Biological Control of Potato Scab in the Field with Antagonistic *Streptomyces scabies*

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ABSTRACT

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Control of potato scab by two suppressive strains of *Streptomyces* (*S. diastatochromogenes* strain PonSSII and *S. scabies* strain PonR) was demonstrated in a 4-year field-pot experiment at Becker, MN, from 1988 to 1992. The suppressive strains were grown on a vermiculite-oatmeal broth base and mixed with scab-conducive soil at 1, 5, and 10% (vol/vol). Corresponding treatments with 1, 5, and 10% carrier only and nonamended controls were arranged to investigate dilution and/or nutrient effects on scab control. Both strains significantly decreased scab on potato tubers of cv. Norchip compared with the nonamended control treatment. Both suppressive strains at the 1% level were not significantly different in scab control from those at the 5 and 10% levels after the first year. There were no consistent differences in the level of disease control provided by strains PonR and PonSSII. For strain PonSSII, percent disease control after inoculation at the 1% level increased every year, whereas disease control with the 10% inoculation was constant. Effects of vermiculite dilution and/or oatmeal broth nutrients at the 10% level on scab reduction were significant during the first 2 years but were not significant during the last 2 years. The suppressive strains did not affect tuber yield and were reisolated from the tubers grown in inoculated soil in all 4 years of the experiment.

Additional keywords: suppressive soil.

Common scab of potato (*Solanum tuberosum* L.) is caused by *Streptomyces scabies* (5,10,13,25,27) and other species of *Streptomyces* (1,3,4,6,7,14,21). Root crops such as beet, carrot, radish, rutabaga, parsnip, and turnip also are susceptible to scab disease (10).

Several methods have been used to control potato scab, including excess irrigation during tuber formation (16); application of green manures (23,28); crop rotation (10); organic amendments (12); chemical control (10,22); soil fumigation (10); low soil pH (10,15,22); use of a biofertilizer containing *S. albidoflavus* strain CH-33 that produces an antibiotic lethal to *S. scabies* (8,9); and potato seed tuber bacterization (24). Genetic resistance to potato scab when incorporated into a potato cultivar is stable over time.

A phenomenon associated with potato scab is the natural decline of the disease in soils repeatedly grown to potatoes (11). Menzies (20) demonstrated that a biological factor was responsible for scab decline in a suppressive soil in eastern Washington. In Minnesota, scab decline was first observed in a plot used to screen potato germ plasm for scab resistance after 23 years of potato monoculture (19). After growing the scab-susceptible cultivars Pontiac, Cobbler, and Russet Burbank in the suppressive plot for 3 years (1985 to 1987) and failing to generate scab disease, attempts were made to find a biological factor responsible for the scab suppression, analogous to that described by Menzies (20). From the lenticels of scab-free tubers (cv. Pontiac), three strains of *Streptomyces* were isolated. These three strains produced antibiotics lethal to pathogenic strains of *S. scabies* in antibiosis tests (18). Only one of the three strains was used in this study because the sizes of inhibition zones produced by the strains against pathogens were similar based on antibiosis tests (18), and methodological constraints made it impossible to investigate all strains at the same time.

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A second strain of *Streptomyces* that produced antibiotics against pathogenic strains of *S. scabies* and that was obtained from the lenticel of a Pontiac tuber grown in a scab-screening nursery at Becker, MN, also was chosen for study. The scab nursery had been planted with potatoes for 13 years at the time this strain was isolated, and a slight scab decline had been noted.

These two suppressive strains were identified as *S. diastatochromogenes* PonSSII and *S. scabies* PonR, respectively (17). The purpose of this study was to evaluate the potential of these two antibiotic-producing suppressive strains of *Streptomyces* to control potato scab disease in field-pot tests.

MATERIALS AND METHODS

The potential of strains PonR and PonSSII to biologically control potato scab disease was evaluated in a 4-year field-pot test at the University of Minnesota Sand Plains Experiment Station, Becker. Soil at the Becker site is a dark, coarse sand with a pH of 5.5 to 6.4. The soil is classified in the Hubbard series and is low in nitrogen, potassium, and organic matter.

Streptomyces strains were stored as spore suspensions in 20% glycerol at –20°C. For preparation of field inoculum, 10 μl of spore suspensions of a strain was diluted in 1 ml of sterile water. The resulting spore suspensions were inoculated onto oatmeal agar (0.01 ml per plate) and incubated for 10 days. Fresh spore suspensions were prepared by pouring 15 ml of sterile water onto the surface of a petri dish and gently scraping the culture with a sterile wire loop. The resulting suspension was taken up with a sterile syringe and used to inoculate vermiculite as described below.

Field inoculum was prepared by growing each suppressive strain individually for 4 weeks in metal trays containing 3,500 cm^3 of vermiculite and 900 ml of oatmeal broth. Oatmeal broth was prepared by autoclaving 20 g of oatmeal and 1 g of casamino acids per liter of deionized water for 20 min. The vermiculite-oatmeal mix was sealed in the trays using a double layer of aluminum foil. The sealed trays were autoclaved for 90 min on each of two successive days at a 24-h interval. Trays then were inoculated with 10 ml of the fresh spore suspensions from each of two petri dishes, using a syringe with a sharp needle. The outer layer of aluminum foil was briefly opened inside a laminar flow hood, and the inoculum was injected into the vermiculite tray by piercing the inner foil layer with the sharp needle. Trays were resealed immediately with the outer layer after inoculation. The trays were incubated at room temperature and shaken weekly to distribute inoculum. PonR and PonSSII had 8 and 6×10^7 CFU per cm^3, respectively.

Soil treatments were prepared by adding inoculum of each strain to scab-conducive soil obtained from a current potato scab test plot at Becker. The inoculum was added to conducive soil and mixed for 4 min in a cement mixer at concentrations of 1, 5, and 10% (vol/vol). The four control treatments consisted of nonamended conducive soil and conducive soil to which only the sterile vermiculite-oatmeal broth blend was added at 1, 5, and 10% (vol/vol). The soil then was placed into 16-liter plastic pots that had the bottoms removed. These pots were set in rows into soil in which potatoes had never been grown. One plant (cv. Norchip certified B-size seed tubers having no visual scab symptoms) was grown in each pot. Additionally, a plant was grown between each pair of pots, giving a within-row plant spacing of 30.5 cm. The distance between rows was 91.4 cm. Pots with inoculated soil remained in place throughout the 4-year experiment. The experimental design was a randomized complete block, one pot per replicate, with 18 replicates per treatment.

At harvest, tubers from each pot were carefully removed using hand trowels and placed in individual bags. Care was taken to minimize soil loss from individual pots. Tubers were later washed and scored for total number of lesions of types 3, 4, and 5 (0 = no scab, 1 = very small superficial lesions, 2 = small superficial lesions, 3 = periderm broken, 4 = pit, and 5 = deep pit) per tuber. Lesions of types 3 to 5 are considered economically important. The two suppressive strains were reisolated each year from progeny tubers and identified based on spore color, spore chain morphology, growth patterns, and pigment production.

Analysis of variance was performed using the statistical program STATISTIX (Analytical Software, St. Paul, MN). Duncan's multiple range test was used to compare treatment means of average lesion numbers of types 3 to 5 per tuber within each year.

TABLE 1. Biocontrol of potato scab by suppressive strains of *Streptomyces* in field-pot tests at Becker, MN, 1989 to 1992

	Lesion number[z]			
Treatment[y]	1989	1990	1991	1992
Nonamended control	7.83 a	28.16 a	10.31 a	8.78 a
1% control	8.14 a	24.94 ab	8.74 ab	8.30 a
1% PonR	4.75 b	12.11 d	5.69 bc	2.22 b
1% PonSSII	4.80 b	11.04 de	2.81 c	1.71 b
5% control	6.41 ab	19.81 bc	10.77 a	7.99 a
5% PonR	1.81 de	11.99 d	4.88 c	2.06 b
5% PonSSII	2.79 cd	7.60 d–f	2.98 c	1.26 b
10% control	4.45 bc	13.84 cd	9.92 a	8.12 a
10% PonR	1.32 de	7.24 d–f	4.52 c	2.53 b
10% PonSSII	1.39 de	4.22 ef	2.46 c	1.16 b
Between pots	0.03 e	0.51 f	2.25 c	1.40 b

[y] 1, 5, and 10% represent the ratio of vermiculite-oatmeal broth inoculum; scab-conducive soil (vol/vol). PonR is a strain of *S. scabies*; PonSSII is a strain of *S. diastatochromogenes*.

[z] Average number of type 3, 4, and 5 scab lesions per tuber from 18 replicates (plants). Lesion numbers of treatments followed by the same letters are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

RESULTS

In 1989, all treatments with the two suppressive strains significantly ($P = 0.05$) reduced the number of scab lesions per tuber compared to the nonamended conducive-soil control and the corresponding 1, 5, and 10% controls (Table 1). However, there were differences in the amount of disease control among the suppressive-strain treatments. Both the 5 and 10% PonR and PonSSII treatments resulted in significantly greater reductions in the number of scab lesions per tuber compared to the 1% inoculum treatment ($P = 0.05$). Differences in the number of scab lesions per tuber between the 1 and 10% control treatments also were significant. The number of scab lesions in the 1 and 5% control treatments and in the nonamended conducive soil did not differ significantly ($P = 0.05$). Tubers from plants grown between the pots in soil in which potatoes had never been grown had the smallest number of scab lesions of any treatment.

In 1990, all PonSSII treatments plus the 1 and 5% PonR treatments significantly ($P = 0.05$) reduced the number of scab lesions compared to the nonamended conducive-soil control and the corresponding controls (Table 1). There was no significant difference in the number of scab lesions per tuber among the levels of either suppressive-strain treatment ($P = 0.05$). Strain PonSSII was not different from PonR at all three inoculum concentrations ($P = 0.05$). The effect of incorporation of sterile vermiculite-oatmeal broth material in reducing the number of scab lesions in the 5 and 10% control treatments compared to the nonamended control was significant ($P = 0.05$). Again, tubers from plants grown between the pots had the smallest number of scab lesions.

Results from the 1991 study indicated that all suppressive strain treatments (except the 1% PonR treatment) produced significant ($P = 0.05$) reductions in the number of scab lesions per tuber compared to the nonamended conducive soil and the corresponding controls (Table 1; Fig. 1). As in 1990, there were no significant differences in the number of scab lesions among the three inoculum doses of either suppressive strain. However, 1991 was the first year of the test in which all four control treatments did not show significant differences in scab incidence ($P = 0.05$). Again, PonSSII did not provide statistically better scab control than strain PonR ($P = 0.05$). The result of growing potatoes between the pots for three consecutive years was 2.25 lesions per

Fig. 1. Biological control of potato scab using *Streptomyces diastatochromogenes* suppressive strain PonSSII in a field-pot experiment at Becker, MN, in 1991. Tubers (left) were harvested from a pot treated with suppressive strain PonSSII at the 1% level of inoculation; tubers (right) were harvested from a pot containing nonamended control (check).

tuber, which was significantly lower than the four control treatments but not significantly different than any of the suppressive-strain treatments ($P = 0.05$).

Results from the 1992 tests indicated that all suppressive-strain treatments significantly ($P = 0.05$) reduced the number of scab lesions per tuber compared to the four control treatments (Table 1). As in 1991, none of the three inoculum concentration levels differed significantly ($P = 0.05$) based on the number of scab lesions per tuber for either suppressive strain. Also, as in the previous year, all four control treatments did not differ significantly from one another in scab incidence ($P = 0.05$). At every inoculum dose, strain PonSSII had fewer lesions than strain PonR; however, the differences were not statistically significant ($P = 0.05$). The number of scab lesions on progeny tubers of plants grown between the pots was significantly lower than the four control treatments but not significantly different than in the suppressive-strain treatments.

The average disease reduction over all inoculum doses for the 4-year test was 73% for strain PonSSII and 64% for strain PonR. Higher inoculum levels of the two suppressive strains resulted in greater scab reduction than did lower levels for the first year (Figs. 2 and 3). The 1% PonSSII treatment reduced scab lesions per tuber by 39, 61, 73, and 80% over the 4 years of the test. It provided scab control similar to the 5 and 10% inoculum levels for the last 3 years (Fig. 2) with significant interaction between years and levels ($P = 0.02$). A similar phenomenon was observed for 1% PonR (Fig. 3). However, the 5 and 10% inoculum levels did not show any clear trend in disease reduction over time, since the interaction between years and levels was not significant ($P = 0.49$).

Disease reduction in the amended control pots compared to the nonamended controls was observed during the first 2 years of the study. This reduction was most notable at the 5 and 10% levels. The difference between the amended (10% level) and the nonamended controls was statistically significant for the first 2 years of the test (Fig. 4). Interaction of years and levels of amended controls was significant ($P = 0.04$).

Tuber yield for each treatment was determined at harvest. In 1989, yields from plants grown in pots in the nonamended conducive soil and from plants grown between the pots did not differ significantly ($P = 0.05$) from any of the treatments with the suppressive strains (data not shown). The relationship between the average number of scab lesions per tuber and tuber yield was not significant, ($r = 0.24$, $P > 0.05$).

In 1990, the 10% PonR treatment significantly enhanced yields compared to the nonamended control and the 1% PonSSII treatment ($P < 0.05$). During the last 2 years of the experiment, no significant yield differences were noted among treatments (data not shown).

The suppressive strains were reisolated from potato tubers grown in pots inoculated with the three levels of inoculum in each of the 4 years of the test. The reisolated strains had similar morphological, physiological, and pairing reactions as the parent strains, *S. diastatochromogenes* strain PonSSII and *S. scabies* strain PonR, respectively.

DISCUSSION

Two nonpathogenic strains of *Streptomyces* were selected and tested for their ability to biologically control potato scab in field-pot tests over a 4-year period. The strains, identified as *S. diastatochromogenes* strain PonSSII and *S. scabies* strain PonR, were selected based on their differential in vitro production of antibiotic(s) against pathogenic strains of *S. scabies*. Both strains provided significant disease control over all 4 years of the experiment. Strain PonSSII had numerically fewer scab lesions than

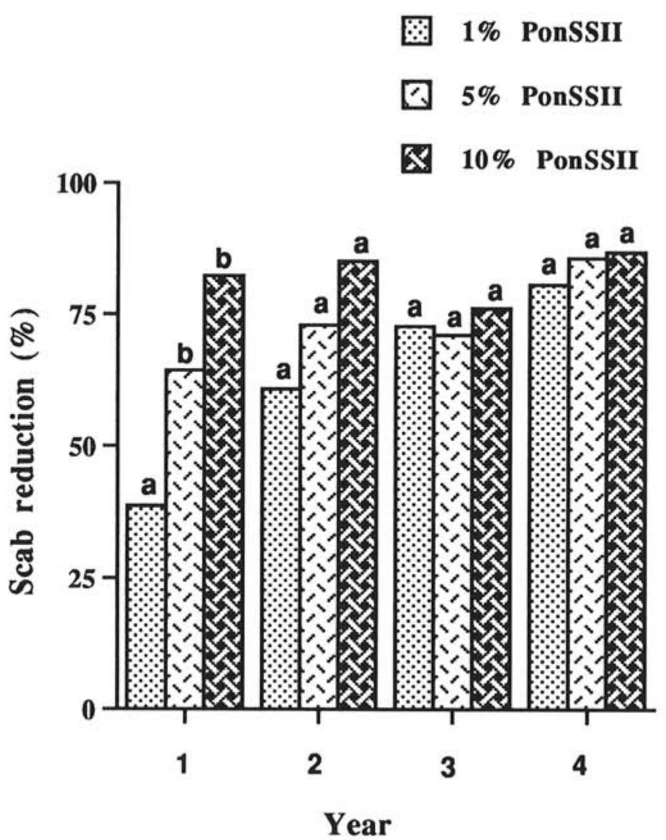

Fig. 2. Effect of *Streptomyces diastatochromogenes* strain PonSSII on scab reduction over a 4-yr period. Scab reduction = (1 − lesion number of a treatment/lesion number of nonamended control) × 100. Columns with the same letters are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

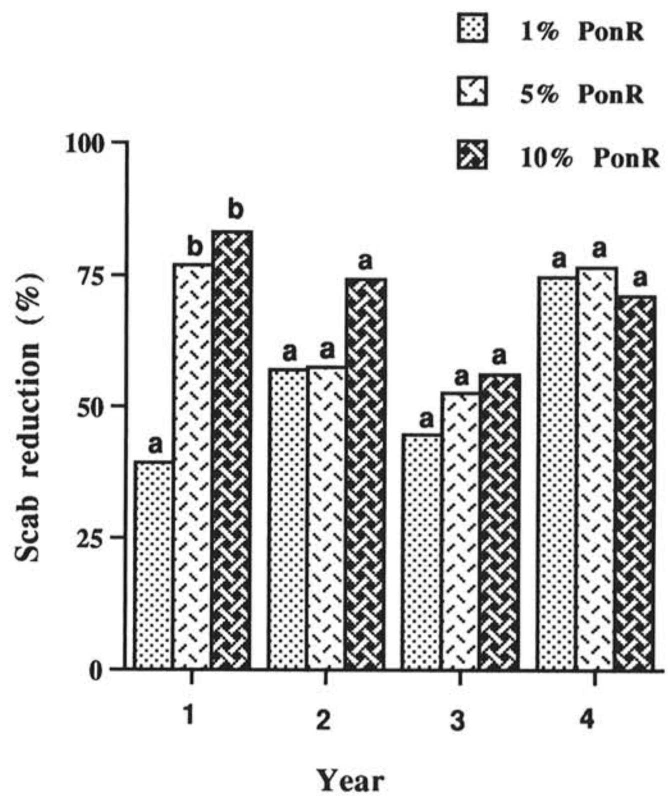

Fig. 3. Effect of *Streptomyces scabies* strain PonR on scab reduction over a 4-yr period. Scab reduction = (1 − lesion number of a treatment/lesion number of nonamended control) × 100. Columns with the same letters are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

strain PonR every year, except 1989, though the differences were not statistically significant. This is correlated with the more vigorous growth and larger inhibition zones of PonSSII against pathogenic strains than PonR in in vitro tests (17).

Two attributes of a successful biological control agent are its ability to survive and increase in soil over time. Both suppressive strains survived and provided significant disease control over the 4 years of this experiment. Although both suppressive strains were reisolated from lenticels of tubers during each year of the test, we do not have quantitative data to indicate they increased in number. The total number of actinomycetes isolated per g of soil in inoculated pots was sometimes greater than in the noninoculated control pots, providing circumstantial evidence for an increase in the suppressive strains over time (data not shown). In large-scale field trials the pathogen populations were reduced in soil inoculated with the suppressive strains (2). Thus, even in cases in which total numbers of *Streptomyces* in inoculated pots were not significantly different from those in noninoculated pots, disease control may reflect increasing populations of the suppressive strains coupled with decreasing pathogen populations over time.

The carrier and nutrient base of a suppressive strain are important to its success as a biocontrol agent. The dilution and/or nutrient effects during the first 2 years of the trial suggest that the vermiculite-oatmeal broth acted either to dilute pathogen inoculum or as a nutrient source for pathogen antagonists in the soil. Soybean as a cover crop and green manure in a potato rotation were reported to prevent an increase in pathogen population by stimulating an increase in populations of *Bacillus subtilis* and saprophytic *Streptomyces* spp. that produced antibiotics against *S. scabies* (28). Thus, the oatmeal broth could have aided the establishment and increase in populations of other microorganisms that helped control scab disease. The dilution and/or nutrient effects were not observed in the last 2 years of the experiment when the amount of scab disease in the 1, 5, and 10% control treatments did not differ significantly from the nonamended conducive soil control (Table 1; Fig. 4). This indicates that the suppressive strains played a more important role in scab control over time than dilution and/or nutrient effects due to application of vermiculite-oatmeal broth.

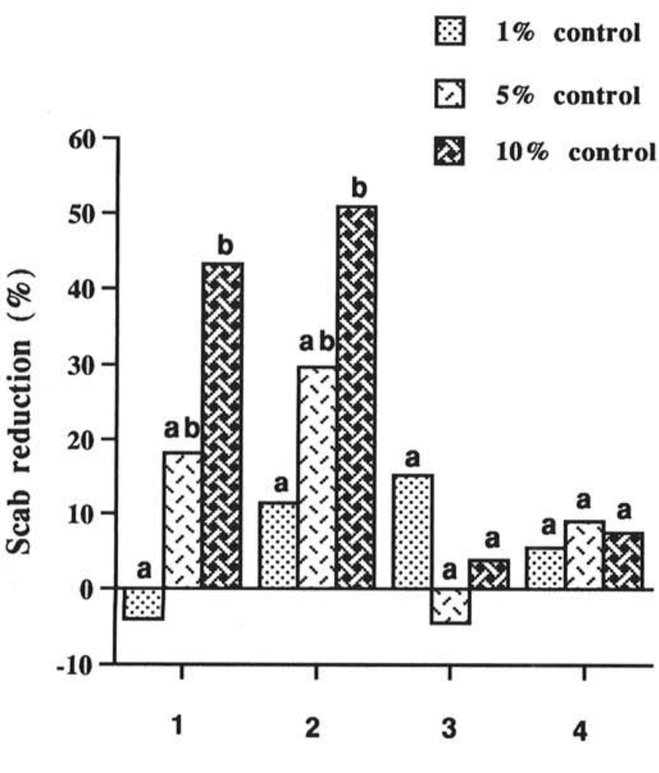

Fig. 4. Effect of vermiculite-oatmeal amendment on scab reduction over a 4-yr period. Scab reduction = (1 − lesion number of a treatment/lesion number of nonamended control) × 100. Columns with the same letters are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Neither suppressive strain at the 1% level showed significant differences in scab control from the treatments at the 5 and 10% levels after the first year. This implies that there was an increase over time of the suppressive strains in the pots inoculated at the 1% level to levels similar to the 5 and 10% inoculations. In potato production fields, the 1% inoculation level is more practical than the 5 and 10% levels. At the end of the 4-year test, inoculation at the 1% level led to disease suppression of 80%. Use of suppressive strains in combination with other control methods, such as crop rotation and resistant cultivars, may improve disease control even further.

The 16-liter plastic pots set into the ground, although different in some respects from a production system, permitted precise quantification of the amount of suppressive strain inoculum that was added to scab-conducive soil. Yield data indicated that the pots did not significantly inhibit tuber yield over the 4-year test. Also, in these tests as in commercial or field tests, common scab did not decrease yield (10). None of the suppressive-soil treatments significantly reduced yield compared to the nonamended conducive-soil control, although increased yields were noted in some cases. This phenomenon is under investigation.

Additional work on the biological control of potato scab is being done to develop the technology needed to deliver the suppressive strains in commercial applications. Studies also are being carried out to select more efficient strains and combinations of strains that are compatible with respect to both antibiotics and rhizosphere colonization. Ideally, a final product will be composed of several complementary suppressive strains to reduce the chances of the development of resistance in the pathogen (26) and to enhance the range of environmental conditions in which biocontrol is successful.

LITERATURE CITED

1. Archuleta, J. G., and Easton, G. D. 1981. The cause of deep-pitted scab of potatoes. Am. Potato J. 58:385-392.
2. Bowers, J. H., Kinkel, L. L., Jones, R. K., and Anderson, N. A. 1993. Development of fatty acid libraries to track *Streptomyces* spp. in soil. (Abstr.) Phytopathology 83:1420.
3. Corbaz, R. 1964. Etude des streptomyces provoquant la gale commune de la pomme de terre. Phytopathol. Z. 51:351-360.
4. Doering-Saad, C., Kämpfer, P., Manulis, S., Kritzman, G., Schneider, J., Zakrzewska-Czerwinska, J., Schrempf, H., and Barash, I. 1992. Diversity among *Streptomyces* strains causing potato scab. Appl. Environ. Microbiol. 58:3932-3940.
5. Elesaway, A. A., and Szabó, I. M. 1979. Isolation and characterization of *Streptomyces scabies* strains from scab lesions of potato tubers. Designation of the neotype strain of *Streptomyces scabies*. Acta Microbiol. Acad. Sci. Hung. 26:311-320.
6. Gordon, R. E., and Horan, A. C. 1968. A piecemeal description of *Streptomyces griseus* (Krainsky) Waksman and Henrici. J. Gen. Microbiol. 50: 223-233.
7. Harrison, M. D. 1962. Potato russet scab, its cause and factors affecting its development. Am. Potato J. 39:368-387.
8. Hayashida, S., Choi, M.-Y., Nanri, N., and Miyaguchi, M. 1988. Production of potato common scab-antagonistic biofertilizer from swine feces with *Streptomyces albidoflavus*. Agric. Biol. Chem. 52:2397-2402.
9. Hayashida, S., Choi, M.-Y., Nanri, N., Yokoyama, M., and Uematsu, T. 1989. Control of potato common scab with an antibiotic fertilizer produced from swine feces containing *Streptomyces*. Agric. Biol. Chem. 53: 349-354.
10. Hooker, W. J. 1990. Common scab. Pages 33-34 in: Compendium of Potato Diseases. W. J. Hooker, ed. The American Phytopathological Society, St. Paul, MN.
11. Huber, D. M., and Schneider, R. W. 1982. The description and occurrence of suppressive soils. Pages 1-7 in: Suppressive Soils and Plant Disease. R. W. Schneider, ed. The American Phytopathological Society, St. Paul, MN.
12. Huber, D. M., and Waston, R. D. 1970. Effects of organic amendment on

soil-borne plant pathogens. Phytopathology 60:22-26.
13. Lambert, D. H., and Loria, R. 1989. *Streptomyces scabies* sp. nov., nom. rev. Int. J. Syst. Bacteriol. 39:387-392.
14. Lambert, D. H., and Loria, R. 1989. *Streptomyces acidiscabies* sp. nov. Int. J. Syst. Bacteriol. 39:393-396.
15. Lambert, D. H., and Manzer, F. E. 1991. Relationship of calcium to potato scab. Phytopathology 81:632-636.
16. Lapwood, D. H., and Adams, M. J. 1975. Mechanisms of control of common scab by irrigation. Pages 123-129 in: Biology and Control of Soil Borne Pathogens. G. W. Bruehl, ed. The American Phytopathological Society, St. Paul, MN.
17. Liu, D. 1992. Biological control of *Streptomyces scabies* and other plant pathogens. Ph.D. thesis. University of Minnesota, St. Paul.
18. Lorang, J. M. 1988. Heterokaryosis and inhibitory reactions among isolates of *Streptomyces scabies* causing scab on potato. M.S. thesis, University of Minnesota. St. Paul.
19. Lorang, J. M., Anderson, N. A., Lauer, F. I., and Wildung, D. K. 1989. Disease decline in a Minnesota potato scab plot. Am. Potato J. 66:531.
20. Menzies, J. D. 1959. Occurrence and transfer of a biological factor in soil that suppresses potato scab. Phytopathology 49:648-653.
21. Millard, W. A., and Burr, S. 1926. A study of twenty-four strains of *Actinomyces* and their relation to types of common scab of potato. Ann. Appl. Biol. 13:580-644.
22. Nuget, T. J. 1956. Soil treatment with PCNB (Terraclor) for control of potato scab. Plant Dis. Rep. 40:428.
23. Rouatt, J. W., and Atkinson, R. G. 1950. The effect of the incorporation of certain cover crops on the microbiological balance of potato scab infested soil. Can. J. Res. 28:140-152.
24. Tanii, A., Takeuchi, T., and Horita, H. 1990. Biological control of scab, black scurf and soft rot of potato by seed tuber bacterization. Pages 143-164 in: Biological Control of Soil-Borne Plant Pathogens. D. Hornby, ed. C.A.B. International, Wallingford, England.
25. Thaxter, R. 1892. The potato scab. Conn. Agric. Exp. Stn. Rep. 1891: 153-160.
26. Vidaver, A. K. 1976. Prospects for control of phytopathogenic bacteria by bacteriophage and bacteriocins. Annu. Rev. Phytopathol. 14:451-465.
27. Waksman, S. A., and Henrici, A. T. 1948. Family II. *Actinomycetaceae* Buchanan and family *Streptomycetaceae* Waksman and Henrici. Pages 892-980 in: Bergey's Manual of Determinative Microbiology, 6th ed. R. S. Breed, E. G. D. Murray, and A. P. Hitchens, eds. The Williams and Wilkins Co., Baltimore.
28. Weinhold, A. R., and Bowman, T. 1968. Selective inhibition of the potato scab pathogen by antagonistic bacteria and substrate influence on antibiotic production. Plant Soil 27:12-24.