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HOST RANGE AND CULTURAL CONTROL OF SOME MICHIGAN ISOLATES OF STREPTOMYCES SCABIES

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Melvyn L. Lacy
Major professor

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HOST RANGE AND CULTURAL CONTROL OF SOME MICHIGAN ISOLATES OF *STREPTOMYCES SCABIES*

by

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ABSTRACT

HOST RANGE AND CULTURAL CONTROL OF SOME
MICHIGAN ISOLATES OF STREPTOMYCES SCABIES

by

Linda Eve Hanson

Several Michigan isolates of Streptomyces scabies were characterized and pathogenic isolates typical of S. scabies were used for further tests. In host range tests, all putative hosts tested developed scab symptoms with several of these isolates.

In naturally infested field soil sweet corn and red clover, incorporated as green manures increased scab levels and reduced marketable yields while oriental mustard (Brassica juncea) reduced scab severity compared to a fallow control. Other green manures had no significant effects on disease severity. In pot tests, no green manure treatment produced scab levels significantly different from that of the fallow control, but mustard produced significantly lower scab levels than did red clover.

No correlation was found between scab levels in green manure treatments and changes in soil pH, pathogen population levels in soil, growth of Streptomyces in plant extracts, or infection of cover crops by Streptomyces. Stimulation of antagonistic micro-organisms, however, may play a role in differences in scab levels.

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbol or Abbreviation

L. = Linaeus
S. = Streptomyces
spp. = species
J/Kg = joules per kilogram
N = normal
M = molar
g = gram
mg = milligram
lb = pound
°C = degrees centigrade
kPa = kiloPascals
ml = milliliter
µl = microliter
min = minute
hr = hour
ft = foot or feet
in. = inches
m = meter
cm = centimeter
mm = millimeter
µm = micrometer
w/v = weight to volume
KV = kilovolts
P/N = positive/negative
cfu = colony-forming units
cv. = cultivar
P = probability
rxn = reaction
No. = number

WA = water agar
PHWA = water agar adjusted to pH≈10
ABWA = antibiotic-amended water agar
YME = yeast extract-malt extract agar
ISSA = inorganic salts-starch agar
GAA = glycerol-asparagine agar
SEA = soil extract agar
PYI = peptone-yeast extract iron agar
CSN = Czapek's sucrose-nitrate solution
NBY = nutrient broth-yeast extract agar
PDB = potato dextrose broth
CMC = carboxymethylcellulose
LSD = least significant difference
SEM = scanning electron microscope
RPM = revolutions per minute
CWT = hundredweight

INTRODUCTION

Potato (Solanum tuberosum L.) is a crop of primarily temperate regions or of high elevations in tropical areas. It probably originated in the Andean highlands of South America, where many species have been cultivated for centuries (53). In world food production, potato ranks fourth (107) or fifth (53). It ranks first among dicotyledonous sources of human food (53). Clearly the diseases affecting such an important crop are of major concern.

One such disease, common scab of potato, has been known in Europe and the Americas for more than 150 years (151). The disease has been reported in Africa, Asia, Australia, Europe, and North and South America and has been recorded in every state in the United States where potatoes are grown. Scab can cause serious yield losses due to decreased tuber quality or marketability, and it may cause some gross yield reductions (53, 64, 110, 125).

The primary cause of common scab is Streptomyces scabies (Thaxt.) Waksman and Henrici, a filamentous bacterium or actinomycete (71). However, other Streptomyces species may cause scab, either on potato or on other crops (12, 45, 72, 73, 137). The pathogen is able to persist in soil for many years, and has been reported in soils in which susceptible root crops have never been grown (34, 91, 93, 139). Spores present in the soil can germinate when exogenous nutrients are available and the growing streptomycetes rapidly produce more spores (93), thus potentially increasing the level of the pathogen in the soil.

Infection of potatoes is believed to occur primarily through tuber lenticels (2, 53, 64, 133), which may be susceptible to infection only

at certain developmental stages (77). Wounds and stomata also may be sites of entry (53, 64, 147). Once inside the tuber, the pathogen induces production of a cork layer around the site of infection. As the potato tuber grows, this cork layer is pushed outward, breaks apart, and is sloughed off, producing the corky lesions typical of the disease. Such lesions can enlarge and eventually can produce any of three major types of scab symptoms. The first type is called surface, common, ordinary, or shallow scab (35, 53, 110, 125, 151). The scabs are superficial, rough, ruptured or corky lesions on the tuber surface which are slightly darker than the healthy skin. The second type is the raised, stud, or tumulus scab (53, 110), which consists of corky or warty growth which protrudes above the level of the healthy plant surface. This type is particularly common on infected beets (Beta vulgaris L.) (92). The third type is the deep or pitted scab (5, 35, 53, 110, 125, 151), which produces distinct, corky, sunken, dark lesions that may coalesce and form deep furrows in the tuber surface. Such lesions may be enlarged by insects or secondary organisms. All three scab types will only develop if the tuber continues to grow after infection (28, 56, 126).

Common scab can also affect various root crops. These include radish (Raphanus sativus L.), rutabaga (Brassica napobrassica (L.) Mill.), turnip (Brassica campestris L.), beet (red and sugar), parsnip (Peucedanum sativum Benth.), and carrot (Daucus carota L.). However, growers in Michigan have reported little or no problem with scab in carrots grown in muck soil in which radish could not be grown due to scab severity (M.L. Lacy, personal communication), and there have been

few reports of scab symptoms on any crops other than radish and potato in Michigan. For example, Michigan Agricultural Experiment Station bulletins for sugar beet do not list scab as a disease affecting sugar beets in Michigan (136).

A number of methods have been used in the past to control common scab, but none has proven economical in Michigan.

The objectives of this study were: (1) to isolate and characterize some Streptomyces spp. causing scab in Michigan; (2) to investigate the host range of some typical isolates; (3) to investigate the use of some green manures as a possible cultural control of common scab of potato in Michigan; and (4) to investigate some possible mechanisms of green manure activity.

LITERATURE REVIEW

Taxonomy of the causal organism:

The primary causal agent of common scab of potato was first isolated and demonstrated to cause scab on potato by Thaxter in 1890 (146). Thaxter tentatively named the pathogen Oospora scabies, and designated it a "bacteria-like fungus" which caused scab on potato tubers and beet roots (147).

Gussow (44) determined that the pathogen was a filamentous bacterium which belonged in the genus Actinomyces and should be called Actinomyces scabies. Millard and Burr (110) surveyed 24 different actinomycete isolates for pathogenicity on potatoes, cultural characteristics and morphology. They concluded that the pathogenic isolates should be divided into 11 different species on the basis of their growth on various media and their spore chain morphology, and that the type of lesion produced depended on the species involved. By the third edition of Bergey's Manual, nine different Actinomyces species were reported to be associated with potato scab (8). Waksman and Henrici (150) subdivided the actinomycetes based on oxygen utilization and spore production. Aerobic forms with spores in chains on aerial hyphae, like the potato scab pathogen, were placed into the genus Streptomyces. Archuleta and Easton (5) reported that they isolated, from deep pitted scab, several different species of Streptomyces which caused lesions when used to infest sterilized soil. They suggested that many different species might be able to infect potato.

Some authors have questioned the pathogenicity of isolates as a valid species distinction. Gordon and Horan (33) suggested that the

Scab resistance:

Selection of scab resistant varieties is highly recommended to control common scab. Many mechanisms of scab resistance have been investigated with limited success. These include: lower levels of natural cork production by tubers (110), russet skins and increased skin thickness (90), small lenticels with more tightly packed cells and earlier periderm suberization (18, 81), persistent viability of peripheral periderm layers (15), high concentrations of chlorogenic acid and related phenolic compounds in potato periderm (25, 63, 135), and low levels of reducing sugars in potato peels (38).

These mechanisms have not gone unchallenged. Darling (18) found no consistent association of russet skin and scab resistance, although other researchers have reported a genetic linkage between russeting and resistance (14). Cooper et al. (15) found no correlation between the rate of periderm suberization and resistance, and Adams (2) found no difference in lenticel anatomy between resistant and susceptible cultivars.

Host range:

S. scabies has a fairly wide range of susceptible host crops. Scab lesions associated with streptomycetes have been reported on the fleshy roots of radish (50, 53, 66, 68, 82, 83, 119, 131, 151), red beet (11, 53, 92, 142, 147, 151), sugar beet (11, 53, 68, 92, 119, 142), turnip (53, 68), rutabaga (53, 68), parsnip (53, 65, 68), carrot (16, 53, 61, 66, 119, 142), mangels (Beta vulgaris L.) (109), cabbage (Brassica oleracea L.) (125, 142) and possibly leek (Allium porrum L.) (119). It can also infect the fibrous roots of crops such as potato (53), celery

(Apium graveolens L. var. dulce (Mill.)) (142), salsify (Tragopogon porrifolius L.) (125) and eggplant (Solanum melogena L.) (68). In addition to crops, the scab organism has been reported to infect the roots of some weeds, including pigweed (Amaranthus retroflexus L.) and black nightshade (Solanum nigrum L.) (68). It can also infect various plant seedlings in artificially infested sterile sand, soil, or soil extract agar (50).

Some conflicting reports about the host range of Streptomyces have been produced. KenKnight (68) reported no infection of carrot, cabbage, or leek in Michigan field tests. Researchers in California suggested that carrot scab is a disease complex that may be of non-pathogenic origin (43). Different types of scab symptoms on mangel were reportedly caused by two different types of Streptomyces, one of which was identified as S. scabies, and the other as a separate species (109). Scab on radish was caused both by a Streptomyces isolate characteristic of S. scabies, and by one that produced straight spore chains, rather than the spiral chains typical of S. scabies (82). Streptomyces spp. causing "netted scab" were also able to produce scab on sugar beet (137). Thus the host range of the pathogen is uncertain, which may be due in part to the uncertain classification of Streptomyces isolates.

Chemical control:

Foliar sprays of plant growth retardants such as daminozide, N-diamethylamino maleamic acid, and N-(dimethylamino) methylsuccinamic acid (97, 98), ethionine (99), and some 2,5 disubstituted benzoic acids (101), which showed in vitro toxicity to Streptomyces, decreased scab severity with no significant yield effects. Other growth retardants

such as 3,5-dichlorophenoxyacetic acid (3,5-D) and 2,4-D also reduced scab severity, but caused decreased yields (100). Nitrate inhibitors such as a urea-formaldehyde mixture have shown scab suppression (122, 138), and other chemicals, such as sodium-N-methyl dithiocarbamate (29) and captafol (96) have shown some possibility as controls for scab.

Hooker (51) and Houghland and Cash (59) simultaneously reported decreased scab severity with soil treatments of pentachloronitrobenzene (PCNB) on organic and mineral soils, respectively. Hooker reported no decreased yield with this treatment, but Houghland and Cash observed some phytotoxicity. The effect of PCNB was confirmed by other authors (20, 23, 29, 104, 115, 122), who reported that both the application method and application rate affected efficacy. However, PCNB was ineffective when soil water potential dropped below -1.6 bars (23), suggesting that PCNB was not directly responsible for the control reported. The effects of PCNB have often been found when "excessive" rates of the fungicide were used (20).

At present, there are no chemicals registered for control of common scab.

Cultural control of common scab:

a: Soil pH

A number of studies showed that lime and alkaline soil conditions were correlated with increased scab levels while scab levels were generally reduced in acid soils (10, 26, 31, 32, 79). Other researchers reported no significant relationship between soil pH and scab severity (143), and one even reported a scab response the opposite of that reported in the majority of the literature, i.e. decreased scab with

increasing pH (between pH 5.9 and 8.6) (34). Sanford (133) reported that a soil reaction in the level which inhibits in vitro spore germination did not completely inhibit development of scab. He reported scab in soils of pH between 4.2 and 4.8, and that the scab tissue had a higher pH level than that of either the surrounding acid soil or the healthy tuber. He interpreted this as evidence that scab development in the host is virtually independent of the soil pH once infection has occurred.

Liming of the soil has been correlated with increased scab severity. The effect might be due to increased soil pH, but some researchers reported that increased scab severity may be related to higher calcium levels (37, 42, 58). Other reports showed no effect on scab with the addition of calcium when soil pH was buffered (9, 116); however, Gries et al. (42) reported that scab severity increased as the Ca:K ratio was decreased, either by increasing K or decreasing Ca at a constant pH. Other researchers have reported no relation between the Ca:K ratio and scab severity (26, 60). Some researchers have suggested that the significant factor in the effect of calcium is the level of Ca in the potato peels, and have correlated high calcium levels in potato peel with scab severity (20, 21, 23, 58, 60).

Sulfur and sulfur compounds inhibited scab levels in some studies (20, 55, 79, 114, 140). Other researchers reported variable results with sulfur applications (27, 68, 80, 127, 159), with the effect being related to the soil type and severity of infestation (27). The amount of sulfur required to give commercial control of scab differed in different areas, and the mode of application of sulfur affected disease

control (55). Decreased disease severity with sulfur might be due to decreased soil pH (55, 79), but only slight pH reductions (0.1-0.4) were detected by Davis et al. (20) following sulfur treatments and no consistent relationship was seen between acidity and control by Duff and Welch (27), suggesting that some other factor than pH may be involved in the activity of sulfur in scab suppression.

Sulfur applications have been reported to reduce yield (80, 114, 140), although other researchers have reported no yield effects (79). This may be due to varietal sensitivity of the potato to sulfur (55). The variable results and possibility of yield reductions limit the desirability of sulfur in controlling scab.

b: Soil nutrients

Levels of nutrients such as nitrogen, phosphorous, iron and potassium in the soil or tuber have been reported to be correlated with scab severity (21, 76). Incidence of scab has been reported to increase with increasing amounts of nitrogen fertilizer (the form was not specified) (76). Other researchers reported no significant effect of such nutrients (143).

Applications of manganese sulfate have been reported to reduce scab severity by some (94, 95, 112); others reported no significant scab control with $MnSO_4$ (7, 113), but saw decreased yield with the treatment (7). Copper sulfate and aluminum sulfate also reportedly decrease scab (41, 112). The mechanism of activity of these compounds has not been determined.

c: Soil moisture:

The relation of scab severity to soil moisture was first demonstrated experimentally by Sanford (132) when a significant reduction in lesion numbers and percent infection of tubers grown under conditions of high soil moisture was found. Subsequently, several other researchers showed similar effects of soil moisture. Wellings and Rosser (155) reported good control with irrigation, and Lewis (84) found that "negligible" infection occurred when soil was irrigated to maintain a water potential greater than 13 J/kg (≈0.13 bars) Lapwood and others (74, 77, 78) found that economical control of infection could be attained with irrigation, particularly at soil water potentials above -0.4 bars. Davis et al. (22,23) reported maximum control above -0.46 bars, with scab severity increasing as water potential decreased. Maximum scab occurred below -0.96 bars.

Reduction of disease severity with irrigation has also been variable. In general, dry soils have been correlated with increased disease, but severe scab has occurred in several soils that were at or near the saturation point (35). Starr et al. (143) reported the highest levels of scab found in plots receiving the most irrigation, and Goto (38) reported that heavy rainfall (taken to indicate high soil moisture) did not reduce scab severity.

Sanford (132) reported a critical period for maintaining soil moisture of 36-56 days after planting. Lapwood et al. (78) reported that the first three weeks after tuber initiation were the most critical. Barnes (7) found the critical period to vary from year-to-year, but found that irrigation must be at or immediately following

tuber initiation. Three weeks of irrigation was usually sufficient for scab control. Lewis (84) found no control if irrigation was applied for only the first two weeks after tuber initiation, but irrigation during the five weeks after tuber initiation controlled scab.

Some of the variability in results may be due to differences in the techniques used to measure soil moisture. Percent soil moisture by weight can vary significantly between different soil types, and it is not always indicative of the amount of water perceived by organisms in the soil. Soil water potential is probably a better expression of soil moisture, as it is as not affected by soil type or structure (103).

Irrigation is widely recommended for scab control, but Davis et al. (22, 23) reported that scab control by irrigation was not sufficient to reduce disease levels to those acceptable for a U.S. no. 1 grade potato. Thus further control methods are desirable.

d: Crop rotations:

Crop rotations have been recommended for control of common scab. In a survey of potato fields, long rotations out of potato were correlated with decreased disease, and small grains preceding potatoes were correlated with low scab levels, legumes were correlated with medium rates, and corn (Zea mays L.) and summer fallow were correlated with the most scab (34). In irrigated fields, short rotations with sugar beets produced severe scab. Longer rotations, especially those in which alfalfa (Trifolium pratense L.) preceded potatoes, had lower scab levels, and two-year rotations with oat (Avena sativa L.), oat and rye (Secale cereale L.), or corn all reduced scab severity to some extent (36). However, in similar fields without irrigation, scab was reduced

by a three year rotation but the crops preceding potato (corn, wheat (Triticum spp. L.), or beans (Phaseolus spp. L.)) had no significant effect on scab severity (156). Hooker (52) reported that, while three to six year rotations with various crops reduced scab compared to continuous potato cropping, there was no clear evidence for extra benefit from any given rotation crop.

Wheeler (158) reported that rotations involving oat-and-alfalfa, corn- and-rye, or alfalfa followed by a rye cover crop decreased scab severity. The corn-and-rye rotation gave a low yield of potatoes, which was attributed to the rye. Decreased yield was not seen with the alfalfa-rye treatment. Rotation involving sweet clover (Melilotus officinalis Lam.) gave the highest disease levels of any of the rotations tested, a level markedly higher than the scab level seen in control fields continuously cropped to potato.

Weinhold et al. (154) found no difference in scab levels over time in one year rotations with barley (Hordeum vulgare L.), cotton (Gossypium sp. L.) or sugar beet, while a three year rotation involving potato, sugar beet and cotton produced a higher rate of scab increase than when potatoes were cropped continuously. Rotation with alfalfa for three years showed no significant effect on scab severity. However, Rich (125) reported that a four year rotation with alfalfa reduced scab severity.

Thus, long term rotations out of potato (four to six years) have been beneficial in scab control, with alfalfa and small grains frequently showing a reduction in scab severity. Other crops have given more variable results.

When potatoes have been continuously grown in certain soils, decrease in disease and eventual evidence of scab suppressive soils have been reported (88, 105). The addition of such soil to infested soil in pots suppressed scab (105). This suppressive effect was lost when soil was steamed, thus these soils appeared to have some biological component that suppressed scab, and might be a potential source of control in the future.

e: Green manures:

Some researchers have reported success with the use of green manures to control common scab; however, results of others have been less successful.

In England, green manure of an unspecified mustard followed by rye gave scab control, as did green rye (grown as a cover crop and incorporated) and grass cuttings (15 tons/acre). Similar scab suppression was reportedly demonstrated in Denmark with lupine (Lupinus sp. L.) as a cover crop (108).

In Minnesota, Sanford (133) reported no significant scab control with green rye at 50 tons/acre in soils of either pH 4.9 or 5.4. However, in a later experiment, green manures (30 tons/acre) of both rye and an unspecified clover reduced the amount of scab (134).

In Kansas, green manures of rye and cowpea (Vigna sinensis Savi) reduced scab, and freshly plowed rye was more effective than cowpea that had been plowed under two to three months earlier (159).

Rouatt and Atkinson (130), in Ottawa, Canada, found that soybean (Glycine max (L.) Merr.) reduced scab incidence while rye and red clover (Trifolium pratense L.) had no significant effect on scab severity.

They suggested that the effect of the soybean treatment was due to changes in the microbial population in the soil and potato rhizosphere with the green manure crops.

Weinhold et al. (154) reported that a green manure crop of barley increased scab incidence, compared to control, while Canadian pea (Pisum sativum L.), used similarly, had no appreciable effect on scab, and soybean prevented increases in scab severity over time but had no effect on scab severity once the disease was present in the soil. Oswald and Lorenz (118) reported similar results with these crops and Rogers (128) found significant control of scab with a green manure of dried grass meal (unspecified grass).

Other researchers reported no suppression of scab with green manures. No decrease of scab was observed in Minnesota with rye (133) and in Nebraska with alfalfa or grass cuttings (35). Most striking of all, KenKnight (68) reported that green manures of both alfalfa and blue grass cuttings (Poa spp. L.) produced significant increases in scab severity in Michigan.

Green manures reportedly reduce soil pH over time, which might have a role in scab suppression, but the amount of this reduction in pH is generally low (144). Sanford (133) reported that green manures of rye produced an initial increase of soil pH, followed by a steady decrease of pH, with soils returning to approximately their initial pH by 30 days after green manure incorporation. This was followed by a decreased pH after approximately 60-70 days. He concluded that "an abnormally large amount of rye would be required to increase the acidity of less acid soils to an effective point for scab control".

Rogers (128) suggested that organic manures might control scab by reducing insoluble manganese to soluble forms toxic to S. scabies. However, he found that dried grass meal, which gave "significant control" of scab, and produced an increase in the concentration of soluble Mn in the soil, did not increase Mn sufficiently to account for the control achieved. The lack of sufficient change in Mn levels for control, and the variability in reports of the effectiveness of Mn as a control agent suggest that it is not a major factor in scab suppression with green manures.

Antagonistic activity by micro-organisms in the soil has been suggested to play a role in several of the control methods for scab, including irrigation (3), rotation crops, and green manures (111, 153). Millard and Taylor (111) showed that no suppression of scab occurred with green manures in sterile soil but addition of a saprophytic Streptomyces to the soil with the pathogen reduced disease severity. Goss (35) was unable to duplicate the effect of this saprophytic organism.

Weinhold and Bowman (153) isolated various bacteria and Streptomyces spp. that were antagonistic to S. scabies from green manure plots, with Bacillus subtilis-type bacteria predominating, but found no consistent difference in antagonist counts among plots with different green manures.

Recently, there has been a report of some success in the use of an antagonistic Streptomyces as a biological control agent for potato scab. A fertilizer infested with an antagonistic Streptomyces species decreased scab severity in greenhouse and field trials in Japan (46,

47). Unfortunately, scab levels and significant differences were not given in these reports, so the effectiveness of this treatment is hard to determine.

No commercially effective controls are yet available for common scab of potato. Current recommendations for control of common scab include planting scab-free seed tubers, maintaining soil pH between 5.0 and 5.3, limiting the use of fresh manure, wood ashes, and lime, using resistant varieties, maintaining high soil moisture levels during and after tuber set, and using crop rotations to reduce scab (19, 48, 49, 53, 57, 125).

MATERIALS AND METHODS

Culture media used:

Water agar (WA), used to isolate Streptomyces from soil and most plant material, contained 15 or 20 g of Difco agar (Difco Labs., Detroit, MI) per liter of distilled water (pH≈7). For isolations from crucifers the pH of the medium was adjusted to 10.0-10.5 with 1N NaOH (PHWA) (82). All media were autoclaved at 121°C at 103.5 kPa pressure.

Antibiotic water agar (ABWA), used for isolations from potato tubers, contained 10 ml of antibiotic stock solution (containing 500 mg nystatin, 50 mg polymixin B sulfate, 10 mg sodium penicillin-G, and 500 mg cycloheximide per 100 ml of sterile distilled water) and 20 g of agar per liter of water (160).

Media used to characterize Streptomyces included: Yeast extract-malt extract agar (YME) (141), containing 4 g yeast extract, 10 g malt extract, 4 g dextrose and 15 g of agar per liter of water, with the pH of the medium adjusted to 7.3 with 1N NaOH; inorganic salts-starch agar (ISSA) (141), containing 10 g soluble starch, 1 g K_2HPO_4, 1 g $MgSO_4$ $7H_2O$, 1 g NaCl, 2 g $(NH_4)_2SO_4$, 2 g $CaCO_3$, 1 mg $FeSO_4.7H_2O$, 1 mg $MnCl_2.4H_2O$, 1 mg $ZnSO_4.7H_2O$, and 15 g agar per liter of water; and glycerol-asparagine agar (GAA) (141), containing 1 g L-asparagine, 10 g glycerol, 1 g K_2HPO_4, 1 mg $FeSO_4.7H_2O$, 1 mg $MnCl_2.4H_2O$, 1 mg $ZnSO_4.7H_2O$, and 15 g agar per liter of water.

Soil extract agar (SEA) (4) contained 15 g agar, 1 g glucose, 0.5 g K_2HPO_4 and 100 ml of soil extract per 900 ml tap water. Soil extract was prepared by autoclaving equal parts of soil and tap water (g:ml) for

30 min, adding approximately 0.5 g of $CaCO_3$, filtering twice through filter paper, and autoclaving the extract for 30 min.

Peptone-yeast extract iron agar (PYI) (141), containing 36 g Bacto-Peptone iron agar (Difco) and 1 g yeast extract per liter of distilled water, was used to detect melanin production.

Carbon utilization media (141) contained 2.64 g $(NH_4)_2SO_4$, 2.38 g KH_2PO_4, 5.65 g $K_2HPO_4.3H_2O$, 1 g $MgSO_4.7H_2O$, 6.4 mg $CuSO_4.5H_2O$, 1.1 mg $FeSO_4.7H_2O$, 7.9 mg $MnCl_2.4H_2O$, 1.5 mg $ZnSO_4.7H_2O$ and 15 g agar per liter of water. After autoclaving, 10 ml of a sterile 10% (w/v) solution of each carbon source (D-glucose, L-arabinose, sucrose, D-xylose, rhamnose, raffinose, I-inositol, D-mannitol, D-fructose, or cellulose) was added aseptically to 100 ml of basal media. Sugars were filter-sterilized with bacteriological filters (pore size 0.45 μm). The 10% (g/ml) cellulose solution was sterilized by autoclaving.

Czapek's sucrose-nitrate solution (CSN) (62), containing 2 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g KCl, 0.5 g $MgSO_4$, 0.01 g $FeSO_4$, and 30 g sucrose per liter of water, was used as a defined medium for bacterial growth. When solid medium was used, 15 g/l of agar was added.

Nutrient broth-yeast extract (NBY), used as a bacterial growth medium, contained 23 g Difco nutrient agar, 2 g K_2HPO_4, 0.5 g KH_2PO_4, 2 g yeast extract, 5 g glucose, and 0.246 g $MgSO_4.7H_2O$ per liter of water. When liquid media was used, 8 g of dehydrated nutrient broth (Difco) was used instead of nutrient agar.

Potato-dextrose broth (PDB) (62), containing 500 ml of broth from 200 g of peeled and sliced potatoes and 20 g dextrose with 500 ml sterile water per liter of media, was used for growing Bacillus.

Streptomyces isolation:

The procedure to isolate and culture Streptomyces from infected tissues, described in detail below, consisted of cleaning the scabby tissue, surface-disinfesting the plant material, sectioning tissue pieces and placing them on culture media, incubating, and streaking cultures on to a nutrient agar.

Cleaning: Potatoes were scrubbed under running tap water with a soft brush for about one min to remove soil. Plants other than potato were washed by hand, placed in beakers, and kept under running tap water for three hours before samples were taken. Samples 0.7-1.0 cm^2 by 0.3-0.7 cm thick were selected and cut from scab lesions.

Surface Disinfesting: Tissue pieces were immersed for 2 min in 10% household bleach (5.25% sodium hypochlorite) to which 2 drops of "Tween 20" per 100 ml of solution were added. Other plant tissues were surface-disinfested as described above, or by immersion for 30 min in tubes of sterile water (10 ml water per tube) in a 60 °C water bath (89). Following surface disinfestation, the dead corky surface material was scraped off, using a sterile scalpel or razor blade, to reduce secondary contaminants.

Plating: Pieces of tissue (0.4-0.8 cm^2) were aseptically cut from beneath lesions and placed, outer surface upward, on media. Potato samples were plated on either 2% WA or ABWA. Samples from crucifers were plated on PHWA (82). All other samples were plated on neutral 2% WA only. Five tissue pieces were placed on each plate.

Incubation: Plates were incubated in the dark at 25°C and examined every three to four days. Visible actinomycete colonies were picked off

with a sterile loop and streaked onto YME for identification (141). Two serial transfers from single colonies were made onto YME to obtain pure cultures. The number of tissue pieces yielding Streptomyces was counted, and percentage yield was calculated. Isolates that showed characteristics of Streptomyces were then characterized, as in the following section.

Characterization of isolates:

Isolates were streaked on to WA, SEA, ISSA, GAA, PYI and YME using a sterile loop. Plates were examined for growth, presence and color of sporulating mycelium, and pigment production after 7, 14, 21, and 28 days. Two known pathogenic Streptomyces scabies isolates (840103 and 8401232) were obtained from Dr. R. Loria (Cornell University) and compared for morphological and cultural characteristics to the isolates obtained experimentally in Michigan. If a pigment with a color other than brown was produced, the response to pH change was tested by adding a drop of 0.05N NaOH or 0.05N HCl to the colored agar and observing for color changes immediately and after 15 min (141). Carbon utilization was tested on carbon utilization media amended with the suggested carbon sources. Each test was replicated on 3 plates.

For light microscopy, 14- or 21-day-old cultures on YME, SEA, and WA were examined at 150 and 645X using a light microscope. Colonies were examined directly on agar plates.

For electron microscopy, samples were prepared using standard techniques for biological specimens (70). YME agar slabs (2-3 cm^2) were cut from plates with a sterile dissecting needle, fixed on ice for 2 hr in 4% glutaraldehyde in 0.2 M sodium phosphate buffer (pH 7.2) and

dehydrated in a sequential ethanol series (25%, 50%, 75%, 95%, and 100%). Specimens were critical point dried with a critical point dryer, mounted on aluminum stubs using adhesive tabs, sputter-coated with gold for 4 min in a sputter coater and examined in a JEOL JSM-35CF scanning electron microscope (SEM) (Japan Electron Optics Lab., Tokyo, Japan) at a working distance of 15mm, an accelerating voltage of 15KV, and a condenser lens setting of 400. Secondary electron images were recorded on Polaroid type 665 P/N film (Polaroid Corp., Cambridge, MA).

Isolates were tested for pathogenicity in the greenhouse. Suspensions of Streptomyces were prepared from 14- to 18-day-old YME cultures which were visibly sporulating. Sterile distilled water (10-15 ml) was poured onto each plate and bacterial spores and mycelium were loosened by gently scraping the surface of colonies over the plate with a sterile L-shaped monel wire. The resulting suspension was poured into a sterile flask and sterile distilled water was added to bring the suspension to 50 ml. Controls were produced by pouring sterile distilled water over uninoculated YME plates.

Potato seed pieces without visible scab lesions were surface disinfested by a 5 min immersion in 10% bleach, allowed to air dry, and planted with the top of each seed piece approximately 2 cm below the soil surface. Plants were fertilized every 2 weeks with Peters 15-30-15 nitrate-based fertilizer (Robert B. Peters Co., Allentown, PA).

In 1988, potato pathogenicity tests were carried out used Shepody potatoes. Soil in 10 in. diameter pots was infested with 50 ml aliquots of approximately 1.2×10^8 cfu/ml by pouring Streptomyces suspensions over planting mix in pots and stirring the soil to distribute the inoculum.

Soil was infested twice for pathogenicity tests to ensure ample inoculum for infection, once when potatoes were planted, and again when potatoes were 10-15 cm tall. Pots were watered daily.

In 1989, Atlantic potatoes were used in 6 in. diameter pots, infested as above with 50 ml aliquots of approximately $5.8x10^7$ cfu/ml or with distilled water. Rather than watering daily, pots were watered every second day to avoid excessive soil moisture and to encourage increased disease incidence (3, 75, 84, 132). When potato tops started to die off, potatoes were harvested, washed under cold running tap water, and examined for scab.

Two Streptomyces isolates from carrot were tested for pathogenicity on Chancellor carrot. For each isolate, 3 pots (6 in. diameter) were steam-sterilized for 2 hr at 121°C, cooled, and infested with 50 ml spore suspensions of approximately $5x10^7$ cfu/ml. Twelve carrot seeds (surface-disinfested for 15 min in 25% bleach) were planted in each pot and thinned to four plants per pot after emergence. Pots were watered daily with tap water and fertilized as indicated above for potatoes. Carrots were harvested after 8-9 weeks and examined for scab. Isolations were made from suspected lesions (see above).

Effect of green manure crops on scab development in the field:

All field work was done at the Michigan State University Botany Farm East in East Lansing, MI, in a sandy clay loam soil with pH of 6.1-6.5. The field had produced severely scabbed potatoes the previous year.

In 1988, a rye winter cover crop was turned under, the soil was disked and rolled, and fertilizer was broadcast at 163 lbs/acre of 6-24-

24 ammonia-based fertilizer. The rye winter cover crop was not grown in 1989, but soil was prepared in the same way, except that 600 lbs/acre 12-12-12 ammonia-based fertilizer was used. The fertilizer program was based on soil tests.

Alfalfa (cv. Big Ten), red clover, and corn (cv. Iochief) were planted at 10 lbs/acre, while rye, oriental mustard (Brassica juncea (L.) Czern et Coss., cv. Cutlass), soybean (cv. Hark), and oat (cv. Mackinaw) were planted at 20 lbs/acre. Soybean and corn were planted in rows in which potatoes were subsequently planted, while the other green manure crops were broadcast over 5x12 ft. plots, within which the rows for the potatoes were contained. Plots were arranged in a randomized complete block design with 4 replicates per treatment.

In 1988, green manure crops were planted on May 12. Green manure crops were rototilled in to the soil on June 14. Atlantic potatoes were planted in two 10 ft. long rows, three feet apart, with a mechanical potato planter on June 17. In 1989, green manure crops were planted on May 4 and incorporated on June 7. Atlantic potatoes were planted on June 12 in two 12 ft rows. In 1988, plots were irrigated by overhead sprinkle irrigation weekly during June and July, after which rainfall supplied sufficient moisture. In 1989, no irrigation was used. Both years, metolachlor and linuron were applied twice each year to control weeds. Chlorothalonil was applied as needed to foliage for fungal disease control. In 1989, paraquat was applied to the plants to kill the vines on September 20. Vines died without treatment in 1988.

In 1988, potatoes were harvested by hand on September 26, and potatoes from 10 ft of row for each plot were weighed, washed, and rated

for scab. In 1989, potatoes were harvested on September 29 with a single row mechanical harvester. Potatoes from 10 ft per row, 2 rows per plot, were gathered and weighed and 50 tubers from each row were washed and rated for scab.

Scab levels were determined using the rating scale of 0=no visible scab, 1=1-5% of the tuber scabbed, 5=5-10%, 10=10-25%, and 25=25% or more of the tuber scabbed (Fig. 1). Each tuber was given a number based on this rating scale, and a scab rating determined for each plot as the average for all tubers. Marketable yield was estimated by multiplying the percent of potatoes with less than 5% scab by the total yield per acre determined from the weight per plot. Significant differences were determined using analysis of variance and Duncan's multiple range test (P=0.05).

Effect of green manure crops on scab development in the greenhouse:

All greenhouse pot tests were carried out using Bacto Professional Planting Mix (Michigan Peat Co., Houston, TX) with a pH of 6.1-6.6. Plants were grown under high intensity sodium vapor lights on a 14 hr daily photoperiod. Plants were sprayed as needed with methomyl, acephate, and cyhexatin to control insects and fertilized as in pathogenicity tests.

Greenhouse potting mix was infested with 50 ml of 5.8×10^7 cfu/ml suspensions of isolate 840103 in 1988 and 87PA32 in 1989, using the method described above for pathogenicity tests, except that the soil was infested only a single time. Green manure crops were planted, with 4 pots per crop, plus 4 pots with no green manure crop and 4 to which no Streptomyces was added. Crops were grown until their maturity equalled

Figure 1. Visual rating scale for scab severity on potato tubers.

that attained in the field (approximately 3 weeks after planting). Plants were then pulled, cut into pieces (3-6 cm long), and incorporated back into the potting mix. In 1988, white mustard (Sinapis alba L., cv. Kirby) was used instead of oriental mustard.

In 1988, green manures were allowed to decay for 2 weeks before Shepody potatoes were planted using 1 seed piece (surface-disinfested with 10% bleach for 5 min) per pot. Pots were watered daily. After 10 weeks, tubers were harvested and rated for scab, using the rating scale described above, and statistical analysis was performed.

In 1989, surface-disinfested Atlantic potatoes were planted 4 days after the incorporation of the cover crops. Pots were watered every second day and exposed to a 12 hour daily photoperiod 3-4 weeks after planting to encourage tuber production (149).

Changes in greenhouse soil pH over time:

Greenhouse potting mix in which soil amendment crops had been grown and incorporated was tested for changes in pH by combining 5-7 g samples from each of the four pots for each treatment after the pot was watered. Soil (15 g) from each treatment was combined with distilled water (30 ml), mixed for 15 min on a magnetic stir plate, and pH was measured with a Chemcadet pH meter (Cole-Parmer Instrument Co., Chicago, IL).

Changes in Streptomyces populations:

Approximately 3 g of greenhouse planting mix in which green manures had been incorporated were gathered and combined within each treatment. Soil was allowed to air dry for 2 days and 1 g was taken from each treatment and suspended in 10 ml of sterile water with 0.05% carboxymethylcellulose (CMC) to improve suspension of soil particles.

Suspensions were agitated on a vortex mixer for 1 min and dilutions (10^{-2} to 10^{-7}) were plated on WA (86) with 3 plates for each dilution. The number of colonies characteristic of the Streptomyces isolate used to infest the soil were recorded after 10-14 days.

Host range:

Crops previously reported to be host crops were tested for symptom production with Streptomyces. These crops included: radish (cvs. Champion and Scarlet Knight), rutabaga (cv. American Purple Top), turnip (cvs. Purple Top White Globe and Tokyo Market Early), parsnip (cvs. Harris Model and Hollow Crown), red beet (cvs. Burpee's Red Ball and Detroit Dark Red), carrot (cv. Gold Pak 263) and sugar beet (cv. E-4).

Crops were planted in 6 in. diameter pots in planting mix infested with 25 ml of 5.8×10^7 cfu/ml bacterial suspension. Isolates 87PA32, 840103, and 87CS11 were used in the spring, and isolate 88CCG2 was included in the summer. In the spring, pots were watered daily while those in the summer were watered every other day until root expansion was evident, after which pots were watered daily. Plants were sprayed and fertilized as described for green manure tests. In the summer the crucifers (except radish) were sprayed with chlorothalonil to control powdery mildew.

In the field, host crops were planted in single 12 ft rows spaced 1 ft apart. Plants were irrigated 1 week after planting, after which rainfall supplied sufficient moisture for growth.

Plants were pulled when sufficient size was attained or when tops started to die. Roots were washed and examined for lesions. Isolations were made from suspected scab lesions to verify infection (see above).

Effects of water extracts from cover crops on growth of S. scabies:

Water extracts were produced from cover crops by grinding 10 g plant material in 100 ml distilled water in a Waring Blendor (Waring Products Co., New York, NY). The resultant slurry was filtered through cheesecloth to remove large particles, filtered once through E&D folded filter paper (Eaton-Dikeman Co., Mount Hollysprings, PA), and twice through Whatman #1 filter paper (Whatman Intl. Ltd., Maidstone, England). Extracts were either sterilized by autoclaving 20 min or by filtration through a bacteriological filter (pore size 0.45 μm).

Equal volumes of sterile water extracts and sterile CSN were combined to make 50 ml. Controls were made by adding 25 ml of a sterile 10% sucrose solution to CSN. Streptomyces isolate 87PA32 was inoculated into test media using a sterile loop to transfer one colony from YME to each medium to be tested. Four flasks were inoculated for each treatment. Liquid cultures were incubated at 20-21 °C on a shaker at 100 RPM for 10-21 days in a darkened room.

Growth was determined by filtering cultures through pre-weighed Whatman glass microfibre filters which were subsequently dried in a drying oven at 85°C for 12 hr and weighed on an electric balance. The weight of the final mycelial mass was the difference between the average weight of dried filter paper (growth media alone) and the average dry weight of the material from culture-containing flasks.

Root infection of green manure crops:

Plants used as green manure crops were tested for root infection by 3 pathogenic Streptomyces isolates (87PA32, 87CS11, and 88CCG2) and by 4

non-pathogenic isolates (88FRCC, 89FRPB, 89MSEP1 and 89MSEP2). Tests were conducted in a similar way to the method described by Hooker (50).

Seeds of oriental mustard, soybean, red clover, alfalfa, oat, rye, corn, and cucumber (Cucumis sativus L., cvs. National Pickling and Wisconsin SMR 58) were surface-disinfested in 25% bleach with a drop of "Tween 20" for 15 min, rinsed twice in sterile distilled water, and placed in sterile petri plates on sterile filter paper saturated with sterile distilled water. Germinated seeds showing no evidence of contamination were transferred to the test boxes when radicles were approximately 1-3 cm long. Cucumbers were included as a negative control, as Hooker (50) had reported no effect on cucumber roots with Streptomyces in pathogenicity experiments.

Inoculum was grown for 21 days on YME in 9 cm petri plates. A suspension was prepared by gently scraping 1/8 of a plate in 10 ml of sterile distilled water, to produce an inoculum level of approximately $1\text{-}3\text{x}10^5$ cfu/ml, and poured into a covered, sterile plastic box (7 cm^2 by 10 cm high). Inoculum was mixed with 75 ml of a modified soil extract agar (50°C) prepared with soil extract from 25 g of soil in 1 liter of distilled water with 13 g agar per liter (50). After the agar hardened, radicles of 9 germinated seeds per box were inserted into the medium, except for corn, which was planted at 5 seeds per box. Boxes were placed about 40 cm below four 40 watt fluorescent light bulbs and plants were grown at 21-25°C under a 16 hr daily photoperiod. Lids were removed after 5-8 days to permit growth of aerial portions, and open boxes were watered with 10 ml of sterile tap water every 2 days. All plants were allowed to grow for 18 days, harvested, washed free of soil

extract agar under warm running tap water, and blotted dry. Roots were collected and the fresh root weights for 10 plants of approximately equal size were recorded using an electronic balance. Samples of the roots were surface-disinfested for 1 min in 10% bleach and plated on 1.5% WA to confirm infection. Root samples (2-3 cm long) were also prepared for scanning electron microscopy as described above for isolate characterization.

Inhibition of S. scabies by antagonists from soil:

In 1989, soil samples were taken from the field plots in which either red clover or oriental mustard had been incorporated. Samples of approximately 10 g were taken every 2 weeks from June 16 until August 25 from each of the 4 replicates per treatment and samples from the replicates were combined. The soil was allowed to air dry for 2 days, and a suspension was prepared, adding 1 g of soil to 10 ml of sterile 0.5% CMC. This suspension was mixed on a vortex mixer for 1 min, diluted in 0.5% CMC, and 0.1 ml from each of the resultant dilutions (10^{-2} to 10^{-6}) were plated on SEA infested with 1 ml of a suspension of $2x10^5$ cfu/ml of pathogenic isolate 87PA32 per liter. Plates were incubated at 25°C and examined for plaques in the lawn of Streptomyces. When plaques were observed, the colony at the center of the plaque was streaked onto SEA, NBY, and YME using a sterile loop. Isolates for antagonist tests were also obtained from lesions on field-grown plants.

Isolates were tested for antagonistic activity on SEA, NBY, and, if filamentous, on YME using two methods. Isolates were inoculated onto agar plates in two strips and challenged with 5 known pathogenic Streptomyces streaked at a 90° angle to the putative antagonist. Test

isolates included 4 potato pathogens (840103, 87CS11, 87PA32, and 87PS21) and the carrot pathogen (88CCG2). Putative antagonists were also tested by placing 5 agar discs containing single colonies of the isolates onto an agar plate previously infested with 87PA32 or 840103. All antagonism test plates were incubated at 25°C and examined daily.

Agar disc test plates were examined for clear plaques, and test plates were examined for inhibition of growth of the test isolates. Isolates which produced positive results for both tests were considered to have antagonistic activity and were tested for production of antibiotics.

Production of water-soluble antibiotics was determined by growing the isolates in liquid media, then harvesting and filter-sterilizing the culture filtrate after 2, 4, 6, 8, or 12 days for the actinomycetes, and 1, 2, 3, 4, or 6 days for Bacillus. In each flask, 50 ml of broth was inoculated with 0.1 ml of a suspension of $6\text{-}8 \times 10^6$ cfu/ml of bacteria. Flasks were kept on a shaker at 100 RPM at 21°C until harvest. Growth of Bacillus was determined by increase in cell numbers determined by dilution plating. Growth of the actinomycetes was determined by dry weight of the mycelial mass. CSN and SEA (without agar) were used for growing all antagonists. PDB was also used for growing Bacillus (153).

The culture filtrate was tested for antibiotics against isolate 87PA32, which had shown inhibition with all of the antagonistic isolates. SEA plates with 2.9×10^3 cfu/ml of 87PA32 were poured (20 ml of media per plate). A sterile #6 cork borer was used to prepare 4 wells per plate, and each well was sealed with a drop of 1.5% WA. Sterile culture filtrate (125 μl) was placed in each well at either

initial strength or at a 10-fold concentration of the initial filtrate, prepared by freeze-drying the filtrate in a lyophilizer and re-suspending the dried material in sterile distilled water at 1/10 the initial volume. Plates were incubated in the dark at 25°C and examined daily for clear plaques in the growth of 87PA32.

Organisms which tested positive for antibiotic production above were grown for 5-7 days in 100 ml of CSN. An initial inoculum concentration of $6\text{-}8 \times 10^6$ cfu/ml was used, and flasks were treated as above. After harvest, the cultures were filtered to remove the bacteria, and a chloroform extraction of the aqueous culture filtrates was performed by adding equal volumes of culture filtrate and chloroform to a separatory funnel, agitating for 2 min, allowing separation for 30 min, and draining off the chloroform fraction. Chloroform was added twice more to each culture filtrate and the chloroform fraction were combined. All fractions from the chloroform extraction were dehydrated using a rotary evaporator. Dried material was re-suspended in distilled water at 1/10 initial concentration, and tested for antibiotic activity using the bioassay described above. Infested plates were also inoculated with the isolates from culture plates to check for loss of antagonistic activity.

Three non-pathogenic isolates from scab lesions were also tested for antagonistic activity using the plate assays, and one was further tested for antibiotic production, as described above.

All statistical analyses were conducted using the statistical computer software MSTAT 4.0 or MSTATC 5.0 (Michigan State University).

RESULTS

Streptomyces isolations:

Streptomyces spp. were isolated from an average of two potato tissue pieces (5 pieces per plate) from each of 15 plates of 2% WA. No Streptomyces were obtained from ABWA, so antibiotic-amended WA was not used for the remainder of the isolations.

Tissue samples (other than potato, red beet, and rutabaga) surface-disinfested with bleach yielded a maximum of 1 Streptomyces isolate out of 5 tissue samples per plate (Fig. 2). Surface-disinfesting by heating yielded Streptomyces from an average of 3 or more tissue samples out of 5 samples per plate. There was also more growth of other bacteria following heat treatment than following bleach disinfestation.

Characterization of isolates:

Several representative Streptomyces isolates were characterized for growth, color, sporulation, and morphology on various media (Tables 1 & 2). Isolates which were pathogenic on potato included: 840103 and 8401232, obtained from Dr. R. Loria (Cornell University), originally from scabby potatoes; 87CS11, isolated from surface lesions on field-infected Shepody potato; 87PS21, isolated from pitted lesions on Shepody potato; and 87PA32, obtained from pitted lesions on Atlantic potato. Isolate 88CCG2, obtained from lesions on Chancellor carrot roots, was not pathogenic on Atlantic potatoes in the greenhouse, but did cause lesions on roots of seven of nine Chancellor carrots grown in infested sterile potting mix.

Isolates which were not pathogenic on potatoes included; 88CCP1, obtained from Chancellor carrot root lesions (this isolate was also not

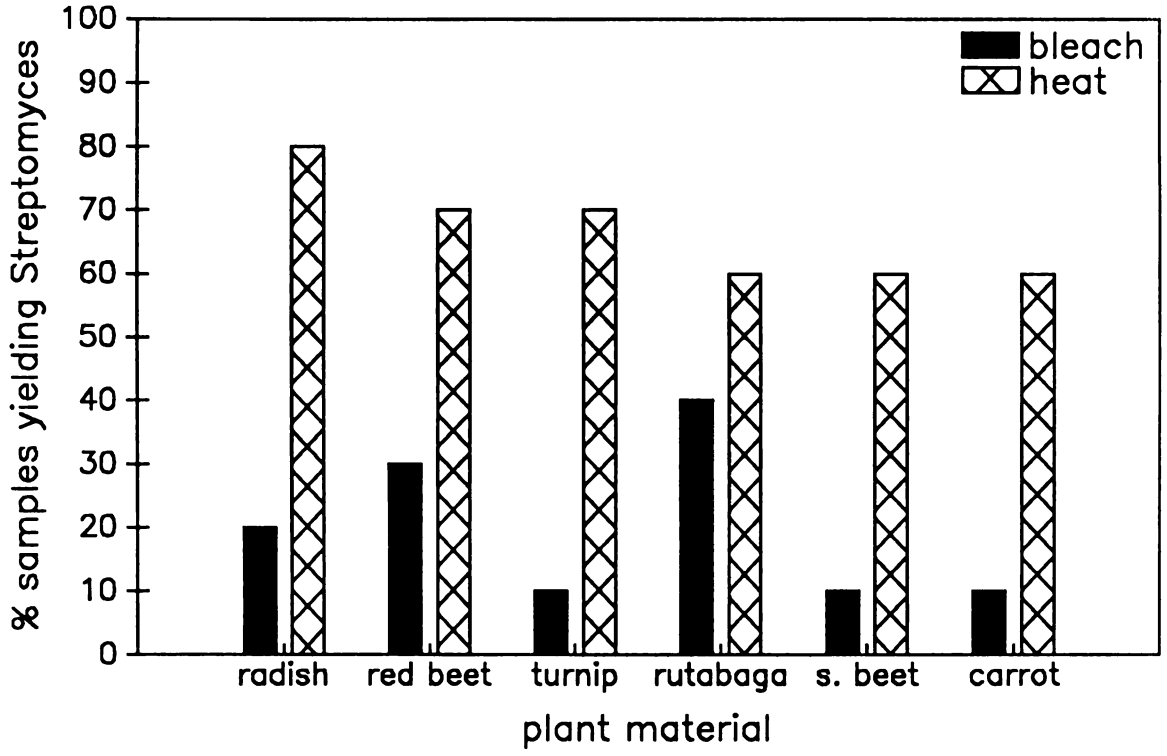

Figure 2. Percent of tissue samples yielding Streptomyces with two surface-disinfestation techniques.

Table 1. Growth of Streptomyces isolates on various media after 14-21 days incubation

Isolate	WA	SEA	ISSA	GAA	PYI	YME
840103	sparse[1]	sparse	light	light	light	heavy
	white[2]	white	white	white	white	white
	white[3]	"	"	"	"	brown
	none[4]	none	none	none	dark brown	none
8401232	sparse	sparse	light	heavy	light	heavy
	white	white to gray	white to gray	white	white	gray
	"	"	"	"	"	brown
	none	none	none	none	dark brown	brown
87CS11	sparse	sparse	light	sparse	none	heavy
	white	white	gray	white to gray		light gray
	"	"	"	"	brown	tan to gray
	none	none	none	none	dark brown	none
87PS21	sparse	sparse	light	sparse	sparse	light
	white to gray	white to gray	white to gray	light gray	white	gray
	"	"	yellow	"	tan	tan
	none	none	yellow	none	none	yellow
87PA32	sparse	light	heavy	light	none	heavy
	gray	gray	gray	gray		gray
	"	"	yellow-gray	tan	brown	brown
	none	none	none	none	dark brown	brown
88CCG2	sparse	light	light	sparse	none	heavy
	dark gray	brown-gray	gray	white		gray brown
	"	"	tan	white to tan	brown	dark brown
	none	none	none	none	dark brown	brown
88CCP1	light	heavy	heavy	heavy	light	heavy
	cream to pink	pink	pink	pink	white	pink
	"	cream	"	"	"	"
	none	none	none	none	none	tan
89MSEP1	sparse	sparse	light	light	none	heavy
	gray	gray	gray-brown	white		gray
	"	"	gray	"	brown	tan
	none	none	none	none	dark brown	none
89MSEP2	sparse	sparse	sparse	sparse	none	heavy
	white	white	white	white		white
	"	"	yellow to tan	yellow	brown	tan to brown
	none	none	none	none	dark brown	none
88FRCC	light	heavy	heavy	heavy	heavy	heavy
	cream	cream	cream to gray	cream	white to pink	cream
	"	"	"	white	tan to reddish	brown
	none	none	none	none	none	red brown
89FRPP	sparse	light	heavy	heavy	sparse	heavy
	gray	gray	gray	gray	white	gray
	"	"	purple	purple	"	"
	red	none	red	red,NaOH rxn	dark gray	purple,NaOHrxn
89FRPB	sparse	light	heavy	light	sparse	heavy
	gray	gray	gray	white	gray-purple	gray
	"	"	purple	red-purple	dark gray	"
	red	none	red-purple,HCl rxn	purple,HCl rxn	none	blue,HCl rxn

[1] aerial growth
[2] color of aerial mycelium
[3] color of substrate mycelium
[4] color of diffusible pigment

pathogenic on carrot); 89MSEP1 and 89MSEP2, isolated from mustard plot soil; 88FRCC, isolated from radish and carrot root lesions; 89FRPP, isolated from rutabaga root and Norkota russet potato lesions; and 89FRPB, isolated from rutabaga root and Atlantic potato lesions.

All of the Streptomyces isolates tested were able to grow on all of the test media, but rate of growth (indicated by amount of aerial mycelia at 14-21 days) varied, with two of the non-pathogens (88FRCC and 88CCP1) showing the greatest growth on all media used (Table 1). All pathogenic isolates produced tan to brown mycelia on YME agar but there were no other growth rate and color characteristics found consistently with all pathogenic isolates. Most pathogenic isolates had gray aerial mycelia on YME, except isolate 840103, which had white aerial mycelia. All pathogenic isolates, except isolate 87PS21, produced melanin pigment on PYI. These characteristics were not unique to the pathogenic isolates, and so could not be used to identify pathogens. Three non-pathogenic isolates also produced brown mycelium, isolate 89MSEP2 produced melanin on PYI, and isolate 89MSEP1 was indistinguishable from pathogenic isolates in growth, color, and melanin production.

Three non-pathogenic isolates produced diffusible pigment colors other than brown on various media. The reddish pigment produced by 88FRCC did not react to a change in pH, but the diffusible pigments from isolates 89FRPP and 89FRPB responded to pH changes. YME containing the purple pigment from 89FRPP turned blue immediately after addition of 1 drop of 0.05 N NaOH, while on GAA the change from red to blue took 15 min. The purple or blue pigments from isolate 89FRPB also reacted to pH changes. On GAA, the purple medium turned red immediately after

addition of 1 drop of 0.05N HCl, and on YME the blue turned to purple, starting as soon as acid was added and changing completely after 15 min.

Carbon utilization by isolates also varied (Table 2). All isolates examined grew well on glucose and fructose and showed little or no growth on cellulose or on the basal medium without any carbon source. All pathogenic isolates also grew well on all of the other sugars tested, including raffinose. Growth on raffinose is considered particularly indicative of S. scabies since few non-pathogenic Streptomyces can use this sugar as a sole carbon source (71). Non-pathogens varied somewhat more than pathogens in carbon utilization. Isolate 88FRCC was the only non-pathogen with the same carbon utilization range as the pathogenic isolates. None of the other non-pathogens grew on raffinose. Isolate 89MSEP1 grew on all remaining sugars while isolate 88CCP1 grew well on glucose and fructose, poorly on sucrose, and not on any other carbon sources. The remaining non-pathogenic isolates had carbon utilization ranges intermediate between these two isolates.

All of the pathogenic isolates produced coiled or spiral spore chains (Table 2, Figs. 3 & 4). Only isolate 88CCP1 of the non-pathogenic isolates also produced coiled spore chains. All of the other non-pathogenic isolates produced straight or flexuous spore chains.

Most of the pathogenic isolates produced smooth-walled spores, as is typical of S scabies (Table 2 and Fig 4); however, isolates 87CS11 and 87PS21 produced rough-walled spores as cultures aged (Fig 4). Isolate 88CCG2 (from carrot) produced echinulate spores (Fig. 4).

Table 2: Characteristics of *Streptomyces* isolates

Isolate	Source	C Utilization	Spore Chains	Spores	Antagonisms	Scab on Potato
840103	Loria	glucose, fructose, sucrose, mannitol, inositol, rhamnose, arabinose, xylose, raffinose	coiled	smooth	not determined	yes
8401232	Loria	glucose, fructose, sucrose, mannitol, inositol, rhamnose, arabinose, xylose, raffinose	coiled	smooth	not determined	yes
87CS11	potato surface scab	glucose, fructose, sucrose, mannitol, inositol, rhamnose, arabinose, xylose, raffinose	coiled	smooth to rough as age	none found	yes
87PS21	potato pitted scab	glucose, fructose, sucrose, mannitol, inositol, rhamnose, arabinose, xylose, raffinose	coiled	smooth to rough as age	not determined	yes
87PA32	potato pitted scab	glucose, fructose, sucrose, mannitol, inositol, rhamnose, arabinose, xylose, raffinose	coiled	smooth	none found	yes
88CCG2	carrot	glucose, fructose, sucrose, mannitol, inositol, rhamnose, arabinose, xylose, raffinose	coiled	echinulate	none found	no
88CCP1	carrot	glucose, fructose, sparse-sucrose	coiled	smooth	none found	no
89MSEP1	soil	glucose, fructose, sucrose, mannitol, inositol, rhamnose, arabinose, xylose	straight	smooth	87PA32, 840103	no
89MSEP2	soil	glucose, fructose, sucrose, mannitol, inositol, arabinose, xylose	straight	smooth	87PA32,840103, 87CS11,87PS21, 88CCG2	no
88FRCC	radish and carrot	glucose, fructose, sucrose, mannitol, inositol, rhamnose, arabinose, xylose, raffinose	straight	smooth	87PA32,840103, 87CS11,87PS21, 88CCG2	no
89FRPP	rutabaga and potato scab	glucose, fructose, inositol, rhamnose, xylose sparse-mannitol, arabinose	flexuous	rough	none found	no
89FRPB	rutabaga and potato scab	glucose, fructose, mannitol, inositol, xylose sparse-sucrose, rhamnose, arabinose	flexuous	rough	none found	no

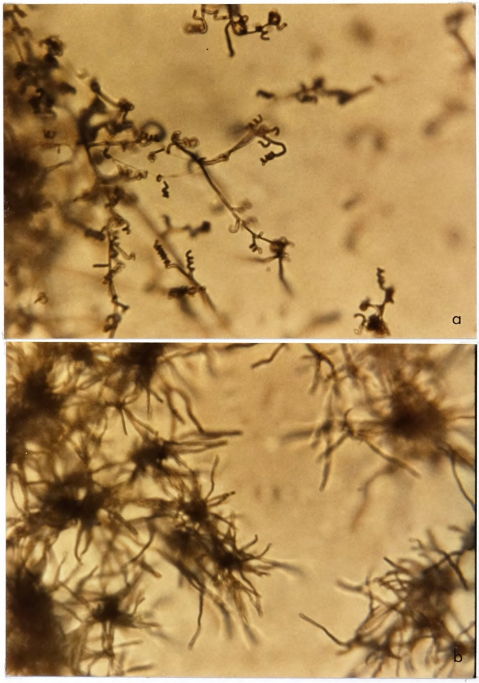

Figure 3. Light micrographs of spore chains of Streptomyces isolates

a. pathogenic isolate (645X)

b. non-pathogen (645X)

Figure 4. SEM photomicrographs of spores and spore chains of typical Streptomyces isolates.

a. 88FRCC (6000X), straight spore chain of non-pathogen

b. 87PA32 (10000X), typical pathogenic isolate

c. 87CS11 (6000X), rough-walled spores

d. 88CCG2 (4000X), echinulate spores

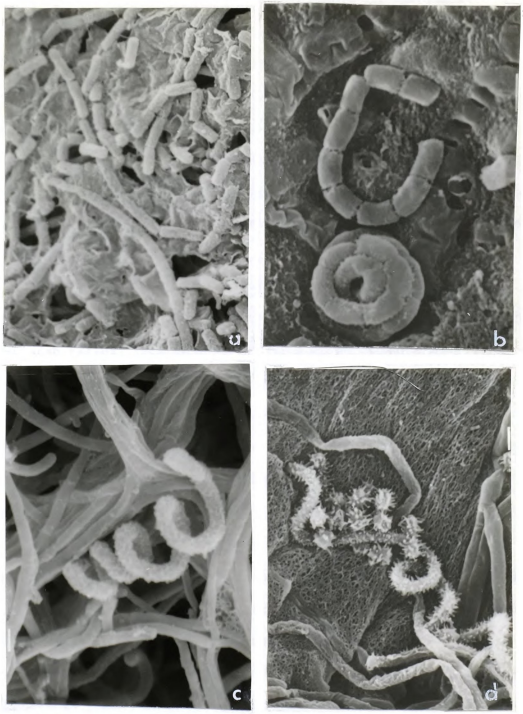

Figure 4.

Effects of green manure crops on scab development in the field:

In 1988, none of the green manure field treatments had a significantly lower scab rating or higher marketable yield than the fallow control; however, the red clover treatment had a significantly higher scab rating and lower marketable yield than the fallow control (P=0.05) (Table 3). The corn treatment also gave a higher scab rating than the fallow control, and the mustard treatment gave the lowest scab rating (Fig. 5) and highest marketable yield, although not significantly different from the fallow control.

In 1989, none of the green manure treatments had a scab rating significantly different from the fallow control (Table 4 & Fig. 5). The highest scab rating, from corn plots, was significantly higher than those from alfalfa, oat, rye, or mustard plots, and the lowest scab rating (from mustard plots) was significantly lower than the scab ratings from soybean, red clover, and corn. The marketable yield from corn plots was significantly lower than that from fallow control, mustard, and rye plots.

When scab ratings and marketable yields were combined over two years, both the corn and red clover treatments had significantly higher scab ratings and lower marketable yields than the fallow control (Table 5 & Fig. 5). Scab levels for the oriental mustard treatment were significantly lower than scab levels for the fallow control, although the marketable yield was not significantly different. The rye and alfalfa treatments were not significantly different from the fallow control in any tests.

Table 3. Effect of green manures on scab incidence and marketable yield in field-grown Atlantic potatoes, summer, 1988

Green Manure	Mean Scab Rating[1]	Marketable Yield (CWT/acre)[2]
red clover (10 lbs/acre)[3]	12.9 A[4]	91 B
corn (10 lbs/acre)	11.4 AB	143 AB
fallow control	9.1 BC	202 A
rye (20 lbs/acre)	8.9 BC	145 AB
alfalfa (10 lbs/acre)	7.9 C	190 A
oriental mustard (20 lbs/acre)[5]	6.8 C	223 A

[1] based on a rating scale where 0=no scab, 1=1-4% of potato scabbed, 5=5-9%, 10=10-25%, and 25=25% or more of the tuber scabbed
[2] estimated weight per acre of marketable potatoes with less than 5% scab (x100 lbs)
[3] seeding rate
[4] means followed by different letters are significantly different by Duncan's Multiple Range test (P=0.05)
[5] Brassica juncea

Table 4. Effect of green manures on scab incidence and marketable yield in field-grown Atlantic potatoes, summer, 1989

Green Manure	Mean Scab Rating[1]	Marketable Yield (CWT/acre)[2]
corn (10 lbs/acre)[3]	14.5 A[4]	55 C
red clover (10 lbs/acre)	12.7 AB	72 BC
soybean (20 lbs/acre)	12.4 AB	92 ABC
fallow control	11.9 ABC	118 AB
oat (20 lbs/acre)	10.6 BC	109 ABC
alfalfa (10 lbs/acre)	10.5 BC	102 ABC
rye (20 lbs/acre)	10.2 BC	141 A
oriental mustard[5] (20 lbs/acre)	9.5 C	142 A

[1] based on a rating scale where 0=no scab, 1=1-4% of potato scabbed, 5=5-9%, 10=10-25%, and 25=25% or more of the tuber scabbed
[2] estimated weight per acre of marketable potatoes with less than 5% scab (x100 lbs)
[3] seeding rate
[4] means followed by different letters are significantly different by Duncan's Multiple Range test (P=0.05)
[5] Brassica juncea

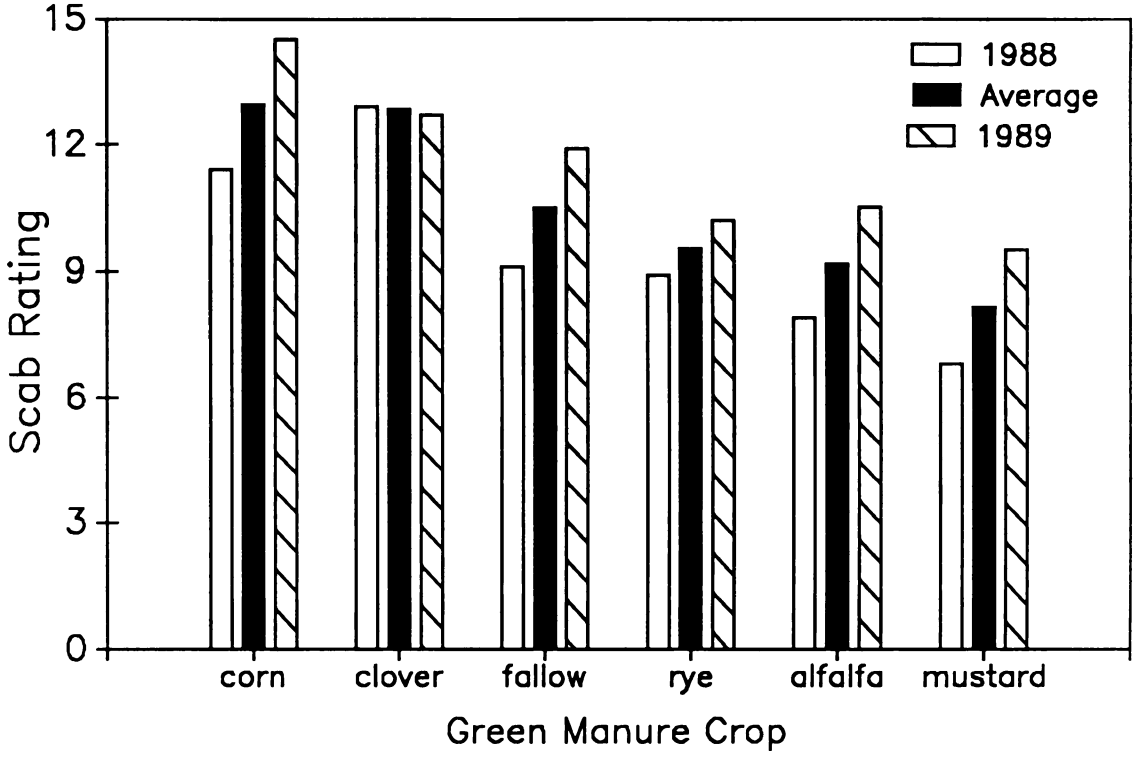

Figure 5. Effect of green manures on scab incidence in field soils over two years

Effect of green manure crops on scab development in the greenhouse:

None of the treatments had a scab level significantly different from the fallow control on either potato variety in the greenhouse (Table 6). On Shepody potato the red clover treatment produced a scab level significantly higher than that from the rye or white mustard treatments. The scab level from the white mustard treatment was also significantly lower than the alfalfa treatment. On Atlantic potatoes, none of the treatments were significantly different from the fallow control, but the red clover treatment produced a significantly higher scab rating than the oriental mustard treatment, which had the lowest scab rating

Changes in greenhouse soil pH over time:

All of the treatments showed an increase in soil pH over time. The soil pH showed some variation among the different green manure treatments (Table 7); however, the pH level remained between 6.6 and 7.3 throughout the test, which is within the range conducive to scab (49, 125, 151).

Changes in Streptomyces population:

All of the treatments had an increase in the number of colony - forming-units (cfu) of Streptomyces per gram of soil after green manure incorporation (Table 8). There was also an increase in cfu for the non-amended fallow control; however, it was lower than that seen in the amended soils. The levels of Streptomyces cfu in all treatments was well above the range reported to give high disease levels in the field (152), and no significant correlation was found by LSD between these levels and scab severity (P=0.05).

Table 5. Average effect of green manures on scab incidence and marketable yield on Atlantic potatoes over 2 summers.

Green Manure	Mean Scab Rating[1]	Marketable Yield (CWT/acre)[2]
corn (10 lbs/acre)[3]	13.0 A[4]	98.8 BC
red clover (10 lbs/acre)	12.8 A	81.5 C
fallow control	10.5 B	159.9 A
rye (20 lbs/acre)	9.5 BC	143.3 AB
alfalfa (10 lbs/acre)	9.2 BC	146.1 AB
oriental mustard[5] (20 lbs/acre)	8.2 C	182.6 A

[1] based on a rating scale where 0=no scab,[1] based on a rating scale where 0=no scab, 1=1-4% scabbed, 5=5-9%, 10=10-25% and 25=25% or more of the tuber scabbed
[2] estimated weight per acre of marketable potatoes with less than 5% scab (x100 lbs)
[3] seeding rate
[4] means followed by different letters are significantly different by Duncan's multiple range test (P=0.05)
[5] Brassica juncea

Table 6. Effect of green manures on scab incidence in greenhouse pot-grown potatoes.

Green Manure	Shepody Scab Rating[1]	Atlantic Scab Rating
red clover	19.5 A[2]	5.38 A
alfalfa	17.8 AB	2.38 AB
fallow control	15.6 ABC	1.88 AB
corn	11.9 ABC	4.00 AB
oat	10.6 ABC	1.25 AB
soybean	10.2 ABC	1.38 AB
rye	7.9 BC	1.25 AB
white mustard[3]	6.9 C	not determined
oriental mustard[4]	not determined	1.00 B

[1] based on a rating scale where 0=no scab, 1=1-4% scabbed, 5=5-9%, 10=10-25% and 25=25% or more of tuber scabbed
[2] means in each column followed by different letters are significantly different by Duncan's Multiple Range Test (P=0.05)
[3] Sinapis alba
[4] Brassica juncea

Table 7. Changes in soil pH over time after incorporation of green manures in greenhouse planting mix.

Green Manure	pH of Soil[1]			
	Week 1	Week 3	Week 5	Week 7
no treatment	6.6	7.1	7.0	6.9
alfalfa	6.8	6.6	6.6	7.0
corn	6.8	7.0	6.9	7.3
fallow control	6.8	7.0	6.9	7.0
oat	6.6	6.9	6.8	7.2
oriental mustard[2]	6.7	6.8	6.8	7.1
red clover	6.8	7.0	7.0	7.3
rye	6.7	6.8	6.9	7.2
soybean	6.9	6.7	6.6	7.1

[1] pH determined for soil from freshly watered pots with 1:2 w/v soil to distilled water
[2] Brassica juncea

Table 8. Number of Streptomyces isolated from greenhouse planting mix over time after incorporation of green manures.

Green Manure	No. of Streptomyces per Gram Soil ($x10^6$)[1]				
	Week 1	Week 3	Week 5	Week 7	Week 9
alfalfa	1.3	7.0	7.4	45	49
corn	1.9	5.0	6.3	35	40
fallow control	1.4	2.2	5.8	15	20
oat	2.0	4.9	10.0	54	53
oriental mustard[2]	1.6	5.9	9.3	46	46
red clover	1.2	5.5	6.2	39	38
rye	1.6	7.2	10.1	19	20
soybean	0.8	5.0	7.3	49	48

[1] per g air dried soil in 1:10 w/v sterile distilled water initial concentration
[2] Brassica juncea

Host range:

In field tests, all of the root crops showed some scab symptoms from which Streptomyces could be isolated (Table 9). Sugar beet, carrot, and rutabaga had the highest disease levels, with 50-53% of the plants showing symptoms for carrot and rutabaga respectively and 93% infection for sugar beet. Red beet had the lowest percent of plants infected (13%). The remaining crops had disease levels intermediate between these levels. Scab severity was not compared as different crops produced different types of symptoms.

In greenhouse tests, the host crops differed in susceptibility to individual isolates of Streptomyces (Table 10). Only one host (Burpee Red Ball beet) showed no symptoms with any of the isolates used. All other host crops developed scab lesions from which Streptomyces could be isolated. Isolate 87PA32 had the broadest host range, inducing lesions on all hosts on which it was tested (except the red beet mentioned above) and generally yielding the highest number of infected plants of any of the isolates. Isolate 840103 showed the narrowest host range, producing small lesions on only 3 hosts (sugar beet, parsnip, and radish). Other isolates showed intermediate host ranges.

Effects of water extracts from cover crops on growth of S. scabies:

Streptomyces showed some differences in growth in water extracts from the green manure crops used (Tables 11-16). Orthogonal comparisons indicated that there were no significant differences in overall growth response of Streptomyces to full strength and one tenth dilutions of green manure crop extracts of decayed plant material (Table 11), indicating that toxic substances were probably not involved in

Table 9. Host range of scab symptoms on field-grown root crops

Host Crop	No. Diseased/No. Harvested	% Diseased
Detroit Dark Red beet	2/15	13
Scarlet Knight radish	4/22	18
Champion radish	3/13	23
Purple Top White Globe turnip	6/20	30
Gold Pak carrot	24/48	50
American Purple Top rutabaga	8/15	53
E-4 sugar beet	14/15	93

Table 10. Host Range of scab symptoms on root crops with different Streptomyces isolates.

	No. Plants With Lesions/Total No. Plants				
Host Crop	87PA32	840103	87CS11	88CCG2	Water
Burpee Red Ball beet	0/4	0/4	0/4	0/4	0/4
Detroit Dark Red beet	2/8	0/8	0/8	0/4	0/8
E-4 sugar beet	3/8	1/8	1/8	1/4	0/8
Gold Pak 263 carrot	3/8	0/8	0/8	3/8	0/8
Harris Model parsnip	2/4	1/4	1/4	1/4	0/4
Hollow Crown parsnip	2/4	1/4	2/4	1/4	0/4
Scarlet Knight radish	4/8	0/8	2/8	0/4	0/8
Champion radish	3/8	1/8	0/8	1/4	0/8
APT rutabaga	2/8	0/8	2/8	0/4	0/8
PT, WG turnip	1/8	0/8	0/8	0/8	0/8
Tokyo Mkt Early turnip	3/4	0/4	1/4	1/4	0/4

APT = American Purple Top
PT, WG = Purple Top White Globe

differences in growth rates. Significant differences were seen in growth rates between sugar water controls and all treatments as a group, and between rye and oat within the two dilutions (Table 11). At both concentrations, growth in corn and oat extracts was significantly lower than in control or rye extracts (Table 12).

There were significantly greater differences in growth of Streptomyces in autoclaved water extracts from decayed plant material than when filter-sterilization was used (Table 13). When extracts were filter-sterilized there were no significant differences between the growth rates of Streptomyces in any of the extracts (Tables 13 & 14). When autoclave sterilization was used, growth in both rye and alfalfa extracts was significantly greater than that in the sugar water control.

When fresh plant material was used, orthogonal comparisons showed no significant differences between the sterilization techniques after 21 days growth (Table 15). With either sterilization technique, growth was highest in the controls, and significantly lower in the corn extract treatments (Table 16).

There was not a significant correlation between scab ratings and growth of Streptomyces in any of the filtered or autoclaved extracts from decayed crop residues. However, significant negative correlations were found ($P=0.05$) between scab ratings from 1989 from both field and greenhouse potatoes and pathogen growth in the autoclave sterilized extracts from fresh (undecayed) plant material. This correlation was not significant for the field potatoes from 1988, or for the combined scab ratings over the two summers.

Table 11. Effect of autoclave-purified extracts from decayed crop residues at full and 0.1 strength on growth of Streptomyces after 14 days as determined by orthogonal comparisons.

	Treatment Codes[1] and Totals[2] (mg)																	
C[3]	fal 98.0	frc 76.4	fco 52.0	fry 82.2	fmu 80.2	fwa 99.4	foa 57.2	fso 50.9	dal 87.1	drc 75.3	dco 42.8	dry 95.3	dmu 77.6	dwa 99.0	doa 60.9	dso 79.3	ss(Q)	F
1	1	1	1	1	1	1	1	1	-1	-1	-1	-1	-1	-1	-1	-1	30.03	1.01
2	-1	-1	-1	-1	-1	7	-1	-1	0	0	0	0	0	0	0	0	389.64	11.89**
3	0	0	0	0	0	0	0	0	-1	-1	-1	-1	-1	7	-1	-1	272.50	8.32*
4	-3	-3	4	4	-3	0	4	-3	0	0	0	0	0	0	0	0	87.01	2.66
5	0	0	0	0	0	0	0	0	-3	-3	4	4	-3	0	4	-3	156.02	4.76*
6	0	0	2	-1	0	0	-1	0	0	0	0	0	0	0	0	0	104.43	3.19
7	0	0	0	0	0	0	0	0	0	0	2	-1	0	0	-1	0	415.36	12.68**
8	0	0	0	1	0	0	-1	0	0	0	0	0	0	0	0	0	156.25	4.77*
9	0	0	0	0	0	0	0	0	0	0	0	1	0	0	-1	0	295.84	9.03**
10	1	1	0	0	-3	0	0	1	0	0	0	0	0	0	0	0	26.67	0.81
11	0	0	0	0	0	0	0	0	1	1	0	0	-3	0	0	1	3.30	0.10
12	1	1	0	0	0	0	0	-2	0	0	0	0	0	0	0	0	326.56	9.97**
13	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	-2	1.20	0.04
14	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33.64	1.03
15	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	34.81	1.06

* significant at the 5% level ** significant at the 1% level

[1] treatment codes: fal=full strength alfalfa; frc=full strength red clover; fco=full strength corn; fry=full strength rye; fmu=full strength white mustard; fwa=full strength sugar water control; foa=full strength oat; fso=full strength soybean; dal=dilute alfalfa; drc=dilute red clover; dco=dilute corn; dry=dilute rye; dmu=dilute mustard; dwa=dilute sugar water control; doa=dilute oat; dso=dilute soybean

[2] total dry weight of Streptomyces per treatment

[3] Contrasts:

1 full strength vs. diluted
2 control vs. extracts, full
3 control vs. extracts, diluted
4 monocots vs. dicots, full
5 monocots vs. dicots, diluted
6 corn vs. other grasses, full
7 corn vs. other grasses, diluted
8 rye vs. oats, full
9 rye vs. oats, diluted
10 mustard vs. legumes, full
11 mustard vs. legumes, diluted
12 forage legumes vs. soybean, full
13 forage legumes vs. soybean, diluted
14 red clover vs. alfalfa, full
15 red clover vs. alfalfa, diluted

Table 12. Effect of autoclave-purified extracts from decayed crop residues at full and 0.1 strength on growth of Streptomyces after 14 days.

Extract	Mean Weight[1](mg)	Mean Weight[2](mg)
water control	47.4 A[3]	49.5 A
alfalfa	44.0 A	43.6 AB
rye	41.1 AB	49.5 A
white mustard[4]	40.1 AB	38.8 AB
red clover	38.2 ABC	37.7 AB
oat	28.6 BC	30.5 BC
corn	26.0 C	21.4 C
soybean	25.5 C	39.7 AB

[1] mean dry weight after 10 days growth in full strength extract
[2] mean dry weight after 10 days growth in 1/10 dilution
[3] means followed by different letters are significantly different by Duncan's Multiple Range Test (P=0.05)
[4] Sinapis alba

Table 13. Effect of filter- or autoclave-purified water extracts from decayed crop residues on growth of Streptomyces after 14 days as determined by orthogonal comparisons.

	Treatment Codes[1] and Totals[2] (mg)																	
C[3]	fal 69	frc 63	fco 133	fry 119	fmu 107	fsw 87	foa 92	fso 97	aal 194	arc 153	aco 83	ary 192	amu 87	asw 93	aoa 109	aso 80	ss(Q)	F
1	1	1	1	1	1	1	1	1	-1	-1	-1	-1	-1	-1	-1	-1	1575	8.23*
2	1	1	1	1	1	-7	1	1	0	0	0	0	0	0	0	0	46	0.24
3	0	0	0	0	0	0	0	0	1	1	1	1	1	-7	1	1	535	2.80
4	3	3	-4	-4	3	0	-4	3	0	0	0	0	0	0	0	0	809	4.22
5	0	0	0	0	0	0	0	0	3	3	-4	-4	3	0	-4	3	0	0.00
6	1	1	0	0	-3	0	0	1	0	0	0	0	0	0	0	0	363	1.90
7	0	0	0	0	0	0	0	0	1	1	0	0	-3	0	0	1	1159	6.06*
8	1	1	0	0	0	0	0	-2	0	0	0	0	0	0	0	0	322	1.69
9	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	-2	2883	15.07**
10	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0.05
11	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	420	2.20
12	0	0	2	-1	0	0	-1	0	0	0	0	0	0	0	0	0	241	1.26
13	0	0	0	0	0	0	0	0	0	0	2	-1	0	0	-1	0	1528	7.99*
14	0	0	0	1	0	0	-1	0	0	0	0	0	0	0	0	0	188	0.98
15	0	0	0	0	0	0	0	0	0	0	0	1	0	0	-1	0	1706	8.92**

* significant at 5% level ** significant at 1% level

[1] treatment codes: fal=filtered alfalfa; frc=filtered red clover; fco=filtered corn; fry=filtered rye; fmu=filtered oriental mustard; fsw=filtered sugar water; foa=filtered oat; fso=filtered soybean; aal=autoclaved alfalfa; arc=autoclaved red clover; aco=autoclaved corn; ary=autoclaved rye; amu=autoclaved oriental mustard; asw=autoclaved sugar water; aoa=autoclaved oat; aso=autoclaved soybean

[2] total dry weight of Streptomyces per treatment

[3] Contrasts:

1 autoclave vs. filter sterilized
2 control vs. extract treatments, filtered
3 control vs. extract treatments, autoclaved
4 monocot extracts vs. dicot extracts, filtered
5 monocot extracts vs. dicot extracts, autoclaved
6 mustard vs. legumes, filtered
7 mustard vs. legumes, autoclaved
8 forage legumes vs. soybean, filtered
9 forage legumes vs. soybean, autoclaved
10 red clover vs. alfalfa, filtered
11 red clover vs. alfalfa, autoclaved
12 corn vs. other grasses, filtered
13 corn vs. other grasses, autoclaved
14 rye vs. oat, filtered
15 rye vs. oat, autoclaved

Table 14. Effect of filter- or autoclave-purified water extracts from decayed crop residues on growth of Streptomyces after 14 days

Extract	Mean Weight[1](mg)	Mean Weight[2](mg)
rye	59.7 A[3]	96.0 A
alfalfa	34.4 A	96.8 A
red clover	31.3 A	76.3 AB
oat	46.0 A	54.7 BC
oriental mustard[4]	53.6 A	43.3 C
water control	43.4 A	46.6 BC
corn	66.3 A	41.5 C
soybean	48.4 A	40.1 C

[1] mean dry weight after 10 days growth in filtered extract
[2] mean dry weight after 10 days growth in autoclaved extract
[3] means followed by different letters are significantly different by Duncan's multiple range test (P=0.05)
[4] Brassica juncea

Table 15. Effect of filter- or autoclave-purified water extracts from fresh crop residues on growth of Streptomyces after 21 days as determined by orthogonal comparisons

	Treatment Codes[1] and Totals[2] (mg)																	
C[3]	fal 454	frc 409	fco 249	fry 376	fmu 379	fsw 481	foa 413	fso 410	aal 451	arc 318	aco 209	ary 405	amu 408	asw 481	aoa 459	aso 398	ss(Q)	F
1	1	1	1	1	1	1	1	1	-1	-1	-1	-1	-1	-1	-1	-1	46	0.68
2	1	1	1	1	1	-7	1	1	0	0	0	0	0	0	0	0	2720	4.04
3	0	0	0	0	0	0	0	0	1	1	1	1	1	-7	1	1	3153	4.68*
4	3	3	-4	-4	3	0	-4	3	0	0	0	0	0	0	0	0	2562	3.80
5	0	0	0	0	0	0	0	0	3	3	-4	-4	3	0	-4	3	683	1.01
6	1	1	0	0	-3	0	0	1	0	0	0	0	0	0	0	0	509	0.75
7	0	0	0	0	0	0	0	0	1	1	0	0	-3	0	0	1	116	0.17
8	1	1	0	0	0	0	0	-2	0	0	0	0	0	0	0	0	105	0.16
9	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	-2	58	0.09
10	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	339	0.50
11	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	3183	4.72*
12	0	0	2	-1	0	0	-1	0	0	0	0	0	0	0	0	0	4682	6.94*
13	0	0	0	0	0	0	0	0	0	0	2	-1	0	0	-1	0	11096	16.46**
14	0	0	0	1	0	0	-1	0	0	0	0	0	0	0	0	0	222	0.33
15	0	0	0	0	0	0	0	0	0	0	0	1	0	0	-1	0	495	0.73

* significant at 5% level ** significant at 1% level

[1] treatment codes: fal=filtered alfalfa; frc=filtered red clover; fco=filtered corn; fry=filtered rye; fmu=filtered oriental mustard; fsw=filtered sugar water; foa=filtered oat; fso=filtered soybean; aal=autoclaved alfalfa; arc=autoclaved red clover; aco=autoclaved corn; ary=autoclaved rye; amu=autoclaved oriental mustard; asw=autoclaved sugar water; aoa=autoclaved oat; aso=autoclaved soybean

[2] total dry weight of Streptomyces per treatment

[3] Contrasts:

1 filter sterilized vs. autoclave sterilized
2 water control vs. extracts, filtered
3 water control vs. extracts, autoclaved
4 monocots vs dicots, filtered
5 monocots vs dicots, autoclaved
6 mustard vs legumes, filtered
7 mustard vs. legumes, autoclaved
8 soybean vs forage legumes, filtered
9 soybean vs. forage legumes, autoclaved
10 red clover vs. alfalfa, filtered
11 red clover vs. alfalfa, autoclaved
12 corn vs. other grasses, filtered
13 corn vs. other grasses, autoclaved
14 rye vs. oat, filtered
15 rye vs. oat, autoclaved

Table 16. Effect of filter- or autoclave-purified water extracts from fresh crop residues on growth of Streptomyces after 21 days

Extract	Mean Weight[1](mg)	Mean Weight[2](mg)
water control	160.2 A[3]	159.0 A
alfalfa	151.3 A	150.2 A
oat	137.5 AB	153.1 A
soybean	136.5 AB	132.5 AB
red clover	136.3 AB	104.1 BC
oriental mustard[4]	126.3 AB	136.1 AB
rye	125.3 AB	134.9 AB
corn	83.0 B	69.5 C

[1] mean dry weight after 21 days growth in filtered extract
[2] mean dry weight after 21 days growth in autoclaved extract
[3] means followed by different letters are significantly different by Duncan's multiple range test (P=0.05)
[4] Brassica juncea

Root infection of green manure crops:

The Streptomyces isolates tested differed in their ability to infect the roots of green manure crops (Table 17). Infection was indicated by reduced root weight, reduced root mass (particularly reduction in the number of fibrous roots), and brown discoloration of roots. Pathogenic isolate 87PA32 produced significant decreases in root weight for all of the plants tested, except for oat, which showed no significant reduction in root weight with any of the Streptomyces isolates. Streptomyces with cultural characteristics similar to those of the original isolate were re-isolated from brown areas on several of these roots, suggesting that infection had occurred, and Streptomyces with coiled spore chains were visible on the root surface with SEM. The other potato pathogen (87CS11) also induced decreased root mass for the plants, except oat and soybean. The carrot pathogen (88CCG2) only

produced significant reduction in root weights with corn and rye. The nonpathogenic isolate 89MSEP2 affected alfalfa, corn, both cucumber varieties, and red clover. Two other nonpathogenic isolates (88FRCC and 89MSEP1) reduced root weight only of red clover.

When the mean reduction in root weight of the different crops with the non-pathogenic isolates was compared to that for the pathogens, only one cucumber variety and red clover showed significant reduction in root weight with the non-pathogens while only oat and soybean showed no significant response to the potato pathogens.

No significant correlation was found between scab ratings in the field and percent weight decrease in roots of green manure crops used in field treatments.

Inhibition of S. scabies by antagonists from soil:

Dilutions from soil from both red clover and mustard plots yielded visible plaques in lawns of pathogenic isolate 87PA32 at a dilution of 10^{-5}. At higher soil concentrations the plates were too overgrown with fungi and bacterial colonies to observe plaques. The number of plaques was greater from the mustard plots than from the red clover plots (Table 18). Only isolates from the mustard soil plaques showed antagonistic activity toward the pathogenic isolates tested in the bioassays.

Soil samples from mustard collected on 6/16/1989 yielded 4 plaques at 10^{-5} dilution. Two of the isolates from colonies in the centers of these plaques showed antagonistic activity in bioassays (Fig. 6). Both isolates had filamentous growth characteristic of actinomycetes. These isolates were designated 89MSEP1, which inhibited the potato pathogens 87PA32 and 840103 in both streak and agar disk tests; and 89MSEP2, which

Table 17. Fresh weight of roots after 18 days growth with different Streptomyces isolates

Culture	Average Fresh Weight of Roots[1]								
	Alfalfa	Corn	Cucumber1[2]	Cucumber2[3]	Mustard	Oat	Red Clover	Rye	Soybean
control[4]	5.72	412.1	169.0	202.9	6.05	68.6	4.66	75.1	207.2
non-pathogens (np)									
88FRCC	5.64	379.2	166.4	181.4	6.58	67.4	3.60*	70.5	191.6
89FRPB	5.90	407.5	161.2	200.2	6.55	70.0	4.70	69.3	206.7
89MSEP1	5.49	356.8	156.7	183.4	6.18	61.3	3.74*	66.8	184.9
89MSEP2	4.65*	268.1**	112.8**	124.8**	5.83	62.3	2.55**	68.0	175.5
np mean	5.42	352.9	149.3	172.5**	6.29	65.3	3.65*	68.7	189.7
carrot pathogen									
88CCG2	5.82	322.2*	165.1	192.6	6.19	62.9	3.79	51.7**	183.1
potato pathogens (pp)									
87CS11	4.10**	324.3*	102.1**	133.9**	3.52**	56.4	2.63**	53.1**	171.1
87PA32	3.44**	298.5**	68.2**	81.8**	3.52**	55.4	1.90**	54.3**	146.4*
pp mean	3.77**	314.9**	85.2**	107.9**	3.52**	55.9	2.39**	53.7**	158.8

[1] average weight in mg from 10 plants per treatment
[2] National Pickling cucumber
[3] Wisconsin SMR 58 cucumber
* significantly lower than control for same crop by LSD (P=0.05)
** significantly lower than control for same crop by LSD (P=0.01)

inhibited all of the isolates tested in streak tests, and 87PA32 and 840103 in agar disk tests.

Soil samples from mustard collected on 7/14/1989 yielded 2 plaques at 10^{-5} dilution, from which one organism was isolated which inhibited isolates 87PA32 and 840103 in both streak and agar disk tests. This isolate was a gram positive, rod shaped bacterium which produced endospores, characteristic of Bacillus.

All three of these antagonists grew in CSN and soil extract, and the Bacillus grew in PDB. None of the culture filtrates inhibited 87PA32 at initial strength, but the 10X concentration of the CSN culture filtrate from isolate 89MSEP1 produced clear zones of inhibition around wells containing filtrates after 6 days growth (Fig. 7). Zones of reduced or delayed sporulation were seen with culture filtrates from 89MSEP2 after 6 days growth, but no clear zones of inhibition were produced with this isolate. No zones of inhibition were seen for any culture filtrates from Bacillus. Bioassays with the original isolates continued to show zones of inhibition for all three antagonists.

After chloroform extraction of aqueous filtrates, no zones of inhibition were seen for any of the isolates around either the concentrated water or the chloroform fractions.

The three nonpathogenic isolates from scab lesions tested included 88FRCC, 89FRPP and 89FRPB. Isolate 88FRCC inhibited all five of the pathogenic isolates in both plate tests, but culture filtrates from 88FRCC did not show any inhibition of the pathogenic isolate. Neither 89FRPP nor 89FRPB showed antagonistic activity in these bioassays.

Table 18. Number of antagonists from soil amendment plots.

Date	Mean Number of Plaques[1]	
	Red Clover	Mustard
6/16	1[2]	4[3]
6/30	0	0
7/14	1[2]	2[4]
7/28	0	0
8/11	0	0
8/25	0	0

[1] on 10^{-5} soil dilution plates
[2] did not show antagonism when isolated
[3] 2 isolates showed antagonism to pathogenic Streptomyces when isolated
[4] 1 isolate showed antagonism to pathogenic Streptomyces when isolated

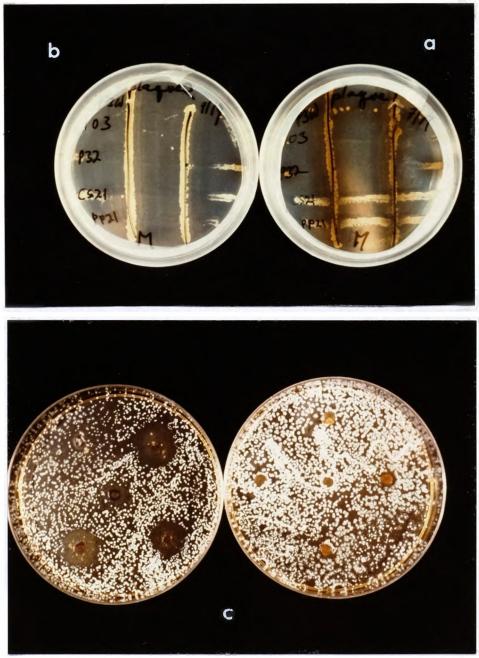

Figure 6. Antagonist bioassay plates
a. 89MSEP1 streak plate
b. 89MSEP2 streak plate
c. agar disk assay

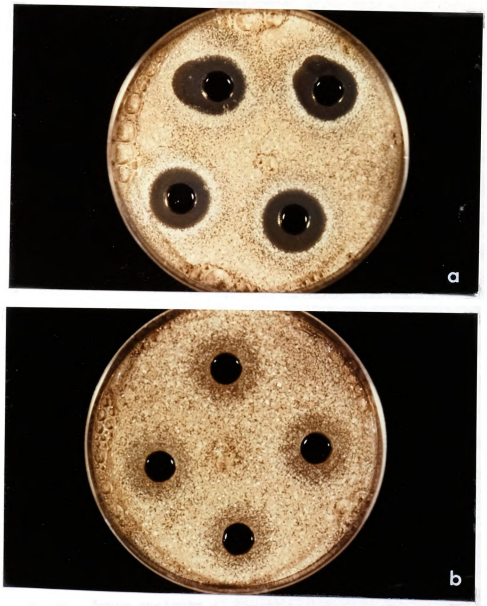

Figure 7. Antibiotic bioassay plates
a. positive
b. reduced sporulation

DISCUSSION

The high levels of contamination and lack of Streptomyces growth with ABWA in the present research suggested that this medium is not useful in isolation of Streptomyces from plant material in Michigan. Better results were obtained with unamended WA.

Most of the Streptomyces isolates obtained corresponded to the description of S. scabies given by Lambert and Loria (71) (i.e. smooth grey spores borne in spiral chains, melanin production, and using all International Streptomyces Project sugars); however, some differences were found in individual isolates. While all of the pathogenic isolates used all of the specified sugars and had coiled spore chains, other characters varied. Isolate 840103 had white aerial mycelium rather than grey, but this might be an artifact due to maintenance of this isolate in culture. Isolate 87PS21 differed from the description of S. scabies in that it did not produce melanin on PYI. The production of melanin may be a plasmid-borne trait and can be lost at a fairly high frequency in culture (39, 40), thus this difference may not be significant enough to separate this pathogenic isolate from S. scabies.

Three isolates differed from S. scabies in the spore surface ornamentation. Spore surfaces of Streptomyces have been classified into four groups: smooth, warty, spiny, and hairy (24, 148), and a fifth category of rugose (with a wrinkled surface, rather than distinct protrusions) has been proposed (24). Isolates 87CS11 and 87PS21 both had smooth spores when cultures were young, but spores became rough (either warty or rugose) as the cultures aged (between 14 and 28 days). Isolate 88CCG2 (carrot isolate) also differed from S. scabies in that it

had echinulate (spiny) spores. Spore surface ornamentation is considered to be a fairly stable morphological characteristic and has been recommended for classification of species within the gray to brown color groups (148). There have been previous reports of pathogenic Streptomyces spp. with echinulate spores (89), and a number of other species have been reported to cause scab. Until more precise species characteristics are defined in this difficult genus, it is probably best to be cautious about reclassification.

Atlantic potatoes are reported to have medium resistance to scab in Michigan (13). However, high levels of disease were found on this cultivar in the current study, indicating that the resistance level is not high.

Corn (a major crop in the midwest) has been recommended as a rotation crop for potato in scab infested soil (19) but high scab levels have been seen in fields where corn was planted (R. Hammerschmidt, personal communication) and rotations with corn have been correlated with high scab severity (35). This is consistent with my results, in which green manures of sweet corn gave increased scab ratings (compared to fallow control) in both field and greenhouse tests. These results suggest that use of sweet corn as a rotation crop for potato, as recommended by Davidson and Byther (19), may be counter-productive.

Red clover and alfalfa were used in my study because legumes are common cover crops. Green manures of red clover increased scab severity in the current study, while alfalfa had no significant effect on scab severity or marketable yield. These results suggest that alfalfa might be a better leguminous cover crop than red clover.

Rye was used because it has been frequently recommended as a cover crop (125), and oat was included as a small grain alternative to rye, since small grains are frequently used as cover crops. Neither of these crops had any significant effect on scab levels in the current research, suggesting that these crops might also be better cover crops than red clover.

Green manures of soybean (a widespread crop in the midwest) showed no significant effect on scab severity in either greenhouse or field experiments. These results were consistent with the work of Weinhold et al. (154), who gave evidence that soybean prevented disease build-up but did not decrease levels already present. However, the results were contrary to those of Rouatt and Atkinson (130), who found decreased scab with soybean green manures. This may have been due to differences in the types of soil, soil micro-flora, or pathogenic isolates in the different areas where research was conducted.

Mustard was included in my research because green manures of mustard suppress several root diseases (85, 124), and mustard was one of the crops reported to be effective in controlling scab by Millard (108). Since Millard used mustard followed by rye, it seemed appropriate to test these crops separately. Green manures of mustard produced the lowest scab rating in all of these experiments without showing any decrease in marketable yield. These results suggest that further studies should be done with mustard, using different varieties, different rates of application, and different decomposition periods to determine the most effective control with this crop. Since a number of other crucifers suppress various fungal diseases (85, 124), related

crops, such as canola or oilseed rape, might be desirable rotation or cover crops to aid in scab control.

It may be beneficial to investigate the effect of mustard and other green manures in combination with other control methods, since the effect of certain control methods may be influenced by other factors. For example: the combination of sulfur with a green manure of rye increased scab suppression (159); the addition of a green manure of grass cuttings counteracted the increased scab severity seen with liming (108); and the effect of the preceding crop in crop rotations has been reported to be significant in irrigated fields (36), but not in dry-land crop rotations (156). This suggests that interaction between control measures might affect the level of control achieved with green manures.

It would also be important to test the effects of these green manure treatments on other potato diseases before recommending widespread use of green manures in scab control. Long term rotations, particularly with alfalfa, have been recommended for control of Rhizoctonia and Fusarium wilt on potato as well as for control of scab (36). This, and the suggestion that rotations with corn increased severity of Fusarium wilt, suggest that effects of crop residues might be similar for these three diseases. However, green manures of rye, clover, and soybean have been reported to increase the amount of Fusarium in soil (130), so these treatments could increase dry rots and wilts caused by this fungus, and thus might not be desirable for use in soils where Fusarium was a problem.

Several possible mechanisms for the effects of green manures on scab severity were tested. The first was the possibility that soil pH

was reduced by green manures to a level inhibitory to scab. In my experiment, no reduction in soil pH occurred. An initial small increase in pH was observed with no subsequent decrease, indicating that reduction in soil pH probably is not involved in green manure activity.

Growth of Streptomyces differed in the various aqueous cover crop extracts, but no consistent correlation was seen with disease severity. This indicated that the green manures probably did not affect disease severity by production of water soluble toxins where scab was suppressed.

The weak negative correlation between Streptomyces growth in the autoclave sterilized extract from fresh plant material and scab ratings from 1989 suggest that autoclaving may cause alterations in extracts, thus this correlation may be an artifact due to heating.

The differences in susceptibility of the root crops to individual isolates of S. scabies might explain the differences in disease levels seen in the field, and the ability to grow some root crops in soils in which others are inhibited by scab. This variation in the host range of pathogenic isolates is an important consideration in selection of possible rotation crops. Previous researchers found that crop rotations involving an alternate scab host (sugar beet) produced the highest scab levels on potatoes of any of 14 rotations tested (36). These results indicated that caution should be used in planting alternate scab hosts in rotation with potato. Further work on the host range of Streptomyces would be valuable to determine crops to avoid in rotations.

Hooker (50) reported that several of the crops used as green manures in this study were susceptible to pathogenic Streptomyces in sterile conditions. Green manure crops were tested for susceptibility to determine if the ability of pathogenic isolates to infect cover crop roots might have a role in scab severity with the crops. No significant correlation was found between susceptibility to the pathogens (indicated by reduced root weight) and scab ratings from the corresponding green manure treatments. However, red clover was more susceptible to the potato pathogens than was alfalfa, which might have some role in differences in disease levels produced with these two crops. The susceptibility of mustard is not surprising since several other crucifers (cabbage, turnip, rutabaga and radish) are susceptible to scab infection.

Hooker (50) reported that, although pathogenic Streptomyces caused symptoms on a large number of crops, nonpathogenic Streptomyces did not adversely affect the roots of these crops in vitro. In contrast to Hooker's results, in my research several nonpathogenic isolates produced reduction in root weight and mass.

Cucumber was included in this study as a negative control since Hooker (50) reported cucurbits were highly tolerant to pathogenic Streptomyces in vitro. My results did not confirm this report, since cucumber showed extensive root damage with both the potato pathogens and the nonpathogenic isolate 89MSEP2. This root damage occurred both on cucumber cultivar Wisconsin SMR 58, and on the cultivar used by Hooker (National Pickling), indicating that the difference in results is probably not due to differences in cultivar susceptibility. Although

these results conflict with Hooker's results in soil extract agar, they are consistent with the stunting and premature death of cucumber seen in soil infested with virulent Streptomyces isolates (54).

These results confirm the need for further work on the host range of S. scabies isolates suggested by the results from infection of root crops. The Michigan isolates from potato were pathogenic on cucumber (on which Hooker's isolates were non-pathogenic) and were non-pathogenic on oat (which Hooker reported was as susceptible as rye to S. scabies) while the carrot isolate had a much narrower range of effect. This suggests that S. scabies from different sources might vary in host range, which might have a role in the differences seen in the effects of green manures in the various areas.

It would be useful to investigate whether the susceptibility of the cover crops in field conditions is similar to that seen in vitro. Hooker (50) reported similar reactions of roots to Streptomyces in sterile soil, sterile sand, and soil extract agar, suggesting that infection in soil conditions is possible. However, root infection in natural soil conditions might vary.

The interaction between the various Streptomyces isolates in association with crop roots should also be investigated. Abraham and Herr (1) reported greater populations of actinomycetes in corn rhizospheres than in soybean rhizospheres and found differences in physiological characteristics of populations with the different crops. No tests were done on the pathogenicity of the actinomyces in these populations, but it is possible that the cover crops might differ in their effect on the relative populations of both pathogenic and non-

pathogenic Streptomyces in the soil, and thus might affect disease levels.

Increases in actinomycete populations following incorporation of crop residues into soil has been reported previously (111, 120, 128), and stimulation of S. scabies populations specifically by green manure incorporation has been reported in sterile soil (111). This is consistent with the increase in numbers of Streptomyces was seen with all of my green manure treatments. The lack of correlation between this increase and disease severity suggested that differences in stimulation of pathogen growth by the green manure crops did not play a role in scab production.

Slightly higher numbers of microbial antagonists were found in field plots in which scab was suppressed than in those in which scab was enhanced. The numbers of antagonists were somewhat low compared to levels detected by Weinhold and Bowman (153), and were small enough that differences may not be significant. The low number of isolates found might be due to the fact that I used SEA (as a nutrient regime similar to what would be in the soil) for screening for antagonists, while other researchers used richer media (87, 102, 153). The use of only one pathogenic isolate of S. scabies in the initial screening for antagonists might also have limited the detection of antagonists, since pathogenic isolates varied in their susceptibility to the antagonists. In the plate tests, all of the pathogens were inhibited by isolate 89MSEP2 while only two of the five pathogens were inhibited by either of the other antagonists. Similar variation in susceptibility of pathogenic Streptomyces other to Streptomyces has been reported by

McQueen et al. (102). These results suggest that it would be useful to screen soil for antagonists using a wider range of pathogenic isolates.

Previous researchers have reported a number of fungi, bacteria, and actinomycetes antagonist to S. scabies (17, 69, 87, 102, 111, 117, 121, 153) and actinomycetes which produce antibiotics show potential as control agents for a number of soil diseases (review in 6, 120, 129, 145). Thus the higher number and activity of antagonistic organisms found in mustard plots than in red clover plots indicated that stimulation of antagonists might play a major role in the suppression of scab by green manures.

Antagonistic micro-organisms, such as the ones found in the present study, might be useful as biological control agents for common scab. However, the use of antagonists as biocontrol agents may be limited because pathogenic isolates differ in susceptibility to antagonists (102, current study). Since antibiotics from antagonists such as Bacillus and some actinomycetes can inhibit growth of several actinomycetes other than S. scabies (30, 67, 87, 121, 153), antagonists used to inoculate soil or seed material might inhibit one another, as well as natural antagonists in the soil, thus reducing the effectiveness of the biocontrol.

The lack of evidence for production of water-soluble antibiotics for two of the three antagonists was unexpected. Weinhold and Bowman (153) reported that PDB supported good antibiotic production with Bacillus subtilis, so the lack of antibiotic production with the Bacillus was probably not due to poor substrate. It is possible that antibiotic production was lost during growth in culture, but plates

inoculated with bacteria from culture plates still showed zones of inhibition, so this is unlikely. It seems more likely that the level of antibiotics in the culture filtrates was still too low for detection with the bioassay used. With the antagonistic Streptomyces spp. it is possible that the media used for antibiotic production were not rich enough or that the carbon source was not appropriate, since carbon sources can influence Streptomyces antibiotic production in vitro (157). While sucrose is a fairly good substrate for antibiotic production from many actinomycetes, and both antagonistic Streptomyces grew on solid CSN and with sucrose as a sole carbon source in carbon utilization studies, glucose is generally the best carbon source for antibiotic production (157). It might be that with different culture media, antibiotics would be found for the other antagonists.

There are a number of other possible mechanisms that might be involved in the effect of green manures on scab severity which should be investigated. Another possible mechanism for the activity of green manures in controlling scab by antibiotics is that crop residues might differ in their suitability as substrates for antibiotic production by soil micro-organisms. Green manure extracts influence the level of antibiotic production by Bacillus in vitro (153) and recovery of antibiotics from sterile soil differs for some actinomycetes when supplied with different organic substrates, including grass, clover, and soybean meal (145). It may be beneficial to test various green manure crops for their suitability as substrates for antibiotic production to determine if there is any correlation between antibiotic production on green manures and scab severity.

The present study did not test for the production of volatile compounds inhibitory to scab during decomposition of green manures. During decomposition, mustard and other crucifers produce volatile sulfur compounds, which are inhibitory some soil-borne plant pathogens (85, 124). Streptomyces are suppressed by some sulfur compounds, so the production of volatile compounds may have a role in suppression of scab by mustard green manures. Ramirez-Villapudua and Munnecke (124) reported that volatile compounds from cabbage residues do not change the total number of actinomycetes in soil, but that there are changes in the types of actinomycetes found. These results indicate a need to investigate the effect of such volatile compounds on scab.

Other plant residues also produce volatile compounds that affect the growth of bacteria and fungi in soil (106). Such compounds might produce differential levels of stimulation for pathogenic Streptomyces or antagonistic organisms. Such stimulation could be involved in the effect of green manures either by encouraging the growth of antagonistic micro-organisms, resulting in scab suppression, or by causing premature germination of the pathogen in the absence of a suitable host.

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