



Suppression of the root-lesion nematode (*Pratylenchus penetrans*) in alfalfa (*Medicago sativa*) by *Streptomyces* spp.

Deborah A. Samac^{1,2,3} & Linda L. Kinkel²

¹USDA-ARS-Plant Science Research Unit, St. Paul, MN, U.S.A. ²Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, U.S.A. ³Corresponding author*

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Abstract

Strains of *Streptomyces* were tested for their ability to reduce population densities of the root-lesion nematode (RLN), *Pratylenchus penetrans*, in roots of alfalfa (*Medicago sativa*) in growth chamber assays. Previously, these strains were shown to suppress potato scab disease, caused by *Streptomyces scabies*, in field experiments and to inhibit *in vitro* growth of a wide range of plant-pathogenic fungi and bacteria. Inoculation with *Streptomyces* at planting significantly reduced RLN population densities in roots of both susceptible and resistant alfalfa varieties grown in either heat-treated or untreated soil. Reductions in RLN population densities were observed 6 weeks after nematode inoculation. Shoot dry matter was not affected by any treatment; root dry weight was reduced in *Streptomyces* plus nematode treatments compared to the nematode inoculation alone in some experiments but was not affected by *Streptomyces* when RLN was absent. Mutant strains not producing antibiotics *in vitro* also reduced RLN population densities in alfalfa roots and all strains maintained high population densities after inoculation into heat-treated soil and on alfalfa roots. These strains may be useful in multi-crop, multi-pathogen management programs to augment genetic resistance to plant diseases.

Introduction

Streptomyces spp. are gram-positive filamentous bacteria that produce and secrete a wide array of biologically active compounds including antibiotics, ionophores, hydrolytic enzymes (proteases, nucleases, lipases and a variety of enzymes hydrolyzing polysaccharides) and enzyme inhibitors. Many species are rhizobacteria that effectively colonize plant roots, influence plant growth and protect plant roots from pathogens. They are resistant to desiccation and nutrient stress, partly as a function of their ability to produce spores. These characteristics make Streptomycetes attractive candidates for biological control agents against soil-borne plant pathogens.

Streptomycetes have been implicated in antagonism of a variety of plant pathogens. In petri-plate

assays, a number of species produce compounds that inhibit the growth of plant-pathogenic fungi (Crawford et al., 1993; Jones and Samac, 1996; Trejo-Estrada et al., 1998; Yuan and Crawford, 1995) and bacteria (El-Shanshoury et al., 1995; Jones and Samac, 1996; Lorang et al., 1995). When inoculated into soil or on seeds, many Streptomycetes protect plants from fungal diseases (Hiltunen et al., 1995; Jones and Samac, 1996; Nemeč et al., 1996; Rothrock and Gottlieb, 1984; Tahvonen and Avikainen, 1987; Yuan and Crawford, 1995), bacterial diseases (El-Shanshoury, 1994; Liu et al., 1995; Liu et al., 1997) and nematodes (Dicklow et al., 1993). In fact, *Streptomyces* spp. have been associated with the development of several naturally-occurring disease-suppressive soils (Dicklow et al., 1993; Lorang et al., 1995; Zuckerman et al., 1989) and with disease suppression associated with the use of some green manures (Papavizas, 1963; Papavizas and Davey, 1960). In Grand Rapids, MN, soils suppressive to potato scab, caused by *Streptomy-*

* FAX No: 651-649-5058;
E-mail: debbys@puccini.cdl.umn.edu

ces scabies, developed after continuous cultivation of potatoes (Lorang et al., 1995). Intensive studies have shown that non-pathogenic antibiotic-producing isolates of *Streptomyces* colonize potatoes grown in this suppressive soil (Liu, 1992). These strains kill pathogenic strains of *S. scabies in vitro* (Liu, 1992; Lorang et al., 1995) and control potato scab both in the greenhouse and in the field (Liu et al., 1995, 1997). The *in vitro* growth of a number of bacterial and fungal pathogens of alfalfa and soybeans was inhibited by potato scab-suppressive strains and these strains protected alfalfa and soybean seedlings from root rot and damping off (Jones and Samac, 1996; Xiao, 2000). This study was undertaken to determine if these strains would also be effective against plant-parasitic nematodes.

Bacteria for use in biological control of plant-parasitic nematodes can be grouped into parasitic bacteria and non-parasitic rhizobacteria (Siddiqui and Mahmood, 1999). Some rhizobacteria detrimental to plant-parasitic nematodes act through production of antibiotics, enzymes and toxins. These compounds may reduce nematode infection of roots by reducing egg hatch, modifying attraction to plant roots and/or through direct antibiosis. A number of Streptomycetes have activity against plant-parasitic nematodes. *Streptomyces avermitilis* produces macrocyclic lactones known as avermectins that have potent antihelminthic and insecticidal activity (Stretton et al., 1987). Avermectin B₁, also known as abamectin, is registered as an insecticide, acaricide, and nematicide in more than 50 countries. Several studies have shown that avermectins are as effective as conventional nematicides in controlling plant-parasitic nematodes (Blackburn et al., 1996; Jansson and Rabatin, 1998). Walker et al. (1966) found that culture filtrates of four isolates of *Streptomyces* spp. were nematicidal to adults and larvae of *Pratylenchus penetrans*, the root-lesion nematode. When inoculated into soil an isolate of *S. costaricanus* from nematode-suppressive soil had broad activity against plant-pathogenic fungi and several nematodes including *P. penetrans* (Dicklow et al., 1993).

Antimicrobial compounds may also have roles in determining the colonization ability and overall competitiveness of biological control agents in the rhizosphere (Pierson et al., 1994; Ryan and Kinkel, 1997). Recently, a number of biological control agents and plant growth promoting rhizobacteria have been shown to induce systemic plant defenses against pathogens and insects (van Loon et al., 1998). In some cases, induced resistance may be mediated by

compounds produced by the inducing organisms (De Meyer and Höfte, 1997; Leeman et al., 1995).

With the removal of several widely-used nematicides from the market, there is an increasing need for methods to control damage to plants from feeding by nematodes. Although genetic resistance to many sedentary nematodes, the root-knot and cyst-forming nematodes, has been introduced into adapted plant varieties, developing varieties with resistance to migratory nematodes has been more intractable. The root-lesion nematode (RLN), a migratory endoparasite with a broad host range, can cause economic levels of damage to many crop species. Surveys have determined that high, damaging populations of the RLN occur in many agricultural areas in the Midwestern and eastern United States (Thies, 1991).

The purposes of this study included testing the effect of antibiotic-producing strains of *Streptomyces* on RLN populations in alfalfa germplasm with varying amounts of resistance to the RLN, and in other forage legume and grass hosts of the nematode. The efficacy of non-antibiotic producing mutant strains was compared to parental strains to examine the role of antibiotics in RLN control. We also investigated the persistence of the introduced strains in heat-treated soil and on alfalfa roots over time.

Materials and methods

Streptomyces inoculum

Antibiotic-producing *Streptomyces* strain 93 and 63 (Liu et al., 1995) and spontaneous non-antibiotic producing mutants of these strains, 93M6 and 63M2 (Schottel et al., 2000), were cultured on oatmeal agar (OMA) plates (Liu et al., 1995). Parent strains were isolated from a naturally-occurring potato scab-suppressive soil in Grand Rapids, Minnesota. Spore suspensions from 10-day-old cultures were inoculated onto sterile vermiculite amended with oatmeal broth (3:1, v/v) (Liu et al., 1995) and cultured at 25 °C for 6–8 weeks. The colony-forming units (CFU) per cm³ of the final vermiculite inoculum were determined by dilution plating onto OMA.

Nematode inoculum

Two populations of *P. penetrans*, originally from Minnesota and Wisconsin field soils and pathogenic on alfalfa, were maintained in sterile axenic culture on sweet corn roots as described previously (Baldrige et

al., 1998). Nematodes were recovered from the cultures using a modified Cornell pie pan and diluted to 50 nematodes/mL in sterile tap water; an equal mixture of both strains was used to inoculate plants.

Plant infection and evaluation of biological control of the RLN

Four experiments were carried out to assess the ability of *Streptomyces* strains to suppress population growth of the RLN in host plants. Alfalfa germplasm used included the variety Baker, susceptible to RLN, and the germplasm MNGRN-16, resistant to RLN (Petersen et al., 1991). In all experiments, plants were grown in 3.8 × 21 cm plastic cone-tainers (Stuewe & Sons, Inc., Corvallis, OR) containing a sand:soil (1:1, v/v) mixture. Seeds were placed on the soil surface approximately 2 cm from the top of the cone-tainer and the *Streptomyces* inoculum was applied around seeds so that each *Streptomyces*-amended cone-tainer received approximately 1–2.5 × 10⁶ CFU/cm³ soil. Control treatments received an equivalent amount of vermiculite amended with oatmeal broth but lacking *Streptomyces*. Seeds were covered with a 0.5 cm layer of the sand:soil mixture, and placed in a growth chamber with a 16 h day-length at 21 °C, approximately 400 μmol m⁻² s⁻¹ irradiance and night temperature of 19 °C. After 1 week, seedlings were thinned to 3 plants/cone-tainer. At both 2 and 3 weeks after planting, 150 *P. penetrans* were injected into the soil around plant roots using a 5-ml pipette. Alfalfa foliage was clipped to within 2 cm of the crown at 8, 11 and 14 weeks after planting, dried in an oven at 35 °C for 7 days, and weighed. To determine nematode populations, roots were removed from soil 14 weeks after planting. Fibrous roots were removed from washed root systems, clipped into approximately 1 cm pieces, and placed in 25 × 100 mm glass petri dishes containing 50 mL tap water. Plates were placed on an orbital platform at 25 °C and shaken at 60 rpm. After 7 days, the water was decanted and the nematode suspension stored at 4 °C until nematodes in aliquots were counted using a stereozoom dissecting microscope. Roots were dried at 35 °C for 7 days and weighed. Each treatment consisted of 20 replicate cone-tainers arranged in a completely randomized manner.

In Experiment 1, we tested the ability of *Streptomyces* strain 93 to reduce population densities of RLN in roots of two alfalfa varieties when grown in a naturally-infested soil. Field soil, obtained from Grand Rapids, MN, with approximately 150 RLN/100

cm³ soil, was screened and mixed 1:1 with sterile sand (yielding approximately 225 RLN/cone-tainer) and used to grow the two alfalfa varieties. Treatments included the natural soil mixture, the natural soil mixture with 300 RLN added as described above, the natural soil mixture with *Streptomyces* strain 93, and the natural soil mixture with additional RLN and *Streptomyces* strain 93. Plant and nematode data was collected as described above.

In Experiment 2, a heat-treated greenhouse soil mixture was used to test *Streptomyces* strains 93 and 63 individually for their effectiveness in control of the RLN in the two alfalfa varieties and to test the persistence of the *Streptomyces* inoculum in soil and on alfalfa roots. The soil mixture consisted of 6 parts Waukegan field soil: 6 parts sand: 5 parts peat: 2 parts composted manure, pH 7.3. Soil was heat-treated by passing live steam under pressure through the soil to obtain 71–82 °C for 30 min. The CFU of *Streptomyces* in soil and on roots of treatments without RLN were measured at 2, 3, 8, 11 and 14 weeks after planting from each of 4 cone-tainers of each alfalfa variety. To estimate CFU in soil, 5 g of soil was sonicated for 15 min in 20 mL sterile water using a water bath sonicator and dilutions of 10⁻³ and 10⁻⁴ were made and plated onto OMA amended with antibiotics (Loria and Davis, 1988). Roots were removed from soil, rinsed briefly to remove rhizosphere soil, the lower 5 cm of the root system removed and sonicated in 40 mL sterile water for 15 min, and dilutions plated as above. Roots were pressed between paper towels to remove water and weighed. Plates were incubated at 30 °C for 6 days and colonies with the color and morphology of strain 93 or 63 were counted. Nematodes were counted *in situ* in control and *Streptomyces* strain 93-treated roots of both alfalfa varieties 3 and 6 weeks after inoculation with RLN, corresponding to 5 and 8 weeks after planting. Whole root systems were stained with acid fuchsin (Byrd et al., 1983), placed between two 6.0 × 7.5 cm glass slides, and the number of eggs, juveniles, and adult nematodes were counted using a stereozoom microscope. Four cone-tainers with a total of twelve plants were evaluated at both sampling dates for each treatment-alfalfa variety combination. Nematodes were also extracted from roots and counted 14 weeks after planting.

In Experiment 3, we tested the ability of *Streptomyces* strain 93 to reduce RLN population densities in five forage legumes and three grasses including the susceptible alfalfa variety Baker, *Medicago truncatula* 'Mogul', *M. polymorpha* 'Santiago', kura clover (*Tri-*

folium ambiguum 'Endura', white clover (*Trifolium repens*) 'Ladino', timothy (*Phleum pratense*) 'Timfor', quack grass (*Elytrigia repens*) 'Western bitter' and oat (*Avena sativa*) 'Starter'. All plant entries except *M. truncatula* and *M. polymorpha* were previously shown to support high population densities of the RLN (Thies et al., 1995). A heat-treated greenhouse soil mixture was used with inoculation of nematodes and *Streptomyces* 93 as described above. RLN population densities and CFU of *Streptomyces* in soil and on roots were determined at harvest. Oats were harvested 8 weeks after planting and the remaining plant species were harvested 14 weeks after planting.

In Experiment 4, we compared non-antibiotic producing mutants, 93M6 and 63M2, to parental strains 93 and 63 for the ability to reduce RLN population densities in the susceptible alfalfa variety Baker using the heat-treated greenhouse soil mixture. *Streptomyces* CFU in soil and on roots as well as RLN population densities were determined at harvest, 14 weeks after planting.

Statistical analysis

Analyses were performed using the generalized linear model procedure in SAS (SAS Institute, 1988). Student's *t*-test was used to separate means.

Results

Biological control of the RLN in alfalfa

The alfalfa germplasm MNGRN-16, with resistance to RLN, was previously shown to support substantially lower population densities of the nematode in greenhouse and field studies compared to the susceptible variety Baker (Thies et al., 1992; Thies et al., 1995). In Experiment 1, we again observed lower populations of the nematode in resistant plants. In addition, we found that treatment with *Streptomyces* 93 significantly reduced the number of nematodes ($P=0.05$) in roots of both the susceptible and resistant varieties compared to the non-amended control (Figure 1A). Nematode population density in Baker plants receiving the *Streptomyces* treatment was 28.8% of the population density in non-inoculated plants and RLN population density in MNGRN-16 plants with *Streptomyces* 93 was 6.1% of the population density in non-inoculated plants. Similarly, when 300 nematodes were added into each cone-tainer, plants receiving the *Streptomyces* amendment supported a significantly

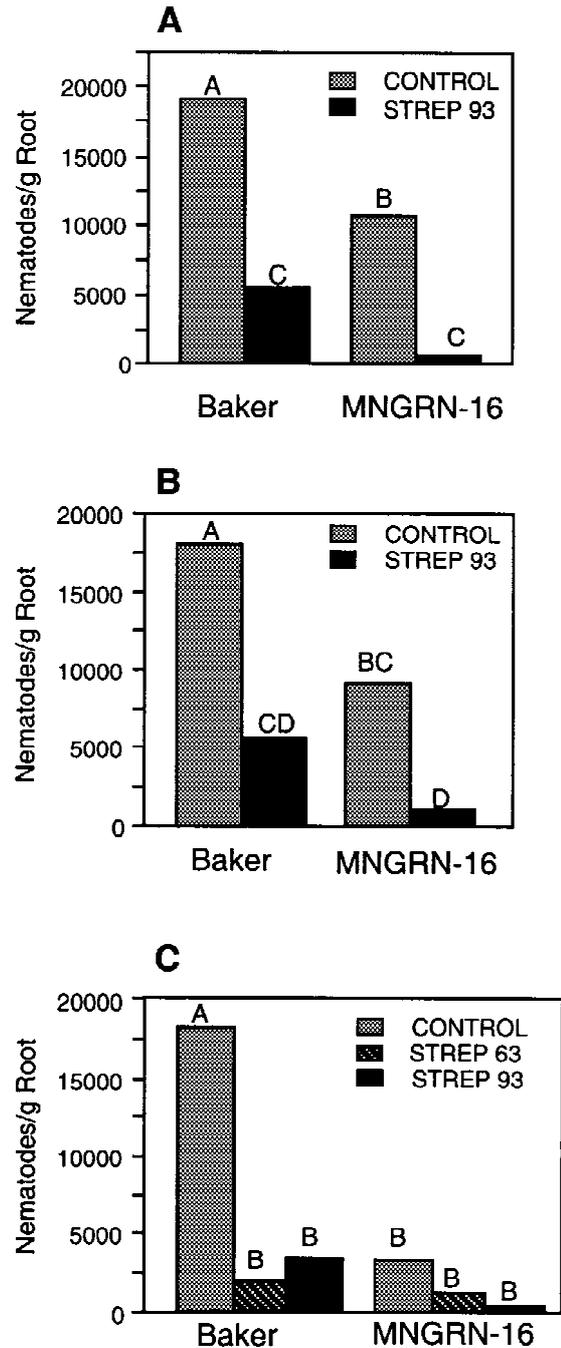


Figure 1. Root-lesion nematodes in fibrous roots of alfalfa plants. Data are means of nematodes per gram dry weight of fibrous roots from 14-week-old plants from the susceptible variety Baker and resistant germplasm MNGRN-16. Bars with different letters are significantly different ($P=0.05$). (A) Untreated field soil; Experiment 1. (B) Untreated field soil with 300 nematodes added to each cone-tainer; Experiment 1. (C) Heat-treated greenhouse soil mix with 300 nematodes added to each cone-tainer; Experiment 2.

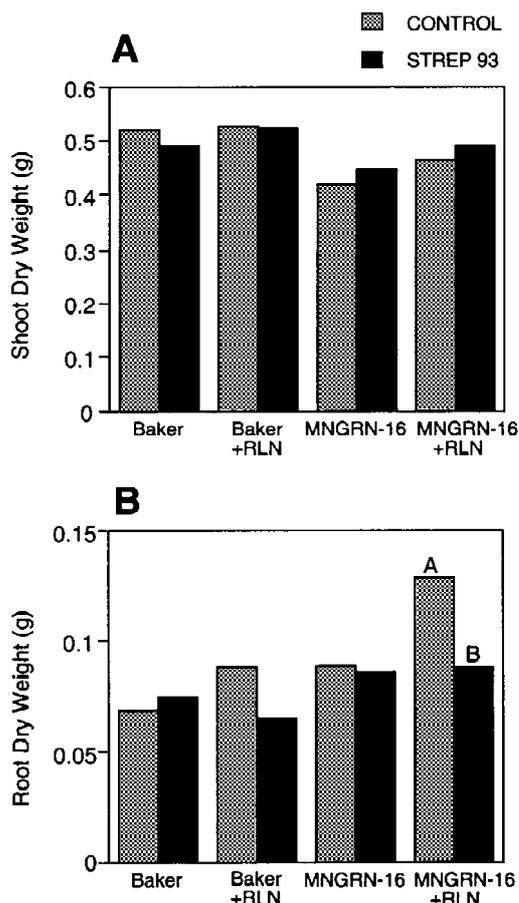


Figure 2. Effect of *Streptomyces* 93 on shoot and root dry weights of alfalfa plants grown in untreated field soil with indigenous nematodes and inoculated with nematodes (+RLN). Paired bars with different letters are significantly different ($P=0.05$). Absence of letters indicates no significant difference between treatments. (A) Shoot dry weight. (B) Root dry weight.

lower population density of RLN than non-amended plants (Figure 1B). Shoot weights of plants in this experiment were not significantly affected ($P=0.05$) by the *Streptomyces* treatment (Figure 2A). Weights of fibrous roots of MNGRN-16 plants were reduced significantly by the combined addition of nematodes and *Streptomyces* although the addition of *Streptomyces* 93 without additional nematodes did not significantly affect fibrous root weight (Figure 2B).

In Experiment 2, heat-treated soil inoculated with 300 RLN/cone-tainer was used to compare the effect of two antibiotic-producing *Streptomyces* strains, 93 and 63, on RLN population densities in roots of plants from the variety Baker and MNGRN-16. The effect of *Streptomyces* strain 93 on RLN populations

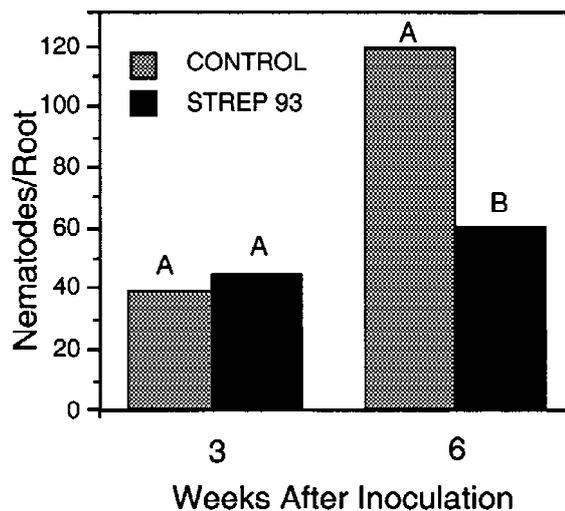


Figure 3. Mean root-lesion nematodes in root systems 3 and 6 weeks after inoculation. Bars with different letters are significantly different ($P=0.05$).

in roots was observed 6 weeks after nematode inoculation. Both varieties responded similarly and the population densities of RLN (adults and juveniles) in roots of the two varieties are combined in Figure 3. At harvest, roots of the resistant variety grown in soil without *Streptomyces* amendment contained 18.4% of the nematode populations in roots of the susceptible variety (Figure 1C). Both strains 93 and 63 had a similar ability to reduce RLN nematode population density in roots of the susceptible variety. RLN populations in the susceptible variety Baker were 18.8 and 10.9% of populations in non-inoculated plants when treated with strain 93 or 63, respectively. The differences in RLN population densities in roots of plants of MNGRN-16 either with or without *Streptomyces* strain 63 or 93 were not significant.

Shoot dry weights from Experiment 2 were measured at 8, 11, and 14 weeks after planting (Table 1). Shoot dry weights were less at 14 weeks than at 8 and 11 weeks. However, compared to the control, shoot dry weights were not significantly influenced by nematode inoculation, *Streptomyces* 93 amendment, or the combination of RLN and *Streptomyces* 93 at any time. Root dry weight was measured at 14 weeks after planting (Table 1). Compared to the control, *Streptomyces* treatment alone had no effect on root dry weight. Nematode inoculation resulted in smaller root weights compared to the control for both varieties. In MNGRN-16 plants the combined RLN and *Strepto-*

Table 1. Effects of root-lesion nematode (RLN) *Pratylenchus penetrans*, antibiotic-producing *Streptomyces* 93, and the combination of RLN and *Streptomyces* 93 on alfalfa shoot and root dry weight over time (Experiment 2)

Plant Variety ^a	Week	Control ^b	Strep 93	RLN	RLN+Strep 93
Shoot dry weight (mg) ^c					
Baker	8	898	ND ^d	920	1010
	11	728	636	718	725
	14	449	397	443	467
MNGRN-16	8	704	ND	811	816
	11	710	729	661	679
	14	495	564	500	553
Root dry weight (mg) ^e					
Baker	14	114 A	103 AB	78 B	100 AB
MNGRN-16	14	162 A	174 A	108 B	106 B

^aVariety Baker susceptible to RLN, MNGRN-16 resistant to RLN.

^bControl= no *Streptomyces* or nematode inoculation.

^cDifferences among treatments in shoot dry weight were not significantly different ($P=0.05$) at any time point

^dND=not determined.

^eValues in a row followed by different letters are significant at $P=0.05$.

myces treatment significantly reduced root dry weight compared to the control.

Colonization of alfalfa roots and soil by Streptomyces
Streptomyces strain 93 survived well in heat-treated soil and on roots when inoculated around the seed at planting. There were no significant differences between the two alfalfa varieties in *Streptomyces* densities in soil or on roots, and data for the two varieties are combined (Figure 4). No microbes having the colony morphology of strain 93 were observed from soil or roots of control non-inoculated treatments. *Streptomyces* populations in soil declined modestly over time, remaining within 0.5 log units of the initial density at the 14 week sampling date (Figure 4A). *Streptomyces* populations on roots showed a somewhat larger (approximately 1 log unit) decline in the first 8 weeks after planting, but subsequently increased between weeks 8 and 14 (Figure 4B).

Root-lesion nematode control in other host plants

Plant species varied widely in the RLN population densities supported in the absence of *Streptomyces* (Figure 5). In this experiment, populations of the RLN in roots of Baker alfalfa plants were much lower at harvest than in the previous two experiments. Large population densities of RLN were recovered from roots of kura clover, *M. polymorpha*, and *M. truncatula*. Small

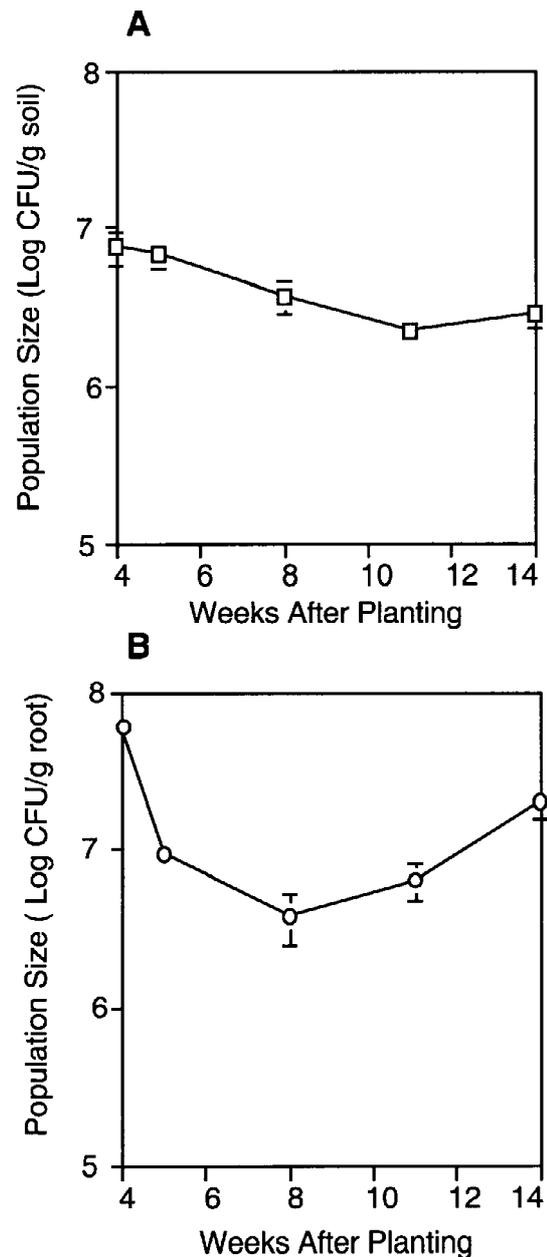


Figure 4. *Streptomyces* colonization of soil (A) and roots (B) over 14 weeks. Error bars denote standard deviations from means.

population densities of RLN were recovered from roots of white clover, oat, timothy and quack grass. Alfalfa plants receiving the *Streptomyces* strain 93 treatment had significantly smaller population densities of RLN than non-inoculated control plants (Figure 5). *Streptomyces* treatment had no significant effect on RLN populations in roots of *M. polymorpha*, kura clover, or white clover. Treatment of oat plants with

Table 2. Variation among plant species in shoot and root dry weight and *Streptomyces* colonization (Experiment 3)

Plant Species	Shoot Dry Weight (g)		Root Dry Weight (g)		<i>Streptomyces</i> colonization ^d	
	RLN ^b	RLN+Strep 93 ^c	RLN	RLN+Strep 93	CFU/g soil	CFU/g root
Baker alfalfa	0.724	0.678	0.212 ^{*d}	0.160 [*]	2.76	4.21
<i>M. polymorpha</i>	0.282	0.368	0.085	0.142	1.77	11.19
<i>M. truncatula</i>	1.471	1.432	0.183	0.175	0.63	2.85
Kura clover	1.092	1.317	0.146	0.172	0.68	3.77
White clover	2.229	2.157	0.376	0.294	1.26	6.10
Oat	1.066	0.996	0.477 [*]	0.274 [*]	7.53	49.88
Timothy	2.200	1.970	12.627 ^{**}	3.931 ^{**}	1.41	5.10
Quack grass	1.685	1.664	0.620	1.410	3.09	8.34

^a Colonization=colony forming units $\times 10^6$. Colonization was assessed in the treatment with *Streptomyces* 93 and RLN inoculation.

^b RLN= root-lesion nematode inoculation only.

^c RLN+Strep 93=RLN and *Streptomyces* 93 inoculation.

^d Between RLN and RLN+Strep 93 treatments, numbers indicated with an * or ** are significantly different at $P=0.05$ or $P=0.01$, respectively.

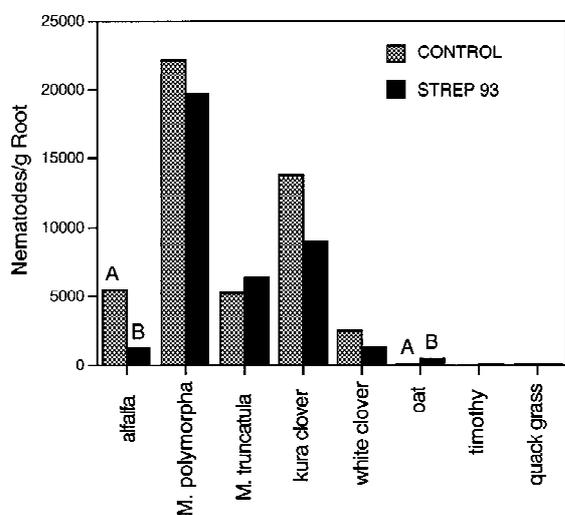


Figure 5. Mean root-lesion nematodes extracted from roots from eight plant hosts. Oats were assayed 8 weeks after planting; all other hosts were assayed 14 weeks after planting. Paired bars with different letters are significantly different ($P=0.05$). Absence of letters indicates no significant difference between treatments.

Streptomyces strain 93 resulted in a small increase in RLN populations.

Shoot dry weights of all host plants were similar in both treatments, RLN alone or RLN and *Streptomyces* 93 amendment (Table 2). Root dry weights of alfalfa, oats and timothy were significantly smaller in the treatment with both RLN and *Streptomyces* 93 compared to the treatment with only RLN.

Population densities of *Streptomyces* on plant roots and in soil were determined at 8 weeks after planting for oats and 14 weeks after planting for the other plant hosts (Table 2). *Streptomyces* densities in soil or on

roots of host plants harvested 14 weeks after planting were not significantly affected by host plant. The roots of the oat plants at 8 weeks after planting had greater *Streptomyces* densities than roots from the other hosts at 14 weeks after planting, and soil from oat plants also had greater *Streptomyces* densities than soil from other hosts. No microbes with the colony morphology of strain 93 were observed from soil or roots of plants inoculated only with RLN.

Effect of non-antibiotic producing mutants on population densities of RLN in alfalfa

Streptomyces parental strains and non-antibiotic producing strains significantly reduced RLN population densities in heat-treated soil as compared with the non-amended control (Table 3). Mutants and parent strains had similar affects on RLN population densities. None of the treatments affected shoot or root dry weight. The population densities of parental and respective non-antibiotic producing strains were similar on roots and in soil (Table 3).

Discussion

The alfalfa germplasm MNGRN-16 was developed by recurrent selection for resistance to RLN (Petersen et al., 1991). Plants from this germplasm support 20%, or fewer RLN than plants from a susceptible variety such as Baker in growth chamber experiments with heat-treated soil (Petersen et al., 1991; Thies et al., 1995). After amending soil with *Streptomyces* strain 93 or 63, we found that plants from the variety Baker supported RLN population densities approximately 30%

Table 3. Biological control of root-lesion nematode (RLN) *Pratylenchus penetrans* by antibiotic-producing and non-antibiotic-producing strains

Treatment ^b	RLN/g root	Shoot Dry Weight (g)	Root Dry Weight (g)	<i>Streptomyces</i> colonization ^a	
				CFU/g root	CFU/g soil
Control	10547 A ^c	2.156	0.069	0	0
RLN+S63	7139 B	2.342	0.085	2.1	0.19
RLN+S63M2	6823 B	2.222	0.082	2.1	0.14
RLN+S93	6328 B	2.487	0.089	1.5	1.1
RLN +S93M6	4315 B	2.378	0.083	1.4	2.4

^a *Streptomyces* colonization=colony forming units $\times 10^5$.

^b Control=RLN inoculation only, S63 and S93 antibiotic producing strains, S63M2 and S93M6 non-antibiotic producing strains.

^c Values followed by a different letter are significantly different at $P=0.05$. Absence of letters in a column indicates no significant difference between treatments.

of those in non-amended plants in untreated soil and population densities 10.9–18.8% of those observed in non-amended plants in heat-treated soil (Figure 1). These results suggest that the *Streptomyces* amendment suppresses nematode reproduction to a similar extent as the protection provided by genetic resistance.

The suppressive effect was observed within 6 weeks after inoculation with nematodes (Figure 3) and continued at least through the end of the experiment at 14 weeks after planting. Because the RLN can infect alfalfa roots at any time in the life of the plant, a biological control agent should be effective over a long time. Nematicides such as carbofuran are effective for only short times and promote crop establishment but do not afford long-term protection of plants (Thies et al., 1992). We found that *Streptomyces* strain 93 populations were fairly stable over the 14 weeks of the experiments. This strain persisted in soil during 4 years of field trials investigating control of potato scab disease (Bowers et al., 1996) and a 3-year field trial for control of RLN on alfalfa (Samac, unpublished). Other *Streptomyces* are also effective colonizers of soil and root systems (Kortemaa et al., 1994; Yuan and Crawford 1995).

The RLN has a very broad host range and will damage a number of forage legumes and grasses. The promising results we obtained for control of RLN in alfalfa prompted us to investigate the effect of *Streptomyces* treatment on population densities of the RLN in other hosts. Grass hosts such as oats, timothy, and quack grass can support large populations of the RLN (Thies et al., 1995). We observed relatively small populations of RLN in these hosts. The legume hosts, however, supported substantially larger populations of RLN. Treatments with *Streptomyces* strain 93 reduced

RLN populations in all legume hosts except *M. truncatula* but reductions were statistically significant only in alfalfa.

In other studies using *Streptomyces* spp. for biological control of plant diseases, both plant biomass increases (El-Shanshoury, 1994; El-Shanshoury et al., 1995; El-Tarabily et al., 1995; Yuan and Crawford, 1995) and decreases (Crawford et al., 1993; Rothrock and Gottlieb, 1984) have been observed. In the four experiments reported here, no difference in shoot dry weights between nematode-inoculated and nematode plus *Streptomyces*-inoculated plants were observed. In two experiments we saw a decrease in fibrous root dry weight of MNGRN-16 plants when RLN and *Streptomyces* 93 were both present compared to the treatment with RLN alone. In two other experiments, no significant difference in fibrous dry root weight was observed after inoculation with *Streptomyces* and RLN. In two other hosts of the RLN, oat and timothy, *Streptomyces* and RLN treatment decreased root biomass compared to *Streptomyces* alone; none of the treatments affected shoot biomass of any of the hosts tested (Table 2). Further experiments are needed to clarify the effects of *Streptomyces* and RLN on root biomass.

Streptomyces strains 93 and 63 produce antimicrobial compounds that are highly effective at inhibiting growth of the potato pathogen *Streptomyces scabies* in *in vitro* assays (Lui et al., 1995). Mutant strains 63M2 and 93M6 either do not produce the compounds active against *S. scabies* *in vitro* or produce greatly reduced quantities of antibiotics (Schottel et al., 2000). Nonetheless, we found that the mutants were as effective as the wild type strains in suppressing RLN in roots of Baker alfalfa plants (Table 3). This suggests that the mechanism that causes growth inhibition of *S. scabies*

can be separated from the mechanism active against RLN. Alternatively, *in vitro* production of compounds inhibitory to *S. scabiei* and/or to RLN may not be correlated with *in vivo* production. There are a number of possible mechanisms for suppression of RLN population densities on alfalfa roots. The RLN-suppressive strains may be producing compounds that directly affect the RLN, or that induce resistance to the nematode in the host plant. The RLN-suppressive strains may also be catabolizing root exudates, or other root-associated compounds that attract RLN. Alternatively, *Streptomyces* strains could suppress RLN populations by reducing space available for nematode feeding.

There is increasing evidence that plant growth promoting rhizobacteria can induce systemic resistance to pathogenic fungi and bacteria; the mechanism(s) of induction is unclear. In alfalfa plants with genetic resistance to RLN, we have found that the constitutive level of the flavonoid phytoalexin medicarpin is 2–10 fold higher than in susceptible plants and medicarpin levels are directly associated with the level of resistance (Baldrige et al., 1998). Purified medicarpin has a nematostatic effect, immobilizing nematodes and protecting roots from penetration (Baldrige et al., 1998). Inoculation of alfalfa with *Streptomyces* may affect nematode populations through a stimulation of flavonoid production by the plants. Production of phenolic compounds and evidence of systemic resistance induced by disease-suppressive strains of *Streptomyces* in alfalfa is under investigation.

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