions (data not published). Tomato cv. Cuore di Bue is particularly sensitive to strong temperature changes.

4. Discussion

The aim of this work was to test the ability of a commercial formulation of *S. griseoviridis* combined or not with soil solarization to control several diseases of greenhouse-grown tomato.

Tomato provides a good example of how the use of biocontrol agents can be introduced into practice as an IPM strategy (Albajes et al., 1999). Examples of integrated methods are provided by the combination of biocontrol agents applied as seed dressing, with soil solarization (Gullino, 1998; Minuto et al., 1995b; Spadaro and Gullino, 2005). Sivan and Chet (1993) combined *Trichoderma harzianum* with soil solarization under field conditions to obtain significant control of Fusarium crown and root rot of tomato and a significant yield increase.

Data obtained over two years (2001 and 2002) demonstrated that *S. griseoviridis* could play a role in integrated control of tomato diseases. In some cases, the effect of biological control was more pronounced early after transplanting (Table 2), but were less evident at the end of the experiment (Table 2). This seemed to confirm the need for information related to the microorganism's ability to survive in a natural soil.

This study shows that biocontrol agents can be effective against corky root rot (Bochow, 1989). *S. griseoviridis* is a root colonizer and stimulates root growth during rhizosphere colonization (Kortemaa et al., 1994). In some cases, stimulated plant growth could explain the enhanced yield

283 results (trial 1 and trial 2) when the antagonist was combined with soil solarization.

284 The effectiveness of Mycostop® against Fusarium wilt of tomato was satisfactory in 2002 (Table 8)
285 when applied to artificially infested soil. It was not effective against Fusarium and Verticillium
286 wilts of tomato in 2001 (Tables 2 and 4).Spraying was a more effective method of application than
287 irrigation, contrary to the results obtained with corky root. In trials carried out previously, strain
288 K61 formulated as Mycostop® had exhibited partial efficacy against *F. oxysporum* f.sp. *basilici*
289 (Minuto et al., 1997), but the same formulation was not effective when tested as a root dip (10^6 cfu
290 ml^{-1}) and soil drench (10^8 cfu ml^{-1}) in glasshouse experiments to control *F. oxysporum* f.sp. *dianthi*
291 on carnation (Garibaldi et al., 1990). Biocontrol efficacy is apparently determined by the method of
292 application of the biocontrol agents to the ecosystem, the number of treatments and the ability of the
293 selected strain to adapt to different environments.

294 The ineffectiveness of *S. griseoviridis* against Fusarium crown and root rot could be explained
295 because *F. oxysporum* f.sp. *radicis-lycopersici* has airborne microconidia that reinfest disinfested
296 soils (Rowe et al., 1977). Soil treatments, therefore, could *a priori* be expected to provide
297 inadequate protection against this pathogen (Rowe and Farely, 1981).

298 The combination of the biocontrol agent with soil solarization generally increased disease control
299 and yield. Soil solarization, as previously documented before (Katan and DeVay, 1991), provided
300 good control of *V. dahliae* and *F. oxysporum* f.sp. *lycopersici*. In trial 1, solarization was also
301 effective against Fusarium crown and root rot, but the result was not confirmed by trial 2. The
302 combination of soil solarization and *S. griseoviridis* was effective against Fusarium and
303 Verticillium wilts and corky root. Previous experiments showed that soil solarization could also be
304 exploited for corky root control (Garibaldi and Tamietti, 1983).

305 In general, no significant differences in fruit yield or quality were observed when solarization and
306 Mycostop® were applied together.

307 Metham sodium fumigation provided a high level of control of *P. lycopersici* and a superior mass
308 and number of fruits per plant produced. The efficacy of *S. griseoviridis*, although encouraging, is

not competitive with the effectiveness of this fumigant or as effective as methyl bromide, chloropicrin, or dazomet. The incidence of Fusarium wilt in the fumigated plots was significantly higher than the other treatments and the control. This may have been because of the low incidence of corky root, but also could have been because metham sodium does not always guarantee a complete protection of the fumigated soil when used at the low dosage of 250 ml m^{-2} of the commercial formulation (32.7 % a.i.).

S. griseoviridis could play a role in integrated control of different soilborne diseases but alone could not control the main soilborne diseases of tomato. Its adoption after a solarization treatment may be used to reduce the period of solarization. Solarization is mainly inconvenient by preventing use of the soil during the hot season, but possesses great potential as an alternative to fumigation for soil disinfestation. Solarization alone controlled at least two serious soilborne pathogens; however, the combination of soil solarization and *S. griseoviridis* was effective against Fusarium and Verticillium wilts and somewhat against corky root even though the biofungicide did not improve control of the individual pathogens. Moreover, at least in one case, the combination of physical and biological methods increased the yield of tomato plants per area unit.

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406 **Tables**

407

408 Table 1 – Experimental protocols for the four trials carried out against different soilborne pathogens

409 on tomato cv. Cuore di bue.

	1st trial	2nd trial	3rd trial	4th trial
Artificial inoculation*	FORL (30 g m^{-2}), FOL (30 g m^{-2}), *Verticillium dahliae* (15 g m^{-2})	FORL (20 g m^{-2})	*Pyrenochaeta lycopersici* (30 g m^{-2})	FOL (35 g m^{-2})
Treatments with *Streptomyces griseoviridis* by irrigation (I) or by spraying (S)	10^7 cfu m^{-2} (S)	10^7 cfu m^{-2} (S) $5x10^6$ cfu m^{-2} (S)	10^7 cfu m^{-2} (I) $5x10^6$ cfu m^{-2} (I) 10^7 cfu m^{-2} (S)	10^7 cfu m^{-2} (I) $5x10^6$ cfu m^{-2} (I) 10^7 cfu m^{-2} (S)
Solarization (26 d)	alone and followed by Mycostop® application	alone and followed by Mycostop® application	---	---
Chemical control	---	---	Metham sodium (76 g a.i. m^{-2})	Benomyl (2 g a.i. m^{-2})
Planting	July 2001	July 2001	April 2002	July 2002
Harvest	December 2001	December 2001	July 2002	October 2002

410 *All artificially inoculated pathogens were propagated on wheat kernels. Dose expressed as mass of

411 infected kernels m^{-2}. FOL: *Fusarium oxysporum* f.sp. *lycopersici*. FORL: *F. oxysporum* f.sp.

412 *radicis-lycopersici*.

413

414 Table 2 – Effect of treatments with *Streptomyces griseoviridis*, soil solarization or their combination
415 on the number of tomato plants infected with *Rhizoctonia solani*, *Fusarium oxysporum* f.sp. *radicis-*
416 *lycopersici* (FORL), *F. oxysporum* f.sp. *lycopersici* (FOL), *Verticillium dahliae* and *Pyrenochaeta*
417 *lycopersici* after two months (left) and six months (right) (trial 1, 2001)

Treatment	% plants infected by (2 months after planting)				% plants infected by (end of the trial)				
	R. solani	FORL	FOL + *V. dahliae*	Total	*R. solani*	FORL	FOL + *V. dahliae*	*P. lycopersici*	Total
Inoculated control	2.1 a*	0 a	12.5 b	14.6	2.1 a*	16.7 b	18.8 b	31.3 B	68.9
S. griseoviridis 10^7 cfu m^{-2} in 0.5 l m^{-2}	2.1 a	0 a	4.2 ab	6.3	2.1 a	20.8 b	12.5 b	18.8 A	54.2
Solarization	0 a	0 a	0 a	0	0 a	2.1 a	2.1 a	27.1 Ab	31.3
Solarization + *S. griseoviridis* 10^7 cfu m^{-2} in 0.5 l m^{-2}	0 a	0 a	0 a	0	0 a	18.8 b	2.1 a	18.8 A	39.7

418 *Means of the same column followed by the same letter do not differ according to Duncan's
419 Multiple Range Test (P=0.05)

420

421 Table 3 – Effect of treatments with *Streptomyces griseoviridis*, soil solarization or their combination
422 on tomato yield (trial 1, 2001)

Treatment	Mass (g)/fruit	n° fruit/plant	Mass (g)/plant
Inoculated control	110.0	6.09 a	661.1 a
S. griseoviridis 10^7 cfu m^{-2} in 0.5 l m^{-2}	110.2	7.64 a	831.6 a
Solarization	123.8	6.02 a	702.2 a
Solarization + *S. griseoviridis* 10^7 cfu m^{-2} in 0.5 l m^{-2}	138.8	5.77 a	781.9 a

423 *See Table 2

424

425 Table 4 – Effect of treatments with *Streptomyces griseoviridis*, soil solarization or their combination
426 on the number of tomato plants infected with *Rhizoctonia solani*, *Fusarium oxysporum* f.sp. *radicis-*
427 *lycopersici* (FORL), *F. oxysporum* f.sp. *lycopersici* (FOL), *Verticillium dahliae* and *Pyrenochaeta*
428 *lycopersici* (trial 2, 2001)

Treatment	% plants infected by				
	R. solani	FORL	FOL + *V. dahliae*	*P. lycopersici*	Total
Inoculated control	0.0 a*	21.2 a	28.8 b	0.0 a	50.0
Solarization	1.9 a	27.0 a	2.9 a	2.9 a	34.7
S. griseoviridis 10^7 cfu m^{-2} in 0.5 l m^{-2}	1.9 a	22.1 a	23.1 b	1.0 a	48.1
S. griseoviridis $5x10^6$ cfu m^{-2} in 0.25 l m^{-2}	4.2 a	25.3 a	24.2 b	1.3 a	55.0
Solarization + *S. griseoviridis* 10^7 cfu m^{-2} in 0.5 l m^{-2}	0.0 a	26.0 a	7.8 a	1.0 a	34.8

429 *See Table 2

430

431 Table 5 – Effect of treatments with *Streptomyces griseoviridis*, soil solarization or their combination
432 on the yield of tomato plants (trial 2, 2001)

Treatment	Mass (g) / fruit	n° fruit / plant	Mass (g) / plant	Mass (g) / m^2
Inoculated control	76.7 a*	3.0 a	241.5 a	270 b
Solarization	79.4 a	2.6 b	201.1 b	359 b
S. griseoviridis 10^7 cfu m^{-2} in 0.5 l m^{-2}	83.4 a	3.0 a	251.6 a	314 b
S. griseoviridis $5x10^6$ cfu m^{-2} in 0.25 l m^{-2}	84.1 a	3.1 a	253.4 a	326 b
Solarization + *S. griseoviridis* 10^7 cfu m^{-2} in 0.5 l m^{-2}	85.5 a	3.2 a	272.6 a	428 a

433 *See Table 2

434

435 Table 6 - Effect of treatments with *Streptomyces griseoviridis* and fumigation with metham sodium
436 on infection of tomato plants with *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL), *F.*
437 *oxysporum* f.sp. *lycopersici* (FOL) and *Pyrenochaeta lycopersici,* and the severity of infection by *P.*
438 *lycopersici* (trial 3, 2002)

Treatment	% plants infected by				Severity (%) in the tomato roots infected
	FORL	FOL	*P. lycopersici*	Total	by *P. lycopersici*
Inoculated control	5.8a*	1.3a	55.3c	62.4	10.3b
S. griseoviridis $5x10^6$ cfu m^{-2} by irrigation[a]	6.3a	1.9a	37.5b	45.7	9ab
S. griseoviridis 10^7 cfu m^{-2} by irrigation[a]	8.2a	0.6a	38.4b	47.2	7.5ab
S. griseoviridis 10^7 cfu m^{-2} by spraying[b]	3.8a	2.5a	46.2bc	52.5	8.2ab
Metham sodium (32.7% a.i.) 76 g a.i. m^{-2}	9.6a	6.3b	7.4a	23.3	5.9a

439 *See Table 2; [a] Five l of water applied per m^2 immediately before inoculation, 2 with and 3
440 immediately after applying the commercial product; [b] 0.5 l of water distributed per m^2 with the
441 biological product.

442

443 Table 7 – Effect of treatments with *Streptomyces griseoviridis* and fumigation with metham sodium
444 on tomato yield (trial 3, 2002)

Treatment	Mass (g) / fruit	n° fruit / plant	Mass (g) / plant
Inoculated control	75.4a*	9b	639b
S. griseoviridis $5x10^6$ cfu m^{-2} by irrigation[a]	71.7a	12b	881b
S. griseoviridis 10^7 cfu m^{-2} by irrigation[a]	77.4a	16b	1268b
S. griseoviridis 10^7 cfu m^{-2} by spraying[b]	76.3a	14b	1087b
Metham sodium (32.7% a.i.) 76 g a.i. m^{-2}	73.6a	34a	2491a

445 *See Table 2; [a,b] See Table 6.

446

447 Table 8 – Effect of treatments with *Streptomyces griseoviridis* and drenching with benomyl on
448 infection of tomato plants with *Rhizoctonia solani*, *Fusarium oxysporum* f.sp. *radicis-lycopersici*
449 (FORL), *F. oxysporum* f.sp. *lycopersici* (FOL) (trial 4, 2002)

Treatment	% plants infected by						
	R.solani		FORL		FOL		Total
Inoculated control	3.3	a*	7.6	a	35.9	b	46.8
S. griseoviridis $5x10^6$ cfu m^{-2} by irrigation[a]	1.9	a	7.6	a	27.6	ab	37.1
S. griseoviridis 10^7 cfu m^{-2} by irrigation[a]	1.4	a	10.0	a	17.0	ab	28.4
S. griseoviridis 10^7 cfu m^{-2} by spraying[b]	0.6	a	14.5	a	13.9	a	29.0
Benomyl (50% a.i.) 2 g a.i. m^{-2}	1.3	a	14.0	a	21.7	ab	37.0

450 *See Table 2; ; [a,b] See Table 6.