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# Green manures and crop sequences influence alfalfa root rot and pathogen inhibitory activity among soil-borne streptomycetes

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**Abstract**

A two-year trial was conducted to determine the effects of green manures and crop sequences on plant disease, streptomycete and bacterial densities, and inhibitory activity of indigenous streptomycetes against four target pathogens. Green manure treatments, buckwheat (*Fagopyrum esculentum* L.), canola (*Brassica napus* L.), sorghum-sudangrass (*Sorghum bicolor* (L.) Moench × *Sorghum sudanense* (Piper) Stapf.), and fallow control were tested in conjunction with three crop sequences in a Phytophthora-infested soil placed in containers. Alfalfa (*Medicago sativa* L.), potato (*Solanum tubersoum* L.), or corn (*Zea mays* L.) was grown in the first year, and alfalfa was grown in all containers in the second year. Compared to fallow controls, alfalfa grown in sorghum-sudangrass- or buckwheat-treated soil had significantly greater stand counts and total biomass, respectively. In addition, alfalfa grown in fallow-treated soils had the greatest Phytophthora root rot as a function of stand count. Crop rotation also had a significant effect on alfalfa root rot and yield. Potato scab disease intensity was greatest on tubers grown in fallow-treated soils, while tubers grown in canola-treated soils had the highest yields (total tuber weight). Green-manure-treated soils tended to have greater streptomycete and bacterial densities than fallow-treated soils. In addition, buckwheat- or sorghum-sudangrass-treated soils had greater proportions of streptomycetes that were antagonistic against the target pathogens than fallow-treated soils. The proportion of antagonists in soil was negatively correlated with alfalfa root rot, and positively correlated with alfalfa stand counts. Inhibitory activity of the streptomycetes was also negatively correlated with potato scab and positively correlated with potato yield. These data suggest that green manures may provide a strategy for increasing pathogen inhibitory activity within the streptomycete community in soil, and, in conjunction with crop rotation, may contribute to the control of a diverse collection of soil-borne plant pathogens on multiple crop species.

*Abbreviations*: GM – green manure, WA – water agar, SCA – starch casein agar, OA – oatmeal agar, PDWA – potato dextrose water agar, PDA – potato dextrose agar, CZB – Czapek-Dox broth, DAP – days after planting.

## Introduction

The control of soil-borne plant pathogens presents a challenge to crop production. In Minnesota, Phytophthora root rot and damping-off of alfalfa, and potato scab, caused by *Streptomyces scabies* (Thaxter) Lambert and Loria (Lambert and Loria, 1989), can both cause significant yield and quality losses (Erwin, 1990; Hooker, 1981; Rhodes, 1990). Control of alfalfa damping-off is especially challenging due to the numerous pathogens involved in the disease complex including *Pythium*, *Aphanomyces*, *Phytophthora*, and *Rhizoctonia* (Altier and Thies, 1995; Handelsman et al., 1990; Holub and Grau, 1990; Schmitthenner, 1964). Although disease resistant varieties of potato and alfalfa are available, resistance is often incomplete or ineffective. Chemical control, when available, may not be suitable due to the economic costs and environmental risks associated with application. Effective control strategies for the suppression of multiple pathogens are desired.

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Organic soil amendments such as green manures (GMs) have been studied as a potential control strategy for soil-borne pathogens of several host plants. Green manures offer substantial benefits to the soil including increased nutrients and organic matter, improved soil structure, and weed and erosion control (Abdallahi and N'Dayegamiye, 2000; Al-Khatib et al., 1997; Blackshaw et al., 2001), and can often be incorporated into a management scheme without interrupting crop production. In addition, GMs have been shown to significantly reduce *Aphanomyces* root rot of pea (Muehlchen et al., 1990; Papavizas, 1966; Williams-Woodward et al., 1997), Phytophthora root rot of alfalfa (Kinkel and Samac, unpublished data), common scab of potato (Millard, 1923; Millard and Taylor, 1927; Rouatt and Atkinson, 1950; Sanford, 1946), Verticillium wilt of potato (Davis et al., 1996), and Rhizoctonia stem canker of potato (Scholte and Lootsma, 1998).

The mechanisms by which GMs may influence plant disease are varied and often unknown. Green manures may influence pathogens directly through the breakdown of glucosinolates (Charron and Sams, 1999) or by releasing fungitoxic compounds such as avenacin, saponins (Deacon and Mitchell, 1985; Engelkes and Windels, 1994) or allyl isothiocyanate (Mayton et al., 1996). Green manures may also affect soil-borne pathogens indirectly by influencing indigenous microbial populations. For example, incorporation of soil amendments has been shown to increase soil microbial activity (Davis et al., 1994), microbial diversity (Lupwayi et al., 1998), as well as densities of bacteria (Henis et al., 1967; Mazzola et al., 2001; Rouatt and Atkinson, 1950), fluorescent *Pseudomonas* spp. (Bulluck and Ristaino, 2002; Conn and Lazarovits, 1999; Mazzola et al., 2001), non-pathogenic *Fusarium* spp. (Davis et al., 1994), mycophagous organisms (Scholte and Lootsma, 1998) and streptomycetes and other actinomycetes (Henis et al., 1967; Kinkel et al., 2001; Mazzola et al., 2001) in soil. These changes in the microbial community may affect pathogen populations through competition, parasitism, predation or antagonism (Hornby, 1983; Millard and Taylor, 1927; Scholte and Lootsma, 1998; Weinhold and Bowman, 1968).

Research in our lab has focused on manipulating the indigenous soil microbial community, specifically the streptomycete community, in an attempt to achieve disease control. Streptomycetes are Gram-positive bacteria that are ubiquitous in soil (Gottlieb, 1973). Their prolific antibiotic production has made them the subjects of numerous studies on the biocontrol of plant pathogenic bacteria, fungi and nematodes. Inoculated streptomycetes have been shown to reduce diseases caused by *Streptomyces* (Liu et al., 1995), *Phytophthora* (Jones and Samac, 1996; Xiao et al., 2002), *Pythium* (Chamberlain and Crawford, 1999; Jones and Samac, 1996; Yuan and Crawford, 1995), *Verticillium* (El-Abyad et al., 1993), *Rhizoctonia* (Chamberlain and Crawford, 1999), *Fusarium* (Chamberlain and Crawford, 1999) and *Pratylenchus* (Samac and Kinkel, 2001). However, control of soil-borne pathogens using inoculated antagonists is often inconsistent and unpredictable (Ryan and Kinkel, in press). Enhancing pathogen inhibitory activities of the indigenous soil microbial community, specifically the streptomycetes, through the incorporation of GM, offers a potential alternative for plant disease control.

The objectives of this research were to determine the effects of GMs and crop sequences on plant disease, streptomycete and bacterial densities, and on pathogen inhibitory activity of the streptomycete community, and to evaluate the relationships between shifts in the inhibitory activity of the streptomycete community and plant disease. To meet these objectives, a two-year trial was conducted in which GM treatments (canola, buckwheat, sorghum-sudangrass and a fallow control) were tested in conjunction with three production crop sequences (alfalfa-alfalfa, corn-alfalfa, and potato-alfalfa) in all possible combinations.

## Materials and methods

In May 2001, sandy loam soil was collected from a naturally Phytophthora-infested field at the University of Minnesota Experimental Station in St. Paul, MN, USA. The field had been used as an alfalfa disease nursery for evaluating alfalfa resistance to Phytophthora root rot for over 30 years, and had a history of substantial root rot. Soil was mixed with perlite (2:1, vol/vol) in a cement mixer and distributed into 60 5-liter pots. Four GM treatments (canola, buckwheat, sorghum-sudangrass and a fallow control) were tested in conjunction with three crop sequences (alfalfa-alfalfa, corn-alfalfa and potato-alfalfa). Each GM-crop sequence treatment was applied to five containers (replicates) in a completely randomized design. Four treatments, the GMs canola (*Brassica napus* L. 'Impact,' 20 seeds/pot), common buckwheat (*Fagopyrum esculentum* L., ten seeds/pot), and sorghum-

sudangrass (*Sorghum bicolor* (L.) Moench x *Sorghum sudanense* (Piper) Stapf., Dekalb hybrid 'FS-5,' 30 seeds/pot) or weed-free fallow were established on May 16, 2001. After approximately seven weeks, aboveground GM plant matter was cut into approximately 1 cm pieces and thoroughly incorporated into the soil of the same container in which it had grown. Soil from fallow treatments was mixed similarly. Approximately 20 days after incorporation, main crops were sown: alfalfa (*Medicago sativa* L. 'Nitro,' approximately 80 seeds/pot), corn (*Zea mays* L. 'Bodacious,' ten seeds/pot), and potato (*Solanum tuberosum* L. 'Kennebec,' two cut seed pieces/pot). Once the corn sprouted, plants were thinned to three plants per pot. Pots were maintained outside except in early spring and fall when they were moved to a greenhouse to prevent frost damage. Balanced fertilizer (20-20-20) was applied to all containers in equal amounts during production crop growth both years of the trial.

Forty-six days after planting (DAP) in 2001, and 47 and 82 DAP in 2002, all alfalfa plants were cut approximately 2 cm from the soil line to simulate conventional harvests. On October 22, 2001, all crops were dug and assessed for disease as described below. The next day each container was replanted to the same GM treatment it had received the previous spring. Green manures grew for approximately 40 days in the greenhouse and pots were then transported outside to overwinter. In the spring of 2002, GM biomass was cut and incorporated as was done the previous spring. Soil from fallow treatments was mixed similarly. Approximately three weeks after incorporation, alfalfa ('Nitro') was sown in all pots at the same rate as the previous year. In the fall of 2002, entire plants were harvested from each container and assessed as described below.

Soil was sampled throughout the growing season in both years of the study: on July 26, September 19, and October 22 in 2001, and on June 4 and July 26 in 2002. For each sampling date, two soil cores (2 cm in diameter $\times$ 15 cm deep) were collected from two random locations in the center of each container and stored at 4 °C until processed as described below.

*Alfalfa health assessment*

The number of alfalfa plants per container was counted 5, 8, 14, 20, 46 and 87 DAP in the first year, and 21, 47, 82, and 117 DAP in the second year to estimate stand vigor. After each simulated harvest, plant matter was bulked by container, dried at 35 °C, and the biomass was determined. At the final harvest, all alfalfa plants were gently removed from the soil. Roots were rinsed with water and assessed for Phytophthora root rot severity as described by Thies et al. (1991). Briefly, plants were rated on a scale from 1 to 5: (1): roots clean, no lesions; (2): small root lesions ($<$ 2 mm); (3): large, non-girdling root lesions and/or branch root tips rotted off; (4): extensive lesions with ends of large tap or lateral roots rotted off 5 cm or more below the crown; (5): tap and lateral roots almost destroyed, plant alive. After disease assessment in 2001, entire plants were bulked by container, dried at 35 °C and the biomass was determined. In 2002, after disease rating, plants were cut near the soil line and separated into root and aboveground sections. Plant sections were bulked per container, dried at 35 °C, and root and aboveground biomass were weighed separately.

*Potato health assessment*

At harvest, all tubers from each container were gently excavated from the soil and rinsed with water. All tubers were weighed, the percent surface area of each tuber covered with scab lesions (percent scab) was estimated, and the number of scab lesions of type 2 or 3 and of type 4 or 5 (2 = small superficial lesions, 3 = periderm broken, 4 = pit, and 5 = deep pit) were counted per tuber as described by Liu et al. (1995).

*Microbial community analyses: soil streptomycete and bacterial populations*

All soil samples were processed to estimate streptomycete densities using a modification of a method described by Herr (1959). Soil samples were dried overnight under three layers of sterile cheesecloth at room temperature. Soil was ground with a sterile spoon until fine, and 5.0 g of the soil were added to 50 ml sterile water and shaken at 175 rpm for 60 min at 4 °C. Soil wash suspensions were dilution plated onto water agar (WA; 10 g bacto agar/liter). Subsequently, plates were overlaid with 5.0 mL of molten starch casein agar (SCA; Becker and Kinkel, 1999) and incubated at 28 °C for five days. This semi-selective double layer agar method (WA/SCA) results in the growth of predominantly streptomycetes whose densities were estimated based on colony morphology. All soil samples from 2002 were also dilution-plated onto oatmeal agar (OA, Lorang, 1995) amended with cycloheximide (50 $\mu$g/mL). Plates were incubated at 28 °C for five days after which the number of culturable bacteria and streptomycetes on each plate was recorded.

Streptomycete and bacterial densities were expressed as the number of colony-forming units per g dry soil (CFU/g).

*Microbial community analyses: streptomycete pathogen inhibitory activity*

The effects of GMs and crop sequences on the antagonistic activity of soil streptomycetes against pathogenic *Streptomyces*, *Verticillium*, *Fusarium*, and *Rhizoctonia* were assessed using a modification of a method described by Herr (1959). Soil dilutions were performed as described previously and plated onto WA. Plates were then overlaid with 5.0 mL of molten SCA for *Fusarium* tests, and 5.0 mL molten WA for all other pathogen tests ($n = 3$ for each soil sample/pathogen combination). All plates were incubated at 28 °C for three days after which streptomycete colonies were visible. Pathogen inhibitory activity was determined by overlaying each soil dilution plate with medium and one of four pathogens: *S. scabies* (strain 87; obtained from D. Liu; isolated from potato in Grand Rapids, MN; Liu, 1992), *R. solani* (ATCC #44660, AG-3, isolated from potato, obtained from N. Anderson), *V. dahliae* (strain VA33A, VCG 4A, isolated from potato, obtained from N. Anderson) or *F. oxysporum* f. sp. *medicaginis* (strain 31Fs, isolated from alfalfa, obtained from D. Samac), as described below.

*S. scabies:* Ten milliliters of SCA were transferred onto each soil dilution plate. Subsequently, 150 $\mu$L of a 20% glycerol suspension containing approximately $10^8$ CFU/mL of *S. scabies* were plated onto the medium. Plates were incubated at 28 °C for three days.

*V. dahliae:* For the soil sampled in July 2001, 10 mL of potato dextrose water agar (PDWA; 2.4 g/L potato dextrose broth, Difco Laboratories, Detroit; 10 g/L bacto agar) were transferred onto each soil dilution plate. Subsequently, 10 mL sterile water were added to a 7-day old plate of *V. dahliae* growing on OA, and spores and mycelia were loosened by scraping with a sterile loop. Next, 150 $\mu$L of the suspension were spread onto each soil dilution plate. Plates were incubated two days at room temperature.

Soil sampled at all other times was processed using a slightly different procedure that was more time efficient and resulted in clearer inhibition zones. Instead of plating the pathogen on top of the medium, 7.5 mL of the pathogen suspension were added to 0.5 L molten PDWA and mixed thoroughly. Ten milliliters of this suspension were transferred on top of each soil dilution plate. Plates were incubated two days at room temperature. Estimates of antagonist densities did not differ significantly between the two different procedures used in this study, though mean inhibition zone sizes measured from the inoculated media overlays were nearly twice as large as zones measured from the overlays in which pathogen suspensions were spread over the medium (data not shown).

*F. oxysporum:* A seven-day old culture of *F. oxysporum* growing on potato dextrose agar (PDA, Singleton, 1992) was cut into approximately 1-cm$^2$ pieces and blended with 100 mL sterile water in a sterile flask of a Waring blender ($3 \times 5$ s, $1 \times 10$ s). Four milliliters of this suspension were added to 100 mL molten PDWA, and 10 mL of the resulting suspension were overlaid on top of each soil dilution plate. Plates were incubated at room temperature for one day.

*R. solani:* *Rhizoctonia solani* were grown as still cultures for seven days at 25 °C in 250 mL flasks containing 25 mL of Czapek-Dox broth (CZB; Difco Laboratories, Detroit, 35 g/L). The entire contents of two flasks were blended in a sterile flask of a Waring blender with 50 mL molten CZB containing 0.5 g bacto agar ($3 \times 5$ s, $1 \times 10$ s). Subsequently, 10 mL of the suspension were transferred onto each soil dilution plate. Plates were incubated at 25 °C for two days.

For all pathogens, streptomycete inhibitory activity was measured by counting the number of streptomycete antagonists per plate (CFU/g soil). Streptomycetes were considered antagonistic if they produced a clear inhibition zone against the target pathogen. The density of antagonists as a proportion of streptomycete density (proportion of antagonists) was expressed as the density of antagonists divided by the streptomycete density (WA/SCA). The diameter of each inhibition zone was also measured.

*Statistical analyses*

Data were analyzed using SAS (release 8.0; SAS Institute, Inc., Cary, NC). Data were subjected to analysis of variance (proc glm), Fisher's least-significant-difference test (LSD), and Pearson correlations. In addition, potato disease data were analyzed by comparing pooled GM treatments (buckwheat, canola,

and sorghum-sudangrass) with fallow treatments using the binomial test and one-sided Wilcoxon rank-sum test to detect differences among treatment groups (DeGroot, 1975). Proportional data were angular-transformed (arcsine square root) prior to analysis to normalize the variance. In cases in which there was a significant effect of crop on the studied soil variable prior to the crop treatment, subsequent analyses were performed using the pre-treatment sample data as a covariate. Changes in microbial community and pathogen inhibitory activity before and after treatment were analyzed; differences among treatments were similar whether considering changes over time or differences at a single time following treatment. Only comparisons among treatments within a sampling time are presented. There were no significant GM-crop treatment interactions unless noted.

The binomial test was used to detect the consistency of Pearson correlation coefficients for relationships among microbial community characteristics over times. Specifically, in cases where multiple independent correlation analyses were performed, the frequency of positive or negative correlation coefficients was counted, and the binomial *P*-value was calculated against the null hypothesis that correlation coefficients were completely random (DeGroot, 1975). The binomial test was also used to detect significant patterns in correlations between microbial community characteristics and plant disease.

## Results

### *Alfalfa health and disease following green manure and crop treatments*

Phytophthora root rot was extensive both years of the trial. Despite an average germination rate of 88%, only about 25% of sown seeds survived to harvest either year of the trial. Those that did survive had extensive root rot lesions. In the first year of the trial, GM treatment (buckwheat, canola, sorghum-sudangrass or fallow) had a substantial effect on alfalfa stand count five ($F_{3,16} = 4.64$, $P = 0.016$) and 46 ($F_{3,16} = 1.75$, $P = 0.197$) DAP. Five DAP, there were significantly more alfalfa plants growing in sorghum-sudangrass-treated soil than all other soils ($P < 0.05$, LSD). Similarly, 46 DAP, significantly more alfalfa plants were growing in sorghum-sudangrass-treated soil than in fallow-treated soil ($P < 0.05$, LSD); canola and buckwheat treatments were intermediate (Table 1).

Green manure treatments had no significant effect on alfalfa stand count in the second year of the trial. However, the previous crop had a substantial effect on alfalfa stand count 82 ($F_{2,48} = 2.23$, $P = 0.118$) and 117 ($F_{2,48} = 3.22$, $P = 0.049$) DAP. At both times, there were significantly more alfalfa plants in soils previously planted to corn than in soils previously planted to alfalfa ($P < 0.05$, LSD); soils previously planted to potato were intermediate (Table 2).

Green manure treatments had a considerable effect on aboveground plant biomass measured 46 DAP ($F_{3,16} = 4.47$, $P = 0.019$) and total plant biomass measured at harvest in 2001 ($F_{3,16} = 2.04$, $P = 0.148$). Alfalfa grown in buckwheat-treated soil had the greatest aboveground biomass and total biomass as compared with alfalfa grown in any other soil (Table 1). In 2002, GM treatments had no significant effect on aboveground plant biomass at any harvest date. However, there was a significant effect of crop treatment on aboveground plant biomass at each harvest time ($F_{2,48} = 5.97$, $P = 0.005$ 47 DAP; $F_{2,48} = 22.08$, $P < 0.001$ 82 DAP; $F_{2,48} = 15.54$, $P < 0.001$ 117 DAP), and root biomass at harvest ($F_{2,48} = 17.78$, $P < 0.001$). On each date, alfalfa grown in soil previously planted to corn had significantly greater aboveground and root biomass than alfalfa grown in soil previously planted to alfalfa or potato ($P < 0.05$, LSD, Table 2).

Green manures had a modest effect on the mean root rot rating per plant in the first year of the trial ($F_{3,16} = 2.72$, $P = 0.079$). Alfalfa grown in buckwheat-treated soil had significantly greater mean root rot ratings than plants grown in canola- or fallow-treated soils ($P < 0.05$, LSD, Table 1). However, root rot disease was positively correlated with stand count at harvest ($R = 0.557$, $P = 0.011$). When disease was expressed as a function of stand density (mean per plant root rot rating divided by the stand count in each container), plants grown in fallow-treated soil had a significantly greater disease rating than those grown in sorghum-sudangrass-treated soil ($P < 0.05$, LSD); alfalfa grown in buckwheat- and canola-treated soils had intermediate disease ratings (Table 1).

In the second year of the trial, alfalfa grown in fallow-treated soil had the greatest mean root rot ratings, although differences were not significant. However, crop treatment had a significant effect on the mean alfalfa root rot rating ($F_{2,48} = 12.53$, $P < 0.001$). Alfalfa grown in soil previously planted to alfalfa had significantly greater mean root rot ratings

*Table 1.* Alfalfa stand count, aboveground biomass, total biomass at harvest, average disease severity, and average disease severity as a function of stand count following green manure treatments in the first year of the study

| | Green manure | | | |
|---|---|---|---|---|
| | Buckwheat | Sorghum-sudangrass | Canola | Fallow |
| Stand count (5 DAP[a]) | 32.2 b | 44.6 a | 34.6 b | 32.2 b |
| Stand count (46 DAP) | 25.6 ab | 28.2 a | 20.8 ab | 15.8 b |
| Aboveground biomass (46 DAP) | 7.8 a | 5.4 ab | 4.4 b | 3.8 b |
| Total biomass | 14.0 a | 10.6 ab | 8.0 b | 8.6 ab |
| Average disease severity[b] | 3.16 a | 2.89 ab | 2.53 b | 2.45 b |
| Average disease per stand count[c] | 0.16 ab | 0.14 b | 0.17 ab | 0.28 a |

Means in each row with different letters are significantly different ($P < 0.05$, LSD)
[a]Days after planting.
[b]Disease severity = 1–5 (1 = healthy roots, no lesions, 5 = tap and lateral roots almost destroyed, plant alive).
[c]Disease severity divided by stand count.

*Table 2.* Alfalfa aboveground and root biomass, stand count, average root rot severity, streptomycete density, and antagonist density following cultivation of alfalfa, corn or potato

| | Previous crop | | |
|---|---|---|---|
| | Alfalfa | Corn | Potato |
| Aboveground biomass (47 DAP[a]) | 7.85 b | 10.15 a | 8.20 b |
| Stand count (117 DAP) | 18.7 b | 25.0 a | 21.3 ab |
| Aboveground biomass (117 DAP) | 9.30 b | 14.95 a | 9.80 b |
| Root biomass (117 DAP) | 7.79 b | 13.62 a | 8.04 b |
| Root rot severity[b] | 3.08 a | 2.02 b | 2.23 b |
| Streptomycete density[c,d] (June 4, 2002) | 33.55 b | 41.28 a | 35.23 ab |
| *S. scabies* antagonist density[c] (July 26, 2002) | 24.43 ab | 21.10 b | 27.98 a |
| *V. dahliae* antagonist density[c] (July 26, 2002) | 4.44 b | 4.53 b | 10.64 a |

[a]Days after planting.
[b]Disease severity = 1–5 (1 = healthy roots, no lesions, 5 = tap and lateral roots almost destroyed, plant alive).
[c]$\times 10^5$.
[d]Estimated from oatmeal agar plates.

*Table 3.* Pearson correlation coefficients and associated P-values describing the relationships between initial streptomycete density (July 2001) and the change in proportion of streptomycetes antagonistic against each target pathogen from July 2001 to October 2001

| | Target pathogen | | | |
|---|---|---|---|---|
| Green manure treatment | *S. scabies* | *V. dahliae* | *F. oxysporum* | *R. solani* |
| Green manure[a] | $R = 0.393$ | $R = 0.339$ | $R = 0.321$ | $R = 0.405$ |
| | $P = 0.008$ | $P = 0.023$ | $P = 0.031$ | $P = 0.006$ |
| Fallow | $R = -0.004$ | $R = 0.408$ | $R = 0.135$ | $R = 0.071$ |
| | $P = 0.989$ | $P = 0.131$ | $P = 0.632$ | $P = 0.802$ |

[a]Buckwheat, canola and sorghum-sudangrass.

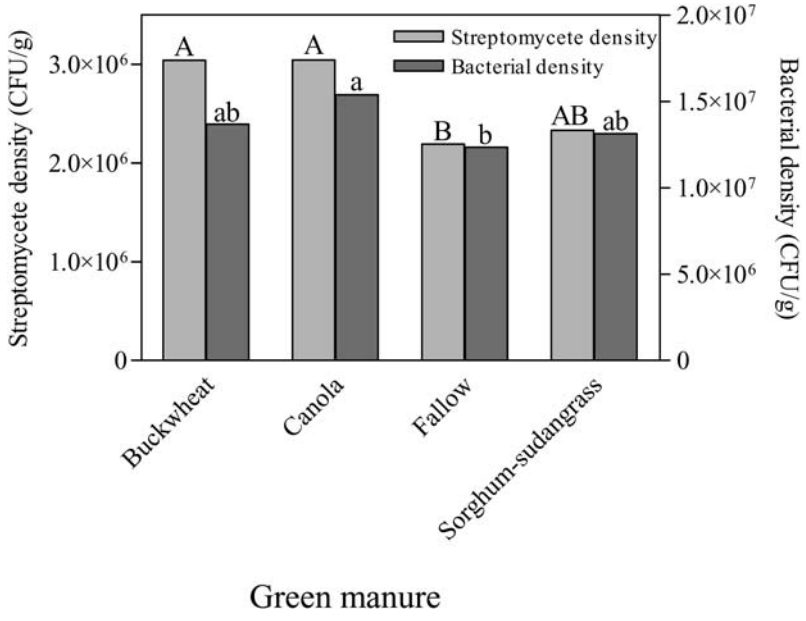


*Figure 1.* Streptomycete and bacterial densities in soil following green manure treatment. Data shown are means of 15 replicates of soil sampled July 2002. For each microbial density, bars with different letters are significantly different ($P < 0.05$, LSD).

than plants grown in soil previously planted to corn or potato ($P < 0.05$, LSD, Table 2).

*Potato health and disease following green manure treatments*

In general, scab disease was very low and infected tubers had only superficial scab lesions. Despite this, GM treatment had a substantial effect on scab severity. Tubers grown in fallow-treated soil had significantly greater disease severity (percent scab) than tubers grown in GM-treated soils (buckwheat, canola and sorghum-sudangrass, $P = 0.043$, Wilcoxon, one-sided exact test), and significantly more scab lesions than potatoes grown in GM-treated soils ($P = 0.010$, Wilcoxon, one-sided exact test). Tubers grown in canola-treated soil had a significantly greater total tuber weight per plant than those grown in buckwheat-treated soils; tubers grown in sorghum-sudangrass- and fallow-treated soils were intermediate ($P < 0.05$, LSD).

*Streptomycete and bacterial densities following green manure and crop treatments*

Streptomycete densities varied significantly over the five sampling dates among all 60 containers ($F_{4,295} = 15.36$, $P < 0.001$). Soil collected in July 2002 had significantly lower streptomycete densities than soil collected at any other time ($P < 0.05$, LSD). Both green manure and crop treatment had significant effects on streptomycete and bacterial densities estimated from OA plates. In particular, GM-treated soils (buckwheat, canola, and sorghum-sudangrass) tended to have greater streptomycete and bacterial densities. There was a significant effect of GM on streptomycete densities ($F_{3,48} = 3.16$, $P = 0.033$) and a marginal effect on bacterial densities ($F_{3,48} = 2.44$, $P = 0.076$) estimated in July 2002; canola-treated soil had significantly greater streptomycete and bacterial densities than fallow-treated soils ($P < 0.05$, LSD, Figure 1). Crop treatment had a significant effect on streptomycete (OA) densities estimated in June 2002 ($F_{2,48} = 3.06$, $P = 0.056$), and bacterial densities estimated in July 2002 ($F_{2,48} = 4.71$, $P = 0.014$); soils planted to corn the first year had greater streptomycete and bacterial densities in June and July, respectively, than soils previously planted to alfalfa ($P < 0.05$, LSD, Table 2).

*Density of pathogen antagonists following green manure and crop treatments*

The density of streptomycetes antagonistic against each of the four target pathogens (*S. scabies*, *V. dahliae*, *F. oxysporum* and *R. solani*) varied over time. Considering all containers, there were significant differences in the density of streptomycetes antagonistic

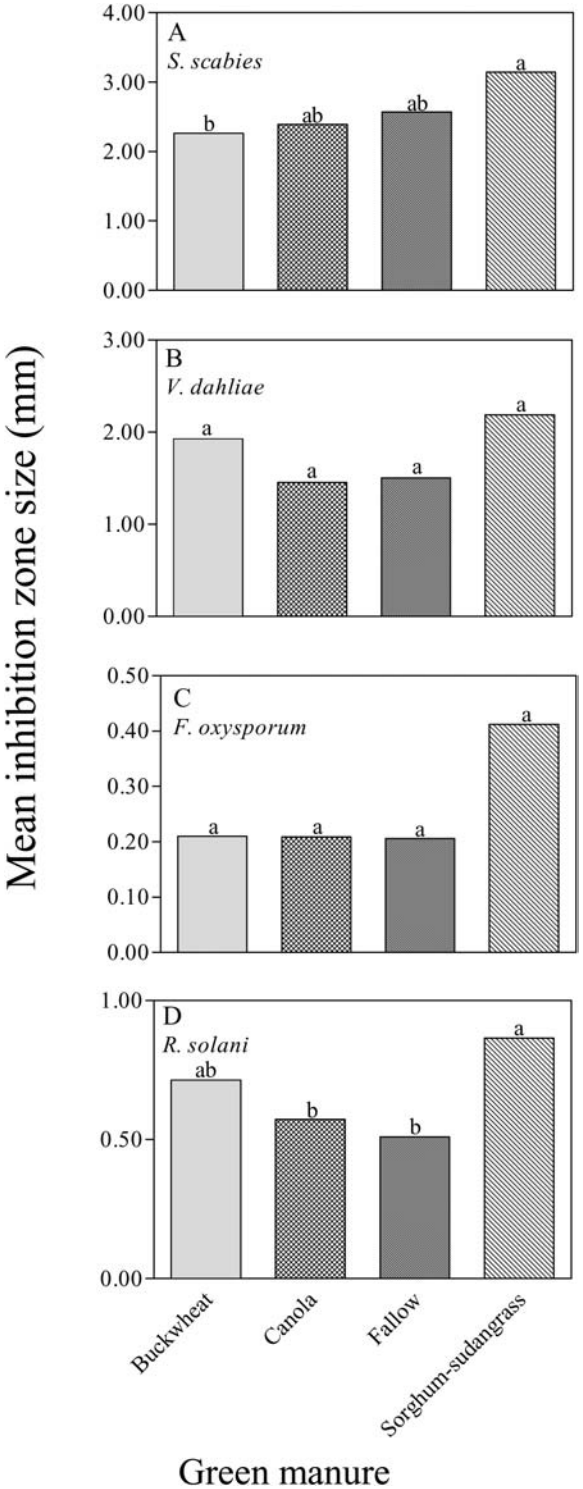


*Figure 2.* Mean inhibition zone size of streptomycetes from soil sampled September 2001 against *S. scabies* (A), *V. dahliae* (B), *F. oxysporum* (C), or *R. solani* (D), among green manure treatments. Data shown are means of 15 replicates per treatment. For each target pathogen, treatments with different letters are significantly different ($P < 0.05$, LSD).

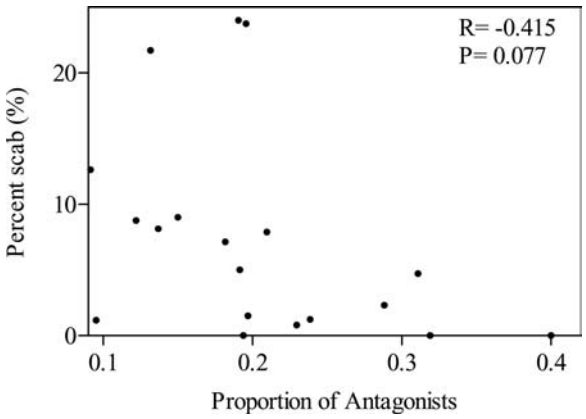


*Figure 3.* Relationship between proportion of streptomycetes antagonistic against *S. scabies* (estimated in July 2001) and percent tuber surface area covered with scab lesions (percent scab) among all containers. Values represent Pearson correlation coefficients and associated *P*-values.

against *S. scabies* ($F_{4,295} = 38.4$, $P < 0.001$), *V. dahliae* ($F_{4,295} = 85.34$, $P < 0.001$), *F. oxysporum* ($F_{4,295} = 37.41$, $P < 0.001$), and *R. solani* ($F_{4,295} = 52.8$, $P < 0.001$) among the five sampling times. For all target pathogens, densities of antagonists peaked at the June 2002 sampling date, and then decreased.

Densities of antagonists against different pathogens varied. Among all containers and sampling dates, more streptomycetes were antagonistic against *S. scabies* ($2.6 \times 10^6$ CFU/g) and *V. dahliae* ($1.2 \times 10^6$ CFU/g) than against *R. solani* ($5.0 \times 10^5$ CFU/g) or *F. oxysporum* ($2.0 \times 10^5$ CFU/g). Despite the relative differences in densities of antagonists of the target pathogens, antagonist densities were consistently positively correlated among pathogens within sampling times. Within each container, the densities of antagonists among all six possible pathogen-pathogen combinations at each of the five sampling times were positively correlated in 24 of the 30 possible correlations ($P = 0.001$, binomial). Thus, soils with relatively high densities of streptomycetes antagonistic against one pathogen are likely to have relatively high densities of streptomycetes antagonistic against other pathogens, indicating the potential for broad range pathogen and disease suppression.

There were few significant or consistent effects of GMs on antagonist densities. In contrast, crop treatment had a consistent effect on the density of streptomycetes antagonistic against *S. scabies*, *V. dahliae*, and *R. solani*; soils planted to potato in the first year of the trial tended to have greater densities of antago-

nists, though significant differences among treatments were not observed until the fall of 2001 (*V. dahliae* and *R. solani*) or the summer of 2002 (*S. scabies*, $P < 0.05$, LSD, Table 2).

*Proportion of pathogen antagonists following green manure and crop treatments*

The proportion of the streptomycete community that was pathogen inhibitory (proportion of antagonists) varied over time. There were significant differences among the five sampling times in the proportion of streptomycetes antagonistic against *S. scabies* ($F_{4,295} = 33.27$, $P < 0.001$), *V. dahliae* ($F_{4,295} = 43.45$, $P < 0.001$), *R. solani* ($F_{4,295} = 28.37$, $P < 0.001$), and *F. oxysporum* ($F_{4,295}$=13.12, P<0.001); proportions of antagonists were greatest in the spring of 2002 for all pathogens. At each sampling date, however, proportions of antagonists of each of the four target pathogens were consistently positively correlated within containers. Among all treatments, each of the 30 possible correlations (six possible pathogen-pathogen combinations, at each of the five sampling dates) was positive ($P < 0.001$, binomial), and 20 of the correlations had P-values less than 0.05. Thus, soils with relatively high proportions of antagonists against one pathogen have high proportions of antagonists of other pathogens, suggesting the potential for broad range pathogen suppression.

In the first year of the trial, buckwheat- and sorghum-sudangrass-treated soils tended to have greater proportions of streptomycetes antagonistic against *S. scabies* and *V. dahliae* than canola- or fallow-treated soils (data not shown). Similarly, sorghum-sudangrass-treated soils tended to have greater proportions of streptomycetes antagonistic against *F. oxysporum* than other soils both years of the trial (data not shown). There were no consistent effects of GM on the proportion of antagonists of *S. scabies*, *V. dahliae* or *R. solani* in the second year of the trial. The effect of crop on the proportion of antagonists was not consistent over time (data not shown). There was a significant GM-crop interaction on the proportion of streptomycetes antagonistic against *F. oxysporum* in October 2001 ($F_{6,48} = 2.34$, $P = 0.047$) and *S. scabies* in June 2002 ($F_{6,48} = 2.76$, $P = 0.022$).

*Inhibition zone size following green manure and crop treatments*

The intensity of pathogen inhibition (mean inhibition zone size per streptomycete) against each target pathogen varied over time. There were significant differences in the mean inhibition zone size against *S. scabies* (2.59 to 4.27 mm; $F_{3,236} = 10.61$, $P < 0.001$), *V. dahliae* (1.39 to 2.70 mm; $F_{3,236} = 16.88$, $P < 0.001$), and *F. oxysporum* (0.25 to 0.38 mm; $F_{3,236} = 4.52$, $P = 0.004$) among all pots over the last four sampling dates. At each sampling time, mean zone sizes against the different pathogens were positively correlated within individual communities (containers). Within each sampling time, correlations between every possible combination of the four pathogen inhibition zones were positive in 27 of the 30 cases (six pathogen-pathogen combinations at five sampling dates, $P < 0.001$, binomial). Furthermore, 26 of the correlations had P-values less than 0.05, suggesting the potential for broad range pathogen suppression by antibiotic-producing streptomycetes.

There were no consistent effects of GM or crop on mean inhibition zone size except in September 2001; streptomycetes from sorghum-sudangrass-treated soils had greater mean inhibition zones against each of the four target pathogens than streptomycetes from other soils (Figure 2). Similarly, on the same date, streptomycetes from soils planted to alfalfa had significantly larger inhibition zones against each of the four target pathogens than streptomycetes from soils planted with corn ($P < 0.05$, LSD, data not shown).

*Relationships between soil microbial community and alfalfa health and disease*

Alfalfa stand counts and total biomass at harvest were consistently negatively correlated with the density of streptomycetes and antagonists. Among all containers, alfalfa stand counts at harvest in both years of the trial were negatively correlated with streptomycete densities estimated in September 2001 and June 2002 ($R = -0.562$, $P = 0.010$ for September 2001; $R = -0.257$, $P = 0.048$ for June 2002). Similarly, streptomycete density estimated in June 2002 was negatively correlated with total biomass at harvest in 2002 ($R = -0.281$, $P = 0.030$). Alfalfa stand counts at harvest in 2001 were also negatively correlated with the density of antagonists of the four target pathogens estimated at three sampling times in 2001 (ten of the 12 time-pathogen correlations were negative, $P = 0.016$, binomial). Similar negative correlations between alfalfa stand counts at harvest and the density of antagonists were observed in 2002 (data not shown). Thus, alfalfa grown in soils with greater streptomycete

and antagonist densities had lower stand counts and total biomass.

In contrast, alfalfa grown in soils with greater streptomycete densities has less root rot, while alfalfa grown in soils with greater proportions of antagonists had greater stand counts and less rot. Among all containers in 2001, ten of the 12 possible correlations between the proportion of antagonists of the four target pathogens at the three sampling dates and alfalfa stand counts at harvest were positive ($P = 0.016$, binomial). Similarly, among all containers, streptomycete densities estimated in September 2001 were negatively correlated with root rot rating ($R = -0.527$, $P = 0.017$). In 2002, Phytophthora root rot ratings were negatively correlated with the proportion of antagonists of the four target pathogens at the two sampling dates (seven of the eight correlations were negative, 0.031, binomial). Thus, alfalfa grown in soils with greater streptomycete densities and proportions of antagonists had less disease.

*Relationships between soil microbial community and potato health and disease*

Potato health was correlated with streptomycete antagonism. In general, measures of pathogen inhibitory activity (mean zone size and proportion of antagonists) were negatively correlated with potato scab disease and positively correlated with potato yield (mean tuber weight per container). Among all containers, percent scab was generally negatively correlated with both the proportion of antagonists and mean inhibition zone size for each of the four target pathogens at the three sampling dates in 2001 ($P = 0.054$ for each, binomial, ex: Figure 3). In contrast, mean tuber weight per container was positively correlated with the proportion of antagonists ($P = 0.054$, binomial, data not shown). Thus, potatoes grown in soils with greater proportions of antagonistic streptomycetes and larger pathogen inhibition zones had less scab and greater yields.

*Relationships between pathogen inhibitory activity and the soil microbial community*

Streptomycetes from soils with greater antagonist densities and proportions of antagonists, but not streptomycete densities, had larger inhibition zones. Among all containers, mean inhibition zone sizes were negatively correlated with the streptomycete density in each of the 20 time-pathogen correlations (four pathogens at five sampling times, $P < 0.001$, binomial). In contrast, among all containers, mean inhibition zone size was positively correlated with both the density of antagonists ($P = 0.001$, binomial) and the proportion of antagonists ($P < 0.001$ for each individual correlation).

In general, soils with relatively low streptomycete densities had greater increases in streptomycete densities following GM incorporation than soils with relatively high streptomycete densities, regardless of GM or crop treatment. For example, among all containers, the change in streptomycete density from October 2001 to June 2002 (19 days after incorporation of the second GM treatment) was negatively correlated with initial streptomycete density ($R = -0.838$, $P < 0.001$). In addition, these correlations were also significant among individual GM and crop treatments ($P < 0.001$ for each GM or crop treatment). Thus, enrichment in streptomycete densities was density-dependent.

In contrast, initial streptomycete densities influenced the changes in streptomycete pathogen inhibitory activity among GM-treated soils, but not among fallow-treated soils. For example, among GM-treated soils, initial streptomycete densities estimated in July 2001 (20 days after GM incorporation) were positively correlated with the change in proportion of streptomycetes antagonistic against *S. scabies* ($R = 0.39342$, $P = 0.0075$), *V. dahliae* ($R = 0.339$, $P = 0.023$), *R. solani* ($R = 0.405$, $P = 0.006$) and *F. oxysporum* ($R = 0.321$, $P = 0.031$) from July 2001 to October 2001. Analogous correlations among fallow-treated soils, however, were not significant (Table 3). Thus, among GM-treated soils, the greatest increases in streptomycete pathogen inhibitory activity were observed among soils with the highest initial streptomycete densities. Similar density-dependent shifts in mean inhibition zone sizes were observed from July 2001 to October 2001 among GM- but not fallow-treated soils (data not shown).

## Discussion

Consistent with previous studies, this work shows that green manures have substantial potential for the management of soil-borne diseases of alfalfa and potato (Kinkel and Samac, unpublished data; Millard, 1923; Millard and Taylor, 1927; Rouatt and Atkinson, 1950; Sanford, 1946). In the first year of the trial, but not the second, alfalfa grown in GM-treated soil (buck-

wheat, canola, or sorghum-sudangrass) had greater stand counts as well as higher yields (aboveground biomass), suggesting protection against the disease complex causing damping-off. In addition, alfalfa grown in fallow-treated soils had greater root rot ratings when the effects of plant density were considered. The lack of significant differences among GM treatments in the second year of the trial likely reflects the very low GM biomass that was incorporated into the soil in the spring of 2002. Much of the GM aboveground biomass was blown from the pots over the winter, and the incorporated biomass was not living. Thus, substantial biomass and/or living GM biomass may be required for effective protection against root rots. This also suggests that the benefits of GM treatments from the previous spring may not be long-lasting, and that one source of inconsistency in the effectiveness of GM in reducing disease intensities may be overwintering biomass.

Because it was unknown which target pathogens would provide the best index of pathogen inhibition and plant disease over time, soil was sampled throughout the trial and four different target pathogens were used for the modified Herr's method. Within samples, streptomycete inhibition of the four pathogens was positively correlated among treatments, indicating the potential for broad-range pathogen suppression and perhaps that fewer target pathogens could be used in studying soil suppression.

Measures of alfalfa health and disease were correlated with pathogen inhibitory activity of the streptomycetes. In particular, the negative correlations between the proportion of antagonists and root rot, and the positive correlations between the proportion of antagonists and alfalfa stand count suggest that the proportion of antagonistic streptomycetes may provide a useful index of the soil pathogen inhibitory activity. Thus, increasing the proportion of antagonists, possibly through the incorporation of GM, may be a significant means by which damping off and Phytophthora root rot may be suppressed and alfalfa yields increased.

Previous research on the control of potato scab disease through the use of GMs has been inconsistent (Millard, 1923; Millard and Taylor, 1927; Rouatt and Atkinson, 1950; Sanford, 1926, 1946; Weinhold et al., 1964). Although disease was very low in this study, potatoes grown in GM-treated soil had significantly fewer scab lesions per tuber as well as lower percent scab compared to potatoes grown in fallow-treated soils. These results are consistent with Weinhold's (1964) experiment in which he demonstrated that soybean GM prevented increases in scab disease in successive potato crops when applied to naturally-infested soils low in disease pressure, but was not effective in suppressing scab disease in successive potato monocultures when the pathogen was already well established. In addition, the negative correlations between the proportion of antagonists and the mean surface area of tubers covered with scab lesions (percent scab) and the positive correlations between the proportion of antagonists and the mean tuber weight per container (yield) indicate that antibiotic-producing streptomycetes may play a key role in disease suppression. More specifically and consistent with the alfalfa data, the proportion of antagonistic streptomycetes may provide a useful index of the pathogen inhibitory activity of the soil. More broadly, these data suggest that increasing the proportion of antagonistic streptomycetes may provide a means by which scab disease can be reduced.

Although the intensity of pathogen inhibition did not vary greatly among crop treatments, antagonist density was consistently greatest among potato-treated soils. In contrast, there were few consistent effects of GM on the density of antagonists or the mean inhibition zone size either year of the trial, though sorghum-sudangrass-treated soils tended to have greater proportions of antagonists than fallow-treated soils. Furthermore, consistent with previous studies, GM-treated soils had greater streptomycete (OA) and bacterial densities than fallow-treated soils (Henis et al., 1967; L. L. Kinkel, unpublished data; Mazzola et al., 2001; Rouatt and Atkinson, 1950).

These data support the hypothesis that density-dependent selection for enhanced competitive abilities may be a key mechanism by which disease suppressive activity is enhanced. In particular, the consistent positive correlations between initial streptomycete densities and the change in pathogen inhibitory activity (mean zone size and proportion of antagonists) over time among GM-treated soils, but not fallow-treated soils, suggests that initial density is an important predictor of the potential effectiveness of GM in enriching pathogen inhibitory activity.

It is likely that a combination of complex microbial changes in the soil community contributes to disease suppression. In particular, differences in disease among crop or GM treatments were not always associated with differences in the streptomycete pathogen inhibitory activity. Other factors that are likely to influence disease intensity include changes in pathogen

densities due to the lack of a susceptible host in the first year of the trial, shifts in the non-streptomycete antagonist populations, and changes in soil edaphic characteristics that may influence pathogen or antagonist growth and activities.

Green manures may provide a practical means by which diseases of multiple production crops can be reduced. In particular, integrating spring or fall GMs into a crop management scheme may be feasible without taking fields out of valuable production. In addition to benefits conferred to the soil such as increased organic matter and nutrients, improved soil structure, and reduced erosion (Abdallahi and N'Dayegamiye, 2000; Al-Khatib et al., 1997; Blackshaw et al., 2001), GMs may also be a practical tool by which plant disease may be suppressed. Despite the extraordinary complexity of microbial communities in agricultural soils, these data suggest that the streptomycete pathogen inhibitory activity within the soil plays a role in pathogen and disease suppression. Furthermore, increasing the streptomycete pathogen inhibitory activity of the soil, specifically the proportion of antagonistic streptomycetes, through the incorporation of GM, may be a means by which broad-range pathogen and disease suppression may be achieved.

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