Plant Disease • 2017 • 101:224-232 • http://dx.doi.org/10.1094/PDIS-07-16-1020-RE

Repeated Applications of a Nonpathogenic *Streptomyces* Strain Enhance Development of Suppressiveness to Potato Common Scab

Lea H. Hiltunen and **Jani Kelloniemi,** Natural Resources Institute Finland (Luke), 90014 University of Oulu, Finland; and **Jari P. T. Valkonen,** Department of Agriculture Sciences, 00014 University of Helsinki, Finland

Abstract

Potato common scab caused by several *Streptomyces* spp. is an important disease with no effective methods of control. Suppressiveness against common scab can develop in soil as a result of long-term potato monoculture and has been associated with nonpathogenic *Streptomyces* spp. To determine whether the development of scab suppressiveness could be enhanced, the effect of repeated applications of an antagonistic *Streptomyces* strain on common scab was investigated in a long-term field trial over 5 years. *Streptomyces* strain 272 applied annually at planting consistently suppressed development of common scab symptoms. On scab-susceptible potato cultivar Bintje, strain 272 reduced disease severity, on average, by 43%; whereas, on the scab-tolerant Nicola, the strain reduced both disease incidence and severity by 43 and 59%, respectively. Regardless of disease pressure, the combined use of strain 272 and the tolerant cultivar reduced the scab coverage to a negligible level. After a single application of strain 272, efficient disease suppression did not persist in the soil to the following growing season. However, when strain 272 was applied in three or more consecutive years, the soil remained suppressive to scab for at least 2 years beyond the last application, suggesting that, with repeated applications, it may be possible to enhance development of scab suppression in soil.

Disease-suppressive soils represent an attractive option for combating diseases that lack effective control methods. In these soils, disease development is reduced even in the presence of a virulent pathogen and susceptible host under environmental conditions conducive for the disease (Cook and Baker 1983). General suppression is thought to be directly related to the total amount of microbial activity in a given soil rather than operating through the action of specific microorganisms, whereas specific suppression involves the activity of an individual or selected group of microorganisms that are antagonistic toward the pathogen (Mazzola 2002). Suppressive soils have been reported for a number of fungal and bacterial plant pathogens and plant-parasitic nematodes (Kinkel et al. 2011; Mazzola 2002, 2007; Weller et al. 2002). However, for most disease-suppressive soils, the microbes and mechanisms involved in pathogen control are unknown. Disease suppression is thought to be a result of complex interactions, both direct and indirect, among the pathogen, host, and beneficial and other rhizosphere microorganisms, as well as chemical and physical properties of the soil environment (Höper and Alabouvette 1996; Janvier et al. 2007; Mendes et al. 2011; Raaijmakers et al. 2009; Weller et al. 2002). *Streptomyces* spp. have been associated with the development of disease-suppressive soils against many fungal and bacterial plant pathogens (Bakker et al. 2010; Hjort et al. 2010; Kinkel et al. 2012; Meng et al. 2012; Postma et al. 2008; Sagova-Mareckova et al. 2015).

Common scab is a harmful disease of potato (*Solanum tuberosum* L.) caused by a number of thaxtomin-producing *Streptomyces* species (Dees and Wanner 2012). It degrades the crop quality by producing superficial, pitted, or raised lesions on the tuber surface and thereby reduces the market value of the crop. It has also been reported to reduce yield (Hiltunen et al. 2005) and affect tuber size (Wanner and Kirk 2015). Although knowledge regarding common scab has expanded considerably over recent years, effective management of the disease remains elusive (Dees and Wanner 2012). Suppressiveness against potato common scab has been observed in some soils as a result of long-term potato monoculture (Lorang et al. 1989; Meng et al. 2012; Menzies 1959) and has been associated with nonpathogenic *Streptomyces* spp. (Bowers et al. 1996; Kinkel et al. 2012; Liu et al. 1995, 1996; Lorang et al. 1995; Meng et al. 2012). Consequently, many efforts have focused on the utilization of nonpathogenic *Streptomyces* spp. or strains as potential biocontrol agents against common scab (Agbessi et al. 2003; Beauséjour et al. 2003; Bowers et al. 1996; Doumbou et al. 1998; Hiltunen et al. 2009; Jobin et al. 2005; Liu et al. 1995; Neeno-Eckwall et al. 2001; Palaniyandi et al. 2013; Prévost et al. 2006; Ryan and Kinkel 1997; Ryan et al. 2004; Schottel et al. 2001; Wanner et al. 2013). Despite some successes, the results have been inconsistent, especially under field conditions.

Although continuous crop monoculture can result in development of suppressiveness to common scab, the duration required may be years, and monoculture may have other adverse effects on potato production. There have been efforts to actively enhance naturally occurring soil suppressiveness against common scab with management practices such as crop rotation, organic amendments, and microbial inoculants (Larkin 2008; Larkin and Halloran 2014; Larkin et al. 2010, 2011; Sun et al. 2015; Tomihama et al. 2016; Wiggins and Kinkel 2005 a,b). However, the effects have been variable and often soil, site, and amendment specific.

Apart from a few studies (Larkin et al. 2010; Peters et al. 2004), only short-term effects on disease reduction and soil microbial community have been investigated; hence, there is little information on the cumulative or long-term effects of microbial inoculants on development of disease suppression and soil indigenous microbial communities. The objective of the present study was to investigate whether the development of suppressiveness to common scab could be enhanced by repeated applications of a nonpathogenic *Streptomyces* strain with a known antagonistic activity to common scab. The specific aims of this study were to (i) determine whether the nonpathogenic *Streptomyces* isolate 272 was able to suppress the development of common scab under field conditions, (ii) investigate whether repeated annual applications of the isolate 272 enhances development of scab suppressiveness in field soil, and (iii) evaluate the persistence of suppressiveness in the soil after the applications of isolate 272 were discontinued.

Materials and Methods

Characterization of *Streptomyces* strain 272. *Streptomyces* strain 272 used in this study was originally isolated from a common scab lesion on a 'Hertha' potato tuber grown in Tyrnävä in northern

Corresponding author: L. H. Hiltunen; E-mail: lea.hiltunen@luke.fi

Accession numbers KX534366, KX534367, KX534368, and KX534369.

Accepted for publication 20 September 2016.

Finland (64 to 65° N) and characterized for morphological and physiological traits (Lindholm et al. 1997). For further characterization, strain 272 was grown on glucose yeast malt extract (GYM) (4.0 g of glucose, 4.0 g of yeast extract, 10 g of malt extract, and 1,000 ml of distilled water, pH 7.2) agar at 28°C for 7 days. The cells were scraped from the plate into an Eppendorf tube and ground with sterile silver sand and a class rod. DNA was extracted using the E.Z.N.A. SP Plant DNA Mini Kit (Omega Bio-Tek, Norcross, GA) according to the manufacturer's instructions. The quality and amount of DNA were determined with NanoPhotometer (Implen GmbH, München, Germany).

The primers developed for the 16S ribosomal RNA gene sequences (Lehtonen et al. 2004) were used to detect *Streptomyces scabies* and *S. turgidiscabies* by polymerase chain reaction (PCR). The PCR products were further subjected to restriction with *Hpy*99I, where cleavage of the internal transcribed spacer (ITS) region of the 16S operon sequence is used to distinguish *S. scabies* from *S. europaeiscabiei* (Flores-González et al. 2008). To further explore the relationship between strain 272 and the scab-causing *Streptomyces* spp., the housekeeping genes *atpD*, *recA*, *rpoB*, and *trpB* were PCR amplified and sequenced using the primers of Labeda (2011). The obtained sequences of strain 272 were aligned with sequences available in public nucleic acid data repositories. Phylogenetic relationships between strain 272 and the closest species were determined using the neighbor-joining algorithm (Saitou and Nei 1987) with 1,000 bootstrap replications. Database accession numbers for the sequences used (for *aptD*, *recA*, *rpoB*, and *trpB*, respectively) were *S. bottropensis*: HQ014980.1, HQ015012.1, HQ015044.1, and HQ014948.1; *S. europaeiscabiei*: HQ014975, HQ015007, HQ015039, and HQ014943; *S. galbus*: JRHJ01000022.1, JRHJ01000006.1, JRHJ01000054.1, and JRHJ01000050.1; *S. scabies*: HQ014966.1, HQ014998.1, HQ015030.1, and HQ014934.1; *S. stelliscabiei*: JPPZ01000636.1, JPPZ01000386.1, JPPZ01000529.1, and JPPZ01000352.1; *S. torulosus*: HQ014961.1, HQ014993.1, HQ015025.1, and HQ014929.1; and *S. turgidiscabies*: HQ014968.1, HQ015000.1, HQ015032.1, and HQ014936.1.

The ability of strain 272 to cause scab symptoms on potato tubers was checked under controlled conditions. Pathogen-free in vitro shoots of 'Siikli' potato obtained from the Finnish Seed Potato Centre Ltd (Tyrnävä, Finland) were micropropagated on Murashige and Skoog medium (Murashige and Skoog 1962). The 5-week-old seedlings were planted in 300-ml pots filled with growing medium containing washed sand (grain size 0.5 to 1.2 mm, OR-6415990100243; Weber, Riihimäki, Finland) and peat (Kekkilä OPM 420 W R8045, Lahti, Finland) (1:4, vol/vol). Strain 272 was grown in GYM broth on a rotary shaker (180 rev min^{-1}) at 28°C for 3 days and the number of CFU was determined by dilution plating on GYM agar. The culture was diluted 50-fold with fresh, sterile GYM broth before it was applied at the base of the micropropagated potato tubers (5 ml/plant). The negative controls were treated with the corresponding amount of sterile GYM broth. *Streptomyces* strain 364 grown in oat meal broth (OMB), as described (Hiltunen et al. 2006), was used as a scab-inducing positive control. The OMB culture was diluted 10-fold with fresh, sterile OMB and used to inoculate sterile vermiculite (167 ml $liter^{-1}$) in autoclave bags, and the inoculum was incubated at 28°C for 2 weeks. The vermiculate inoculum was thoroughly mixed with the growing medium (1:10, vol/vol) and placed in pots before planting a micropropagated potato in each pot. All the plants were grown in a growth cabinet at 21°C (day) and 18°C (night) under a 16-h photoperiod (215 μmol m^{-2} s^{-1}) and 40% relative humidity. Plants were watered twice per week with tap water (25 to 30 ml/pot) and once per week with a 0.13% (vol/vol) liquid fertilizer (NPK 13-7-20, Green Care Puutarhan kesä; Yara, Vlaardingen, The Netherlands). There were 14 replicate plants in each treatment. Tubers were harvested after 7 weeks and the percentage of tubers with scab lesions was recorded.

To further verify that *Streptomyces* strain 272 is not pathogenic, it was used to inoculate radish seedlings (*Raphanus sativus* L.) using a modified assay, as previously described (Dees et al. 2013). Seed ('French Breakfast 3') were disinfected in 0.5% sodium hypochlorite solution for 1 min and then rinsed with three changes of sterile distilled water. After drying in a laminar air flow, the seed (12 seeds/plate) were placed on an 8-day-old strain 272 culture growing on oat meal agar (OMA). OMA was prepared as described for OMB (Hiltunen et al. 2006), with the addition of agar (18 g $liter^{-1}$). Seed germinated on OMA without bacteria were used as a negative control, whereas those germinated with the pathogenic *Streptomyces* strain 364 (Hiltunen et al. 2005), originally identified as *S. scabies* (Kreuze et al. 1999) and later assigned to *S. europaeiscabiei* (Hiltunen et al. 2014), were used as a positive control. All plates were incubated at room temperature (23°C) for 8 days. The experiment was done twice on duplicate plates and the number of germinated and healthy seedlings was recorded.

Field experiments in 2009 to 2011. A field experiment was set up in a grower's field in Lumijoki (64°85′ N, 25°17′ E), Northern Finland, to investigate the ability of strain 272 to suppress development of common scab and to enhance build-up of suppressiveness. The field (sandy soil, pH 6.5 to 7.1) was naturally infested with scab-causing *Streptomyces* spp. and had documented problems with common scab (Hiltunen et al. 2011).

The inoculum of strain 272 for the field applications was produced by growing the strain in GYM broth with shaking (180 rev min^{-1}) at 28°C for 3 to 5 days. The number of CFU was determined by dilution plating on GYM agar. For use as field inoculum, the culture broth was diluted 10- or 100-fold with water, resulting in 10^3 and 10^4 CFU ml^{-1} (2009 and 2010) or 10^4 and 10^5 CFU ml^{-1} (2011), respectively. The efficacy of strain 272 was compared with the commercial biocontrol product Mycostop (Verdera Oy, Espoo, Finland), which contains *S. griseoviridis*, originally isolated from Finnish peat soil (Tahvonen 1982) as an active ingredient. Mycostop was suspended in distilled water at 0.01% (wt/vol) according to the manufacturer's instructions, resulting in 10^4 CFU ml^{-1}.

The field was divided into four blocks surrounded by guard rows and separated from each other by bare strips of soil (6 m). The experiment included two cultivars: the scab-susceptible 'Bintje' and the scab-tolerant 'Nicola'. Both cultivars were planted in each block as four replications, which were nested within the blocks. Each block contained one of the four treatments: (i) application of strain 272 at low concentration, (ii) application of strain 272 at high concentration, (iii) application of Mycostop, and (iv) untreated control (Table 1). The plot size for each cultivar was 5 by 1.6 m (two rows) containing 35 plants. In order to observe the cumulative effects of the applications, the treatments were applied in the same blocks similarly in 2009, 2010, and 2011. Care was taken not to move the soil between the blocks.

In all years, potato tubers were planted using a machine planter (Kuppi-Juko; Juko Oy, Mynämäki, Finland), with spacing of 28 cm apart. The treatments were applied by spraying the seed tubers planted in furrows with freshly prepared bacterial solutions at the rate of 100 ml m^{-2} using a knapsack sprayer (Birchmaijer Power LG; Birchmeier Sprühtechnik AG, Stetten, Switzerland). Fertilization and pesticide applications followed the practices typical to the region. Fertilizer (Perunan Y1, NPK 8-5-19, at 750 kg ha^{-1}; Kemira GrowHow Oyj, Helsinki, Finland) was applied at planting. During the growing season, herbicides were applied at the following rates: a single combined application of rimsulfuron (7.5 g ha^{-1}) and metribuzin (105 g ha^{-1}); when necessary, a further application of rimsulfuron (5 g ha^{-1}). Late blight was controlled with seven or eight

Table 1. Number of CFU for the low (272_L) and high (272_H) application rates of *Streptomyces* strain 272 and Mycostop (Myco) in the field experiments in 2009, 2010, and 2011

	Application rate (CFU m^{-2})		
Treatment code	**2009**	**2010**	**2011**
Control	0	0	0
Myco	1E+06	1E+06	1E+06
272_L	1E+05	1E+05	1E+06
272_H	1E+06	1E+06	1E+07

applications: two applications combining fenamidone (0.15 liter ha^{-1}) and propamocarb (0.75 liter ha^{-1}) or mancozeb (1.2 kg ha^{-1}) and dimethomorph (0.18 kg ha^{-1}), three applications of fluazinam (0.16 liter ha^{-1}), one or two applications of mandipropamid (0.14 liter ha^{-1}), and a single application of syatsofamidi (80 g ha^{-1}). Haulm was killed shortly before harvest, either mechanically or using diquat (0.4 liter ha^{-1}). All of the management measures were first carried out in the untreated control block and then in the treated blocks, starting from the low application rate of strain 272. The equipment and machinery were cleaned between the blocks.

Progeny tubers were harvested about 13 weeks after planting. A tuber sample of 5 kg/plot was taken for assessment of scab type and coverage. Superficial scab lesions were predominant in all samples. Tubers were graded into six categories according to the surface area covered by scab: 0 = no visible symptoms and <1, 1 to <10, 10 to <25, 25 to <50, 50 to <75, or ≥75% of surface covered by scab lesions. The weight of the tubers in each category was recorded. Disease severity was expressed as the average percentage of tuber surface covered by scab, which was calculated as a weighted mean using the mid-values of the categories. Disease incidence was expressed as the percentage of tubers with scab symptoms in each plot. Tubers with diameter over 30 mm and with less than 25% of surface area covered with scab were considered as marketable yield.

Follow-up experiments in 2012 and 2013. Scab suppressiveness in the soil treated with antagonists in 2009 to 2011 was observed for two further years (2012 and 2013). The plots that did not receive any antagonist applications in 2009 to 2011 (control) and those treated with the higher application rate of strain 272 were included in the follow-up experiments so that half of the each plot was treated again with strain 272, while the other half was left untreated. Thus, the experiments included four treatments with the following applications of strain 272: (i) previously untreated plots left untreated in 2012 (control), (ii) previously untreated plots treated with strain 272 in 2012 (272/1), (iii) plots treated with the higher application rate of strain 272 during the previous 3 years but left untreated in 2012 (272/3), and (iv) plots treated with the higher application rate of strain 272 during the previous 3 years and further treated with strain 272 in 2012 (272/4) (Table 2). Each treatment included four replications (plots). In 2013, no treatments were applied. The follow-up experiment was conducted as described above for the 3-year field experiment, with the exception that only the scab-susceptible cultivar (Bintje) was included.

Statistical analysis. The χ^2 test of homogeneity was used to determine whether there were differences in number of healthy seedlings between isolates in the radish seedling test. Data from the pathogenicity test on micropropagated potato was analyzed using analysis of variance after log or square-root transformations. In the field experiment data, treatment effects on scab incidence, scab severity, and yield were analyzed separately for each year using mixed models, with replications as a random factor nested within the biocontrol treatments. Log transformations of scab severity data and arcsine transformations of scab incidence were used when necessary to meet the assumptions of mixed models. The data for Bintje and Nicola were analyzed separately due to the known difference in scab resistance between these cultivars. Tukey's multiple comparison tests using multiplicity-adjusted P values (Wright 1992) were conducted to determine significant differences between means. The χ^2 test was performed using SPSS 20.0 for Windows (SPSS, Inc., Chicago) and all other statistical analysis were carried on with mixed and univariate procedures of SAS Proprietary Software 9.3 (SAS Institute Inc., Cary, NC).

Results

Relationships of *Streptomyces* strain 272 and strains causing common scab. PCR carried out with the primers designed for detection of *S. scabies* amplified a product of an expected size from strain 272, in contrast to the primers designed for detection of *S. turgidiscabies*. The ITS1 sequence of strain 272 included in the PCR amplicon was not cleaved with *Hpy*99I, indicating that strain 272 does not belong to *S. scabies* but might be a strain of *S. europaeiscabiei*. Sequences of the housekeeping genes *atpD*, *recA*, *rpoB*, and *trpB* in strain 272 (National Center for Biotechnology Information GenBank accession numbers KX534366, KX534367, KX534368, and KX534369, respectively) were closest to corresponding sequences of the *S. europaeiscabiei* type strain CFBP 4497T (Fig. 1).

Avirulence of strain 272. Inoculation of the growing medium with *Streptomyces* strain 272 or pathogenic strain 364 did not affect the number of tubers produced by the micropropagated plants (P = 0.097). However, there were differences between bacterial strains in the number of tubers displaying symptoms characteristic of common scab ($P < 0.001$). No symptoms were observed on tubers grown in the uninoculated growing medium or in the medium inoculated with strain 272, whereas over 70% of the tubers grown in the medium inoculated with pathogenic *S. europaeiscabiei* strain 364 displayed typical common scab symptoms (Table 3).

All radish seed (100%, n = 48) germinated on OMA medium containing strain 272, whereas 94 and 96% of the seed germinated on OMA with *S. europaeiscabiei* strain 364 or without any *Streptomyces* strain, respectively. The difference in the number of healthy seedlings was significant between strains 272 and 364 (χ^2, df = 2, $P < 0.001$). Most (98%) of the seedlings grown with strain 272 were healthy, showing normal growth, similar to those grown on OMA without bacteria. In contrast, 69% of the seedlings grown with strain 364 showed symptoms of stunting, necrosis, and hypertrophy.

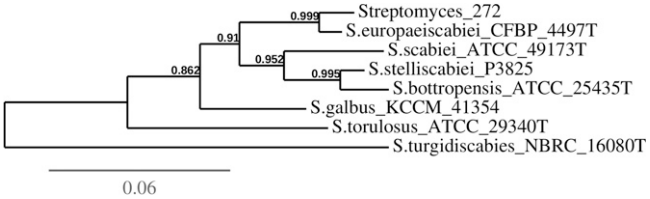

Fig. 1. Phylogenetic analysis on concatenated sequences of the genes *aptD*, *recA*, *rpoB*, and *trpB* (total 2,111 nucleotides) of *Streptomyces* spp. using the neighbor-joining method (Saitou and Nei 1987). The six concatenated sequences most closely related to *Streptomyces* strain 272 are shown. The more distantly related *Streptomyces turgidiscabies* was included to root the tree. Numbers at branches represent bootstrap values of 1,000 replicates (1,000 = 1.000). Scale indicates Kimura units in nucleotide substitutions per site (Kimura 1980).

Table 2. Application rates of *Streptomyces* strain 272 in the follow-up experiments (2012 and 2013) and the treatment history in previous years (2009 to 2011)

	Application rate (CFU m^{-2})				
Treatment code[z]	2009	2010	2011	2012	2013
Control	0	0	0	0	0
272/1	0	0	0	1E+08	0
272/3	1E+06	1E+06	1E+07	0	0
272/4	1E+06	1E+06	1E+07	1E+08	0

[z] Numbers 1, 3, or 4 after strain 272 refer to the total number of antagonist applications.

Table 3. Effect of nonpathogenic *Streptomyces* strain 272 and pathogenic *Streptomyces europaeiscabiei* strain 364 on tuber production and incidence of common scab on micropropagated potato plants (Siikli) grown in a climate chamber[z]

Strain	Total number of tubers produced/plant	Number of symptomatic tubers/plant
Control	3.0 a	0 b
272	2.7 a	0 b
364	1.9 a	1.4 a

[z] Data are means from 14 plants. Means marked with different letters are significantly different ($P < 0.05$, Tukey).

Reduction of common scab by strain 272 in the field. Common scab incidence varied between years and cultivars over the 3 years when strain 272 was applied to the field plots. On scab-susceptible Bintje, the incidence of common scab was high (>94%) in all years (Fig. 2); whereas, on scab-tolerant Nicola, the scab incidence was more variable, being 48, 96, and 44% in the untreated control plots in 2009, 2010, and 2011, respectively (Fig. 3). The scab lesions were predominantly superficial regardless of the cultivar, year, or severity of the symptoms. Hence, disease severity reflected the average percentage of tuber surface covered by scab. On both cultivars, disease severity varied between years. Both disease incidence and severity were highest in 2010, corresponding to the warm and dry conditions during early tuber formation (July) (Table 4), which are known to enhance the development of scab symptoms (Loria et al. 1997).

On scab-susceptible Bintje, there were significant differences between treatments in scab incidence in 1 year (2009, $P = 0.008$; 2010, $P = 0.274$; and 2011, $P = 0.222$) and in scab severity in all 3 years (2009, $P < 0.001$; 2010, $P < 0.001$; and 2011, $P = 0.002$); whereas, on scab-tolerant Nicola, there were differences between treatments in both scab incidence (2009, 2010, and 2011, $P < 0.001$) and scab severity (2009, $P < 0.001$; 2010, $P < 0.001$; and 2011, $P = 0.005$) in all 3 years.

On Bintje, the applications of *Streptomyces* strain 272 reduced disease incidence on average by 8, 1, and 11% and disease severity by 59, 44, and 45% in 2009, 2010, and 2011, respectively, when compared with untreated controls; however, differences were not always statistically significant (Fig. 2). There were no differences in the efficacy between the two application rates of strain 272 used. In contrast, the commercial biological control agent Mycostop did not suppress common scab disease on Bintje in any year. Although each treatment was applied in the same plot in three consecutive years, there was no indication of a cumulative effect in soil suppressiveness.

In Nicola, scab incidence and severity were reduced by strain 272 with at least one of the two application rates in all 3 years, apart from disease severity in 2011, when the common scab incidence was generally low (Fig. 3). On average, disease incidence was reduced by 77, 34, and 19% and disease severity by 86, 77, and 15% in 2009, 2010, and 2011, respectively. Regardless of the disease pressure, the high application rate of strain 272 always reduced disease severity to a negligible level. The high application rate of strain 272 was more effective in reducing disease incidence and severity than the low application rate in 2 of 3 years (Fig. 3). There was no indication of a cumulative effect of the repeated applications of strain 272 on disease incidence or severity. On scab-tolerant Nicola, Mycostop also showed efficacy in controlling common scab by reducing the disease incidence and severity in 2009 and 2011 (Fig. 3).

Development of scab suppressiveness. Following the repeated applications of strain 272 in 2009 to 2011, the development and persistence of scab suppressiveness in soil was observed for two further years (2012 and 2013). In 2012, half of the untreated control plots and half of the plots previously treated with the higher application rate of strain 272 were treated again with strain 272, while the other half was left untreated (Table 2). Differences between treatments were evident in terms of both disease incidence ($P < 0.001$) and severity ($P < 0.001$). Suppression of common scab was equal in all the plots treated with strain 272 regardless of the number or timing of the applications (Fig. 4). A single application of strain 272 in the beginning of the growing season (2012) (Fig. 4, treatment 272/1) was adequate to reduce disease incidence and severity. On the other hand, when the plot had been treated previously, omitting application of strain 272 in the beginning of the growing season (Fig. 4, treatment 272/3) did not reduce disease suppression.

Although no further applications of strain 272 were performed in the growing season 2013, differences between treatments were still

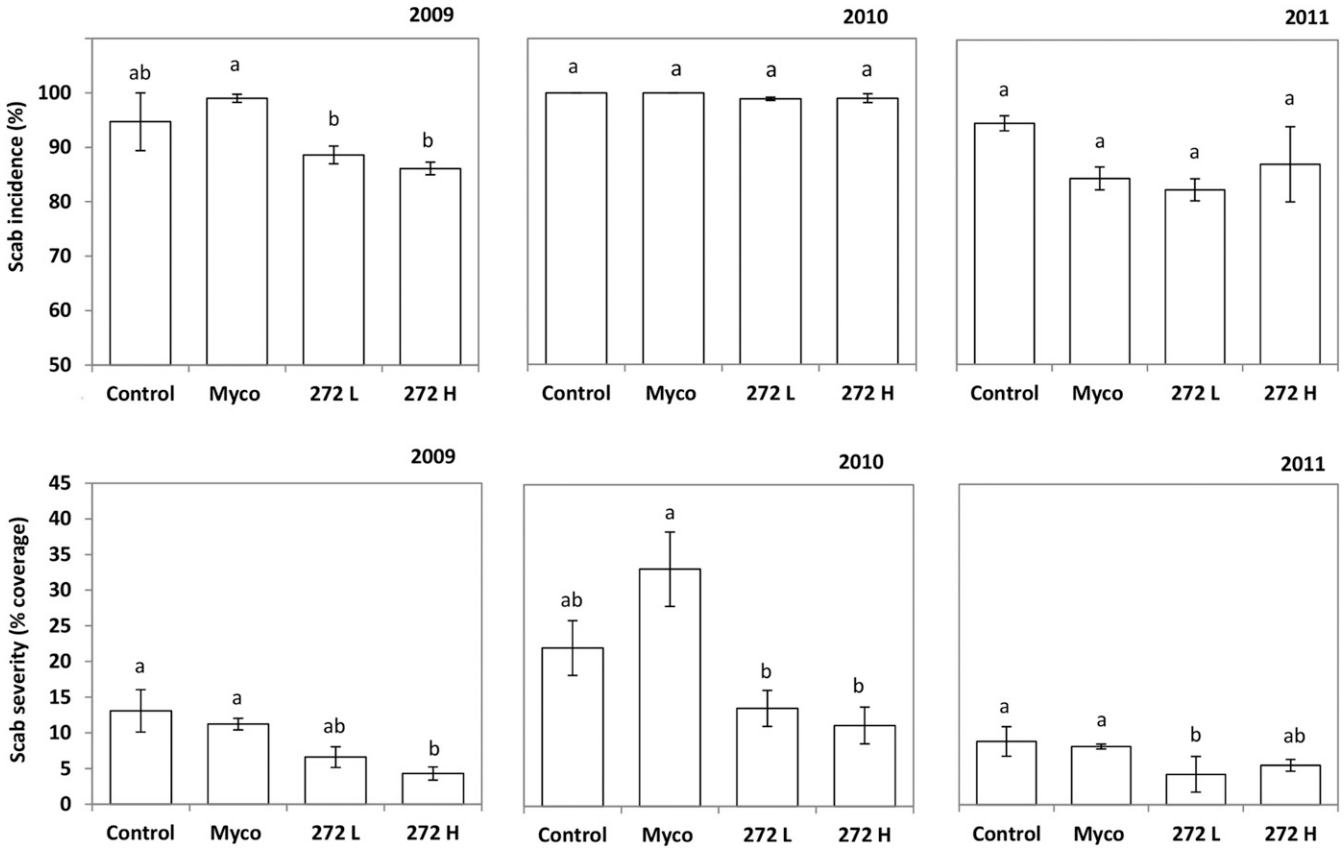

Fig. 2. Effect of annually repeated applications of a low (272_L) or high (272_H) concentration of the nonpathogenic *Streptomyces* strain 272 or Mycostop (Myco) on percentage of tubers with scab symptoms (scab incidence) and average tuber surface area covered with scab (scab severity) on scab-susceptible Bintje in 2009, 2010, and 2011. Bars represent means ± standard error of four replicates per treatment. Means marked with different letters are significantly different ($P < 0.05$, Tukey).

evident in both disease incidence ($P < 0.001$) and severity ($P = 0.004$) (Fig. 5). Scab was suppressed in plots in which strain 272 had been applied three or four times during the past consecutive growing seasons (Fig. 5, treatments 272/3 and 272/4). When strain 272 was only applied once, scab was not reduced significantly although, in this case, the trend of scab suppression also was evident (Fig. 5, treatment 272/1). Furthermore, scab suppression was still observed 2 years after the last application of strain 272 (Fig. 5, treatment 272/3) and was equal to that observed after 1 year since the last application (Fig. 5, treatment 272/4).

Yield. Applications of strain 272 did not affect the total yield or marketable yield of either of the two cultivars in the first 2 years (2009 and 2010) of the experiments (Table 5). In the third year (2011), the high application rate of strain 272 was associated with reduced total yield of Bintje, and both the high and low application rate reduced the total yield and marketable yield of Nicola. However, in all years and on both cultivars, the proportion of the marketable yield was equal or higher with the application of strain 272 compared with the untreated control; the only exception was Nicola in 2011 (Table 5).

In the first year (2012) of the follow-up experiments, repeated applications of strain 272 seemed to have a negative effect on the yield (Table 6). However, in the final experimental year (2013) on both cultivars, the total and marketable yields were increased in the plots where strain 272 had been previously applied.

Discussion

The results of this study showed that *Streptomyces* strain 272 applied at planting consistently suppressed development of common scab symptoms in the field, and annually repeated applications resulted in suppressiveness that lasted in soil at least 2 years beyond the last application. The responses of the two tested cultivars varied. On scab-susceptible Bintje, strain 272 reduced the disease severity (on average by 43%) whereas, on scab-tolerant Nicola, the strain reduced both disease incidence and severity (by 43 and 59%, respectively). Regardless of the disease pressure, the combined use of strain 272 and the tolerant cultivar reduced the scab coverage to a negligible level in all 3 years. In previous field studies, the use of *Streptomyces* inoculants for control of potato common scab has given inconsistent results (Hiltunen et al. 2009; Ryan and Kinkel 1997; Ryan

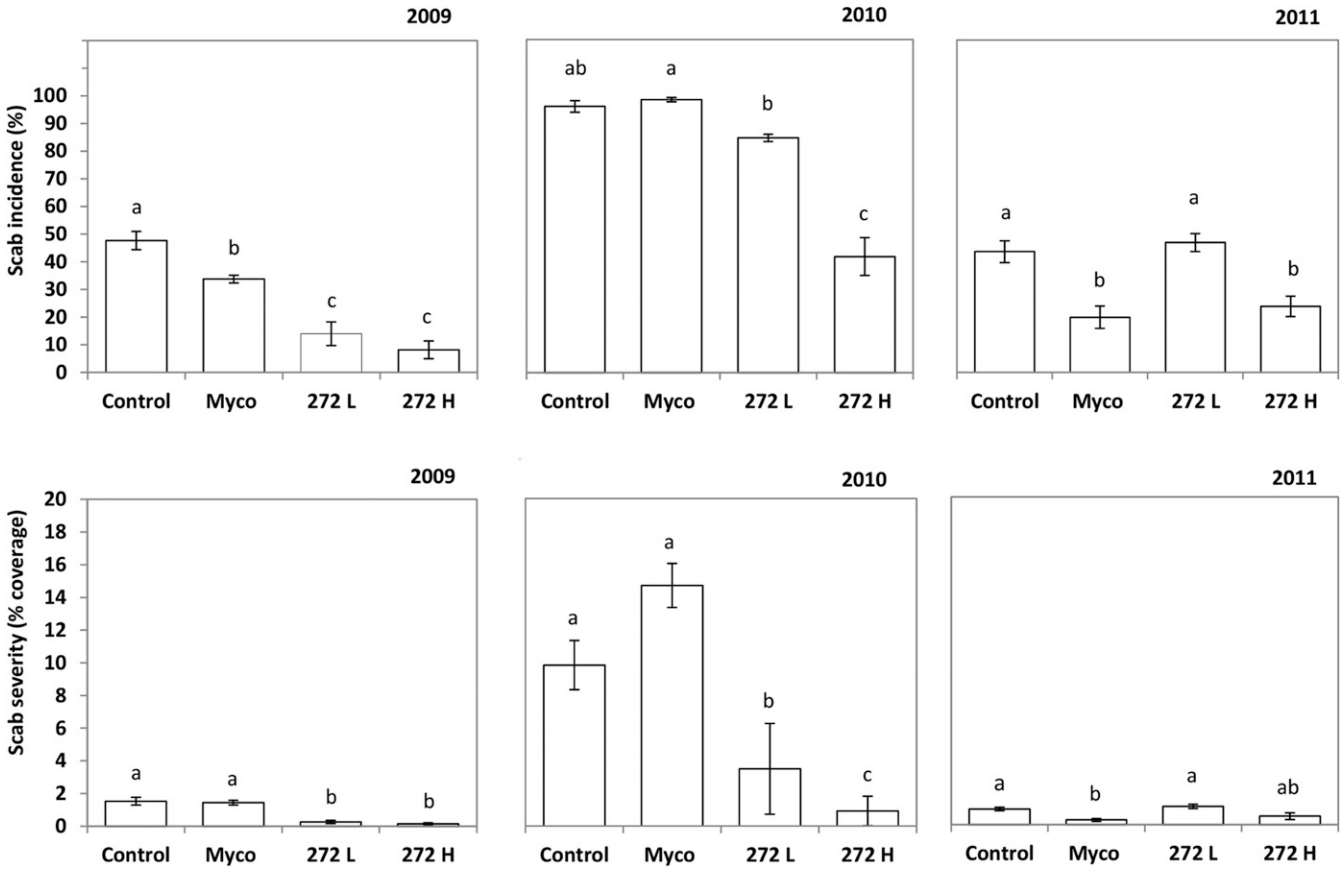

Fig. 3. Effect of annually repeated applications of a low (272_L) or high (272_H) concentration of the nonpathogenic *Streptomyces* strain 272 or Mycostop (Myco) on percentage of tubers with scab symptoms (scab incidence) and average tuber surface area covered with scab (scab severity) on scab-tolerant Nicola in 2009, 2010, and 2011. Bars represent means ± standard error of four replicates per treatment. Means with different letters are significantly different ($P < 0.05$, Tukey).

Table 4. Planting and harvest dates, monthly precipitation, and effective temperature sum (Tbase = 5°C) for the field experiments in 2009 to 2013[z]

			Precipitation (mm)					Effective temperature sum (°C)				
Year	Planting	Harvest	May	June	July	Aug	Sep	May	June	July	Aug	Sep
2009	10 June	16 Sep	40	14	93	74	63	133	242	324	299	178
2010	10 June	14 Sep	31	42	75	85	72	193	222	424	273	124
2011	1 June	9 Sep	37	61	141	72	66	122	324	410	288	180
2012	7 June	6 Sep	67	50	87	49	56	97	211	343	263	120
2013	29 May	3 Sep	28	108	59	26	33	210	321	327	306	180

[z] Abbreviations: Aug = August and Sep = September.

et al. 2004; Sun et al. 2015; Wanner et al. 2013), emphasizing the involvement of multitudinous environmental and soil factors in the development of disease suppression (Wanner et al. 2013; Whipps and Gerhardson 2007).

A single application of strain 272 was suppressive to common scab. However, after a single application, efficient disease suppressiveness did not persist in the soil to the following growing season, although the trend of suppression could be observed. When the applications were repeated in three consecutive years, suppressiveness was observed for 2 years after the last application, and may have lasted even longer beyond termination of the experiment. Hence, multiple applications of the antagonist may be necessary to enhance the development of persisting scab suppression in the field soil. However, there was no evidence of a cumulative effect of the repeated applications in terms of biocontrol efficacy, suggesting that strain 272 is a successful competitor once established and the soil microbiome reaches equilibrium relatively soon.

Although strain 272 clearly enhanced the development of suppressiveness to common scab, the mechanism of disease suppression remains unclear. Actinomycetes are known to employ a variety of mechanisms of biological control, including antibiosis, competition, production of extracellular proteins, and induction of host resistance (Doumbou et al. 2001; Palaniyandi et al. 2013; Raaijmakers et al. 2009). In our preliminary studies, strain 272 seemed to have distinct but modest direct effects, either through antibiosis or competition, on the scab pathogens *S. turgidiscabies* and *S. europaeiscabiei* (unpublished data), which are considered to be the main scab-causing pathogens in Finland (Hiltunen et al. 2014; Kreuze et al. 1999; Lehtonen et al. 2004). Further research is needed to unravel the importance of different mechanisms of disease suppression employed by strain 272 in soil environments, including possible indirect effects through induced host resistance or impact on indigenous soil microbial populations.

In this study, disease severity was determined using the average percentage of tuber surface covered by scab and the type of lesions

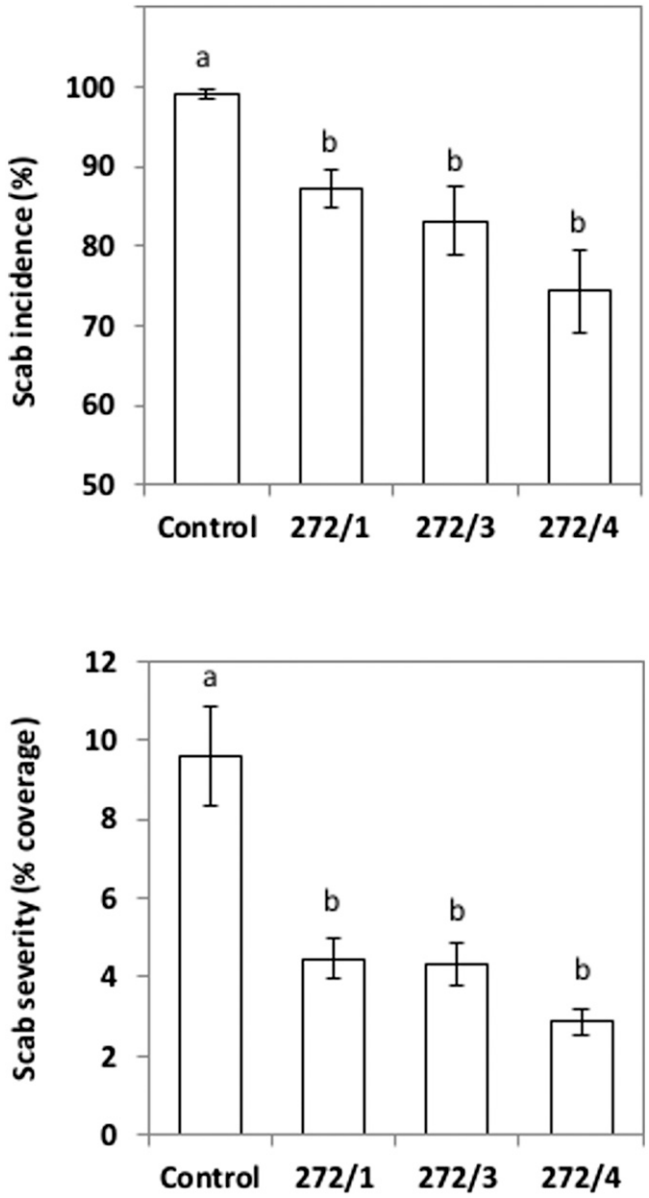

Fig. 4. Percentage of tubers with scab symptoms (scab incidence) and average tuber surface area covered with scab (scab severity) on Bintje potato in 2012 in response to one (272/1), three (272/3), or four (272/4) annually repeated applications in 2009 to 2012 of the nonpathogenic *Streptomyces* strain 272. Bars represent means ± standard error of four replicates per treatment. Means with different letters are significantly different ($P < 0.05$, Tukey).

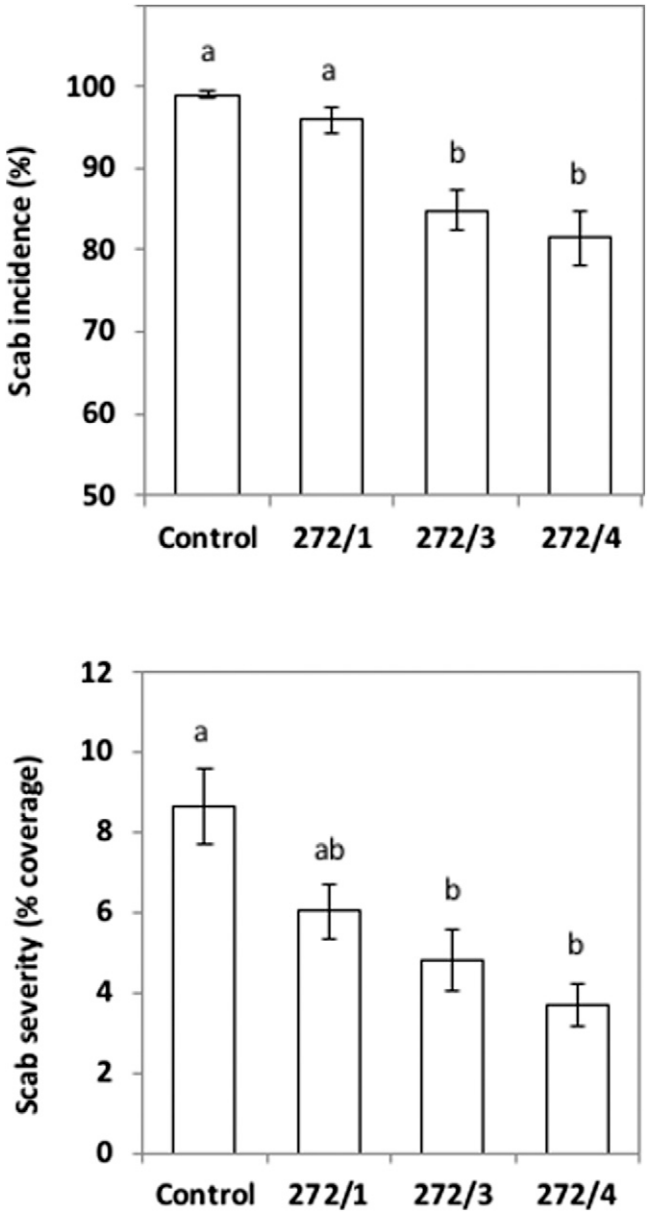

Fig. 5. Percentage of tubers with scab symptoms (scab incidence) and average tuber surface area covered with scab (scab severity) on Bintje potato in 2013 in response to one (272/1), three (272/3), or four (272/4) annually repeated applications in 2009 to 2012 of the nonpathogenic *Streptomyces* strain 272. No applications were made in 2013. Bars represent means ± standard error of four replicates per treatment. Means with different letters are significantly different ($P < 0.05$, Tukey).

(i.e., superficial, erumpent, or deep-pitted) (Kreuze et al. 1999). Because the scab lesions were predominantly superficial, disease severity reflected, in practice, the percentage of tuber surface coverage by scab. On scab-susceptible Bintje, antagonist treatments did not affect the disease incidence but reduced the disease severity whereas, on scab-tolerant Nicola, both disease incidence and severity were reduced in 2 of the 3 years when treatments with strain 272 were carried out. The difference in resistance to scab between the cultivars could be due to their different ability to support populations of *S. scabies* and other actinomycetes on the tuber surface (Keinath and Loria 1989). This indicates that, on the susceptible cultivar, where a high number of pathogenic *Streptomyces* populations may be present on the tuber surfaces, the antagonist is not able to reduce initial infections through lenticels, wounds, stomata, or directly but can slow down multiplication of the pathogen and prevent lesion expansion. Cultivar dependence of biocontrol efficacy has been observed previously (Ryan et al. 2004) and found to be associated with differences among cultivars in "conduciveness" to biocontrol.

Our study indicated that the effect of the antagonist application rate on disease suppression was cultivar dependent. On the susceptible cultivar, higher application rates of strain 272 did not improve the control efficacy whereas, on the tolerant cultivar, the higher application rate tended to be more effective than the lower rate. In our previous studies carried out in a controlled environment (Hiltunen et al. 2009), increasing the application rate increased the control efficacy of the two *Streptomyces* strains used, even on the susceptible cultivar. However, the effectiveness differed between strains, because one of the strains was effective in concentrations 100-fold lower than the other one. Furthermore, the effectiveness of the suppressive strain was dependent on the initial inoculum level of the pathogen in growing medium (Hiltunen et al. 2009). In the current study, a 10-fold difference between the application rates of antagonist may have been too small to cause any perceivable differences on a susceptible cultivar, because scab-susceptible cultivars are thought to support high population levels of pathogenic *Streptomyces* spp. (Keinath and Loria 1989). Comparison of the results of the present study with those of previous studies is difficult due to varying inoculum types and units (e.g., CHU g^{-1} of soil, CFU per seed piece, or CFU ml^{-1} of soil) used to express the application rates (Ryan and Kinkel 1997; Sun et al. 2015; Wanner et al. 2013). Clearly, more information is needed regarding to which extent the efficacy of the application rate is dependent on the inoculant strain, the potato cultivar, and the level of pathogen inoculum.

Strain 272 did not have any effect on the total or marketable yield in the first 2 years of the experiments but all the antagonist applications seemed to reduce the yield in 2011 and 2012. This may reflect the effects of the experimental design and the weather conditions rather than treatment responses. The replications were placed within the blocks in order to contain the biocontrol applications in defined parts of the field and prevent the spread of the biocontrol organisms to other parts. However, the latter parts of the growing seasons of 2011 and 2012 were wet and the part of the experimental area where the blocks treated with strain 272 were situated was most severely affected. No adverse effect of the antagonist treatment on yield was observed during the last experimental year (2013), which indicates that factors other than the biocontrol applications played a role in 2011 and 2012. This is also supported by the fact that, in our pathogenicity tests, strain 272 did not have any harmful effects on growth of radish seedlings or tuber production of micropropagated potato.

Utilization of microorganisms to control plant diseases is an environmentally acceptable alternative to chemical pesticides and, for some pathogens such as the *Streptomyces* spp. causing common scab, no chemical control is available. Although, in many previous studies, nonpathogenic *Streptomyces* strains have shown considerable promise in controlling common scab, their effectiveness in field trials has been unpredictable. Against that background, the consistency of the scab-suppressive impact of strain 272 used in this study is remarkable. A single application of strain 272 at planting consistently suppressed development of common scab symptoms. When strain 272 was applied in three or more consecutive growing seasons, the soil remained suppressive to scab for at least 2 years beyond the last treatment, suggesting that, with repeated applications, it may be possible to enhance development of scab suppression in soil which, under natural conditions, is a slow process. This approach is applicable in practice if the potato crop is grown in the same place for several years, which is sometimes the case. However, for situations when potato is grown in rotation with other crops, further research is required to find out whether soil suppressiveness to common scab could be enhanced by applying strain 272 on crops preceding potato.

Table 6. Effect of one (272/1), three (272/3), or four (272/4) annually repeated applications of the saprophytic *Streptomyces* strain 272 on the total and marketable yield of Bintje potato in the follow-up experiments in 2012 and 2013

	Yield[z]					
	2012			2013		
	Total	Marketable		Total	Marketable	
Treatment	t ha^{-1}	t ha^{-1}	%	t ha^{-1}	t ha^{-1}	%
Control	30.8 a	29.5 a	96 a	31.9 b	28.8 b	90 b
272/1	31.5 a	29.2 a	93 ab	33.2 ab	31.7 ab	95 a
272/3	18.3 b	16.8 b	92 b	37.1 a	35.5 a	96 a
272/4	21.9 b	20.4 b	93 ab	33.5 ab	32.1 ab	96 a
P	<0.001	<0.001	0.047	0.025	0.011	0.004

[z] For each year, means marked with different letters are significantly different ($P < 0.05$, Tukey).

Table 5. Effect of annually repeated applications of a low (272_L) or high (272_H) concentration of *Streptomyces* strain 272 or Mycostop (Myco) on total and marketable yield of Bintje and Nicola in 2009, 2010, and 2011

		Yield[z]								
		2009			2010			2011		
		Total	Marketable		Total	Marketable		Total	Marketable	
Cultivar	Treatment	t ha^{-1}	t ha^{-1}	%	t ha^{-1}	t ha^{-1}	%	t ha^{-1}	t ha^{-1}	%
Bintje	Control	41.3 a	34.8 a	84 b	30.3 a	21.1 ab	68 bc	33.2 a	30.4 a	92 ab
	Myco	39.1 a	31.7 a	81 b	29.8 a	17.2 b	57 c	27.2 ab	21.9 b	81 b
	272_L	40.2 a	37.4 a	93 ab	29.8 a	26.6 a	88 ab	27.5 ab	26.3 ab	95 a
	272_H	41.8 a	40.5 a	97 a	30.0 a	28.2 a	93 a	25.8 b	24.4 ab	94 a
	P	0.486	0.102	0.009	0.918	0.005	0.002	0.025	0.027	0.018
Nicola	Control	41.2 a	40.2 a	98 a	32.2 a	29.9 ab	91 a	31.6 a	30.6 a	97 a
	Myco	39.1 a	38.4 a	98 a	31.3 a	24.9 b	76 b	21.7 b	19.7 b	91 b
	272_L	38.6 a	37.7 a	98 a	31.0 a	30.8 a	98 a	24.5 b	22.9 b	93 ab
	272_H	39.2 a	38.4 a	98 a	32.4 a	32.3 a	98 a	20.8 b	18.9 b	90 b
	P	0.300	0.362	0.820	0.592	0.012	<0.001	0.001	<0.001	0.003

[z] For each year and cultivar, means marked with different letters are significantly different ($P < 0.05$, Tukey).

Acknowledgments

We thank T. Uotila, A. Kankaala, T. Väyrynen, and E. Leiviskä for their assistance with the field experiments. Financial support from Oiva Kuusisto Foundation is gratefully acknowledged.

Literature Cited

Agbessi, S., Beauséjour, J., Déry, C., and Beaulieu, C. 2003. Antagonistic properties of two recombinant strains of *Streptomyces melanosporofaciens* obtained by intraspecific protoplast fusion. Appl. Microbiol. Biotechnol. 62: 233-238.

Bakker, M. G., Glover, J. G., Mai, J. G., and Kinkel, L. L. 2010. Plant community effects on the diversity and pathogen suppressive activity of soil streptomycetes. Appl. Soil Ecol. 46:35-42.

Beauséjour, J., Clermont, N., and Beaulieu, C. 2003. Effect of *Streptomyces melanosporofaciens* strain EF-76 and chitosan on common scab of potato. Plant Soil 256:463-468.

Bowers, J. H., Kinkel, L. L., and Jones, R. K. 1996. Influence of disease-suppressive *Streptomyces* on the native *Streptomyces* community in soil as determined by the analysis of cellular fatty acids. Can. J. Microbiol. 42:27-37.

Cook, R. J., and Baker, K. F. 1983. The Nature and Practice of Biological Control of Plant Pathogens. American Phytopathological Society, St. Paul, MN.

Dees, M. W., Sletten, A., and Hermansen, A. 2013. Isolation and characterization of *Streptomyces* species from potato common scab lesions in Norway. Plant Pathol. 62:217-225.

Dees, M. W., and Wanner, L. A. 2012. In search of better management of potato common scab. Potato Res. 55:249-268.

Doumbou, C. L., Akimov, V., and Beaulieu, C. 1998. Selection and characterization of microorganisms utilizing thaxtomin A, a phytotoxin produced by *Streptomyces scabies*. Appl. Environ. Microbiol. 64:4313-4316.

Doumbou, C. L., Hamby Salove, M. K., Crawford, D. L., and Beaulieu, C. 2001. Actinomycetes, promising tools to control plant diseases and to promote plant growth. Phytoprotection 82:85-102.

Flores-González, R., Velasco, I., and Montes, F. 2008. Detection and characterization of *Streptomyces* causing potato common scab in Western Europe. Plant Pathol. 57:162-169.

Hiltunen, L. H., Alanen, M., Laakso, I., Kangas, A., Virtanen, E., and Valkonen, J. P. T. 2011. Elimination of common scab sensitive progeny from a potato breeding population using thaxtomin A as a selective agent. Plant Pathol. 60: 426-435.

Hiltunen, L. H., Kelloniemi, J., and Valkonen, J. P. T. 2014. First report of *Streptomyces europaeiscabiei* causing common scab on potato in Finland. Plant Dis. 98:1267.

Hiltunen, L. H., Laakso, I., Chobot, V., Hakala, K., Weckman, A., and Valkonen, J. P. T. 2006. The influence of thaxtomins in different combinations and concentrations on growth of micropropagated potato shoot cultures. J. Agric. Food Chem. 54:3372-3379.

Hiltunen, L. H., Ojanperä, T., Kortemaa, H., Richter, E., Lehtonen, M. J., and Valkonen, J. P. T. 2009. Interactions and biocontrol of pathogenic *Streptomyces* strains co-occurring in potato scab lesions. J. Appl. Microbiol. 106:199-212.

Hiltunen, L. H., Weckman, A., Ylhäinen, A., Rita, H., Richter, E., and Valkonen, J. P. T. 2005. Responses of potato cultivars to the common scab pathogens, *Streptomyces scabies* and *S. turgidiscabies*. Ann. Appl. Biol. 146:395-403.

Hjort, K., Bergström, M., Adesina, M. F., Jansson, J. K., Smalla, K., and Sjöling, S. 2010. Chitinase genes revealed and compared in bacterial isolates, DNA extracts and a metagenomic library from a phytopathogen-suppressive soil. FEMS Microbiol. Ecol. 71:197-207.

Höper, H., and Alabouvette, C. 1996. Importance of physical and chemical soil properties in the suppressiveness of soils to plant diseases. Eur. J. Soil Biol. 32:41-58.

Janvier, C., Villeneuve, F., Alabouvette, C., Edel-Hermann, V., Mateille, T., and Steinberg, C. 2007. Soil health through soil disease suppression: Which strategy from descriptors to indicators? Soil Biol. Biochem. 39:1-23.

Jobin, G., Couture, G., Goyer, C., Brzezinski, R., and Beaulieu, C. 2005. Streptomycete spores entrapped in chitosan beads as a novel biocontrol tool against common scab of potato. Appl. Microbiol. Biotechnol. 68:104-110.

Keinath, A. P., and Loria, R. 1989. Population dynamics of *Streptomyces scabies* and other actinomycetes as related to common scab of potato. Phytopathology 79:681-687.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111-120.

Kinkel, L. L., Bakker, M. G., and Schlatter, D. C. 2011. A coevolutionary framework for managing disease-suppressive soils. Annu. Rev. Phytopathol. 49:47-67.

Kinkel, L. L., Schlatter, D. C., Bakker, M. G., and Arenz, B. E. 2012. *Streptomyces* competition and co-evolution in relation to plant disease suppression. Res. Microbiol. 163:490-499.

Kreuze, J. F., Suomalainen, S., Paulin, L., and Valkonen, J. P. T. 1999. Phylogenetic analysis of 16S rRNA genes and PCR analysis of the *nec1* gene from *Streptomyces* spp. causing common scab, pitted scab and netted scab in Finland. Phytopathology 89:462-469.

Labeda, D. P. 2011. Multilocus sequence analysis of phytopathogenic species of the genus *Streptomyces*. Int. J. Syst. Evol. Microbiol. 61:2525-2531.

Larkin, R. P. 2008. Relative effects of biological amendments and crop rotations on soil microbial communities and soilborne disease of potato. Soil Biol. Biochem. 40:1341-1351.

Larkin, R. P., Griffin, T. S., and Honeycutt, C. W. 2010. Rotation and cover crop effects on soilborne potato diseases, tuber yield, and soil microbial communities. Plant Dis. 94:1491-1502.

Larkin, R. P., and Halloran, J. M. 2014. Management effects of disease-suppressive rotation crops on potato yield and soilborne disease and their economic implications in potato production. Am. J. Potato Res. 91:429-439.

Larkin, R. P., Honeycutt, C. W., Griffin, T. S., Olanya, O. M., Halloran, J. M., and He, Z. 2011. Effects of different potato cropping system approaches and water management on soilborne diseases and soil microbial communities. Phytopathology 101:58-67.

Lehtonen, M. J., Rantala, H., Kreuze, J. F., Bång, H., Kuisma, L., Koski, P., Virtanen, E., Vihlman, K., and Valkonen, J. P. T. 2004. Occurrence and survival of potato scab pathogens (*Streptomyces scabies*) on tuber lesions: Quick diagnosis based on a PCR-based assay. Plant Pathol. 53:280-287.

Lindholm, P., Kortemaa, H., Kokkola, M., Haahtela, K., Salkinoja-Salonen, M., and Valkonen, J. P. T. 1997. *Streptomyces* spp. isolated from potato scab lesions under Nordic conditions in Finland. Plant Dis. 81:1317-1322.

Liu, D., Anderson, N. A., and Kinkel, L. L. 1995. Biological control of potato scab in the field with antagonistic *Streptomyces scabies*. Phytopathology 85: 827-831.

Liu, D., Anderson, N. A., and Kinkel, L. L. 1996. Selection and characterization of strains of *Streptomyces* suppressive to the potato scab pathogen. Can. J. Microbiol. 42:487-502.

Lorang, J. M., Anderson, N. A., Lauer, F. I., and Wildung, D. K. 1989. Disease decline in a Minnesota potato scab plot. Am. Potato J. 66:531.

Lorang, J. M., Liu, D., Anderson, N. A., and Schottel, J. L. 1995. Identification of potato scab inducing and suppressive species of *Streptomyces*. Phytopathology 85:261-268.

Loria, R., Bukhalid, R. A., Fry, B. A., and King, R. R. 1997. Plant pathogenicity in the genus *Streptomyces*. Plant Dis. 81:836-846.

Mazzola, M. 2002. Mechanisms of natural soil suppressiveness to soilborne diseases. Antonie van Leeuwenhoek 81:557-564.

Mazzola, M. 2007. Manipulation of rhizosphere bacterial communities to induce suppressive soils. J. Nematol. 39:213-220.

Mendes, R., Kruijt, M., Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J. H. M., Piceno, Y. M., DeSantis, T. Z., Andersen, G. L., Bakker, P. A. H. M., and Raaijmakers, J. M. 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332:1097-1100.

Meng, Q. X., Yin, J. F., Rosenzweig, N., Douches, D., and Hao, J. J. 2012. Culture-based assessment of microbial communities in soil suppressive to potato common scab. Plant Dis. 96:712-717.

Menzies, J. D. 1959. Occurrence and transfer of a biological factor in soil that suppresses potato scab. Phytopathology 49:648-652.

Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Neeno-Eckwall, E. C., Kinkel, L. L., and Schottel, J. L. 2001. Competition and antibiosis in the biological control of potato scab. Can. J. Microbiol. 47: 332-340.

Palaniyandi, S. A., Yang, S. H., Zhang, L., and Suh, J. W. 2013. Effects of actinobacteria on plant disease suppression and growth promotion. Appl. Microbiol. Biotechnol. 97:9621-9636.

Peters, R. D., Sturz, A. V., Carter, M. R., and Sanderson, J. B. 2004. Influence of crop rotation and conservation tillage practices on the severity of soil-borne potato diseases in temperate humid agriculture. Can. J. Soil Sci. 84: 397-402.

Postma, J., Schilder, M. T., Bloem, J., and van Leeuwen-Haagsma, W. K. 2008. Soil suppressiveness and functional diversity of the soil microflora in organic farming systems. Soil Biol. Biochem. 40:2394-2406.

Prévost, K., Couture, G., Shipley, B., Brzezinski, R., and Beaulieu, C. 2006. Effect of chitosan and a biocontrol streptomycete on field and potato bacterial communities. BioControl 51:533-546.

Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., and Moënne-Loccoz, Y. 2009. The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil 321:341-361.

Ryan, A. D., and Kinkel, L. L. 1997. Inoculum density and population dynamics of suppressive and pathogenic *Streptomyces* strains and their relationship to biological control of potato scab. Biol. Control 10:180-186.

Ryan, A. D., Kinkel, L. L., and Schottel, J. L. 2004. Effect of pathogen isolate, potato cultivar, and antagonist strain on potato scab severity and biological control. Biocontrol Sci. Technol. 14:301-311.

Sagova-Mareckova, M., Daniel, O., Omelka, M., Kristufek, V., Divis, J., and Kopecky, J. 2015. Determination of factors associated with natural soil suppressivity to potato common scab. PLoS One 10:e0116291.

Saitou, N., and Nei, M. 1987. The neighbour-joining method, a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.

Schottel, J. L., Shimizu, K., and Kinkel, L. L. 2001. Relationships of in vitro pathogen inhibition and soil colonization to potato scab biocontrol by antagonistic *Streptomyces* spp. Biol. Control 20:102-112.

Sun, P., Otto-Hanson, L., Arenz, B. E., Ma, Q., and Kinkel, L. L. 2015. Molecular and functional characteristics of streptomycete communities in relation to soil factors and potato common scab. Eur. J. Soil Biol. 70:58-66.

Tahvonen, R. 1982. Preliminary experiments into the use of *Streptomyces* spp. isolated from peat in the biological control of soil and seed-borne diseases in peat culture. J. Sci. Agric. Soc. Finl. 54:357-369.

Tomihama, T., Nishi, Y., Mori, Y., Shirao, T., Iida, T., Uzuhashi, S., Ohkuma, M., and Ikeda, S. 2016. Rice bran amendment suppresses potato common scab by increasing antagonistic bacterial community levels in the rhizosphere. Phytopathology 106:719-728.

Wanner, L. A., and Kirk, W. W. 2015. *Streptomyces*—From basic microbiology to role as a plant pathogen. Am. J. Potato Res. 92:236-242.

Wanner, L. A., Kirk, W. W., and Qu, X. S. 2013. Field efficacy of nonpathogenic *Streptomyces* species against potato common scab. J. Appl. Microbiol. 116: 123-133.

Weller, D. M., Raaijmakers, J. M., Gardener, B. B., and Thomashow, L. S. 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu. Rev. Phytopathol. 40:309-348.

Whipps, J. M., and Gerhardson, B. 2007. Biological pesticides for control of seed- and soil-borne plant pathogens. Pages 479-501 in: Modern Soil Microbiology, 2nd ed. J. D. Van Elsas, J. K. Jansson, and J. T. Trevors, eds. CRC Press, Boca Raton, FL.

Wiggins, B. E., and Kinkel, L. L. 2005a. Green manures and crop sequences influence alfalfa root rot and pathogen inhibitory activity among soil-borne streptomycetes. Plant Soil 268:271-283.

Wiggins, B. E., and Kinkel, L. L. 2005b. Green manures and crop sequences influence potato diseases and pathogen inhibitory activity of indigenous streptomycetes. Phytopathology 95:178-185.

Wright, S. P. 1992. Adjusted *P*-values for simultaneous inference. Biometrics 48: 1005-1013.