Etiology of Radish Scab and Its Control Through Irrigation

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ABSTRACT

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A streptomycete cultured from scabbed radishes produced typical scab lesions on radishes grown in artificially infested muck soil, and was reisolated from these lesions. On a number of growth and indicator media, this isolate differed morphologically and in growth characteristics from streptomycetes that had been isolated from scabbed potatoes. Radishes grown in a tiled, relatively dry field plot had significantly greater scab than those grown in an untiled, relatively wet plot. Radishes grown under irrigation applied at 2- to 3-day intervals were consistently less scabbed than those that had received only ambient rainfall or no rainfall. Incidence of scab was significantly lower in plots irrigated for the first 2–3 wk after planting than in plots irrigated for only the first week.

Radish scab occurs in most radish (*Raphanus sativus* L.)-growing areas and can be an economically important disease in commercial radish production (7,16). Symptoms of radish scab appear as circular white lesions, 0.5–1.5 cm in diameter with raised edges and sunken centers on the surface of expanding hypocotyls. Although scab lesions are generally restricted to surface tissues and cause little more than cosmetic damage to the root, infected radishes with even a single lesion are unsalable.

Streptomyces scabies (Thaxt.) Waksman & Henrici, the cause of common scab of potato, is also reported to be the cause of similar scab diseases on a number of root crops including radish (5), but diagnoses are often based on symptomatology alone. Since streptomycetes are a common member of the microflora in most soils, their isolation from the surface of scabbed plant tissues without further pathogenicity testing is of questionable value. Hooker (4) infected seedling roots of a number of plants with a streptomycete isolated from potato, but the resulting symptoms were atypical compared to those that developed on naturally infected plants. The isolation of a streptomycete from scabbed radishes and its subsequent inoculation into healthy plants to produce typical disease symptoms has not been reported.

Although there are no reported attempts to control radish scab, potato scab has been successfully controlled by the use of resistant cultivars (19), soil acidification (1,3) soil treatment with the fungicide pentachloronitrobenzene (2,15), and strategically timed irrigation (8,21).

Results of cultivar trials have indicated little variation between radish cultivars in susceptibility to scab, and no highly resistant cultivars have been identified (11). Preliminary investigations showed that low soil pH could not be maintained for more than 7–14 days in an organic peat soil with the addition of elemental sulfur or manganese sulfate (10). At high concentrations of these amendments, phytotoxicity became evident as stunted growth and reduced yields. Although PCNB significantly reduced the incidence of scab, the likelihood of obtaining a registration for PCNB on radish is low because radishes are in the ground for a much shorter period than potato, and residues of PCNB would be unacceptable. Irrigation appeared to be the most promising control method that was evaluated. In 1979, scab levels were significantly lower on radishes in field plots that had received supplemental irrigation than those that had received only normal rainfall (10).

The objectives of this study were to isolate the causal organism from scabbed radishes, to verify its pathogenicity, and to investigate the use of irrigation for controlling radish scab.

MATERIALS AND METHODS

Isolation of streptomycetes. Two procedures were used to isolate streptomycetes from radish scab lesions.

The whole-lesion method. The radishes were rinsed in water and lesions ≤2 mm in diameter were removed with a thin layer of underlying tissue 1–2 mm in diameter. The lesions were placed, outer surface upward, on 1.5% water agar that had been adjusted to pH 10.0–10.5 by adding 0.1 M NaOH after sterilization. Any streptomycete growing from the plated lesions within 7–10 days was transferred for further study.

The crushed-lesion method. Infected radishes were washed in running tap water for 5–10 min. Lesions ≤5 mm in diameter were removed and ground in 0.1 M phosphate buffer (pH 8.5) with a mortar and pestle. The resultant slurry was then serially diluted in sterile 0.1 M phosphate buffer and 0.1 ml of each dilution was added to a petri plate with 15 ml of colloidal chitin agar (12) that had been prepared with 0.1 M phosphate buffer instead of water. Plates were gently swirled, then incubated at room temperature for 7 days when individual colonies were visible.

Streptomycetes were isolated from scabbed potatoes (obtained from a local Michigan grower) by using colloidal chitin agar culture medium as reported by Taylor (20).

Two strains of *S. scabies*, isolates #3352 and #10246, originally obtained from scabbed potatoes, were obtained from the American Type Culture Collection (Rockville, MD) and used for comparative studies.

Pathogenicity tests. In greenhouse pathogenicity experiments, radishes were grown in wooden boxes 121 cm long, 25 cm deep, and 30 cm wide at the top, tapering to 2.5 cm at the bottom. A Carlisle peat soil (pH 6.5) was obtained from a local radish grower and steamed for 12–16 hr prior to filling the boxes. Radishes received 16 hr of light (22,000 lux) per day and the air temperature was maintained at 27 ± 5 C days and 21 ± 5 C nights. Radish seeds of cultivar Red Prince were planted 3–5 cm apart in a single row down the center of each box. A soluble fertilizer (20-20-20, N-P-K) was applied as a drench at planting and again 2 wk later.

The isolates were also tested for pathogenicity on greenhouse-grown potato plants grown in 20-cm-diameter clay pots in a sandy-loam soil that had been steamed for 12–16 hr. Plants received 16 hr

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of light per day and the air temperature was maintained at 27 ± 5 C days and 21 ± 5 C nights. Healthy tubers of *Solanum tuberosum* L. 'Yukon Gold' were first surface sterilized with 5% formaldehyde for 5 min then rinsed three times with sterile distilled water. Tubers were cut into seed pieces containing at least two eyes and planted at a depth of approximately 5 cm.

Inoculum was prepared by growing lawns of streptomycete isolates on starch agar plates (pH 8.5, 10 gm of soluble starch, 8 gm of nutrient broth [Difco, Detroit, MI], and 15 gm of agar in 1 L of water) or potato-dextrose agar (Difco) for about 21 days and then scraping the agar into a blender with distilled water. This mixture was homogenized and incorporated into the top 7 cm of soil in a growing box or 20-cm-diameter clay pots where it was allowed to dry for 3 days before the seed pieces were planted. Colony-forming unit (cfu) concentrations were determined from the agar-water homogenate by making serial dilutions and replating. Scabbed peelings from naturally infected potatoes were homogenized with distilled water and incorporated into the top 7 cm of soil which was allowed to dry for 3 days before planting. For radish pathogenicity studies, two boxes of infested soil were prepared for each streptomycete isolate tested and the entire experiment was repeated three times. For potato pathogenicity studies, five pots of infested soil were prepared for each streptomycete isolate that was tested.

Characterization of streptomycete isolates. Tyrosinase activity and the ability to hydrolyze starch were determined by using tyrosine casein-nitrate agar (13) and Sx agar (18), respectively. Reactions in litmus milk (Difco) were also determined. Potato plugs were autoclaved for 15 min at 138 kPa (20 psi) then inoculated with test strains and placed in petri plates with moistened filter paper. These were kept in fluorescent room light at 21 C for 7 days then were observed for pigment formation. Colony characteristics of the streptomycetes were observed on water agar, potato-dextrose agar (Difco), and starch agar.

Specimens for electron microscopy were prepared by fixing 1-cm-diameter plugs from 10-day-old starch agar cultures in a 1:1 mixture of 2% OsO_4 and 0.2 M phosphate buffer (pH 7.2) for 2 hr followed by dehydration in an ethanol series. Specimens were critical-point dried, gold coated, and examined in a JEOL JSM-35C scanning electron microscope.

Soil moisture experiments. Soil moisture experiments were conducted at a commercial radish farm near Gregory, MI, which had a recent history of severe scab. The homogeneous Carlisle peat soil (pH 6.0–6.5) was prepared for planting by using standard commercial procedures. Seeds were planted by using a V-belt-type, hand-pushed planter except where noted. Soil moisture potentials (− bars) were determined from percent soil moisture by weight using a previously generated linear regression equation based on values obtained from a Ceramic Plate Extractor (Soil Moisture Equipment Corp., Santa Barbara, CA). Soil samples were taken every 2–3 days during the experiments at a depth of 6–8 cm. In some experiments, rain shelters of clear, corrugated Fiberglas with open sides (2.5 × 2.5 m) were set up 1 m above the ground to block rainfall. At harvest, radishes were collected from the center 1 m^2 of sheltered plots.

In 1979, a field trial was set up in two adjacent fields in which scab had been observed in previous years. The fields differed in that one was tiled while the other was not. Twelve cultivars were planted in six 4.5-m replicate rows with 10 seeds per 30 cm of row and a row spacing of 45 cm. A single completely randomized plot was planted in each field. After 28 growing days, the radishes were hand-pulled, washed, topped, weighed, counted, and the number of lesions per radish and number of scabbed radishes were determined.

In 1980, two consecutive preliminary experiments were conducted in which three levels of soil water potential were maintained. Low soil water potential was achieved by using rain shelters while high soil water potential was maintained by frequent (2–3 days) irrigation. The intermediate moisture level plot received only ambient rainfall. Seeds of cultivar Scarlet Knight were planted using commercial production equipment at about 16 seeds per 30 cm of row and a row spacing of 15 cm. The first experiment was harvested after 33 growing days and the second after 36 growing days. Radishes were processed and assessed for disease as described previously.

In 1981, 57 radish cultivars were grown under two moisture regimes (three replicates per treatment). One group of radishes received only ambient rainfall while the other was irrigated with an overhead system that delivered 2–3 cm of water every 2–3 days. Each replicate row was 4.5 m long with 10 seeds per 30 cm and a row spacing of 45 cm. After 28 growing days, the radishes were hand-pulled, washed, topped, weighed, counted, and the number of scabbed radishes was determined.

In a second experiment, cultivar Scarlet Knight was planted with commercial production equipment at about 16 seeds per 30 cm of row and a row spacing of 15 cm. Irrigation was applied every 2–3 days over periods varying from 1–4 wk after planting in 1-wk increments. One group received no irrigation. After irrigation treatments were completed, rain shelters as described previously were set up 1 m above the ground to block rainfall. Each treatment was replicated three times and radishes were harvested from 1-m^2 plots underneath the shelters after 28 growing days. Radishes were processed and assessed for disease as described previously.

Percentages for statistical analyses were tranformed by using the arc sine transformation.

RESULTS

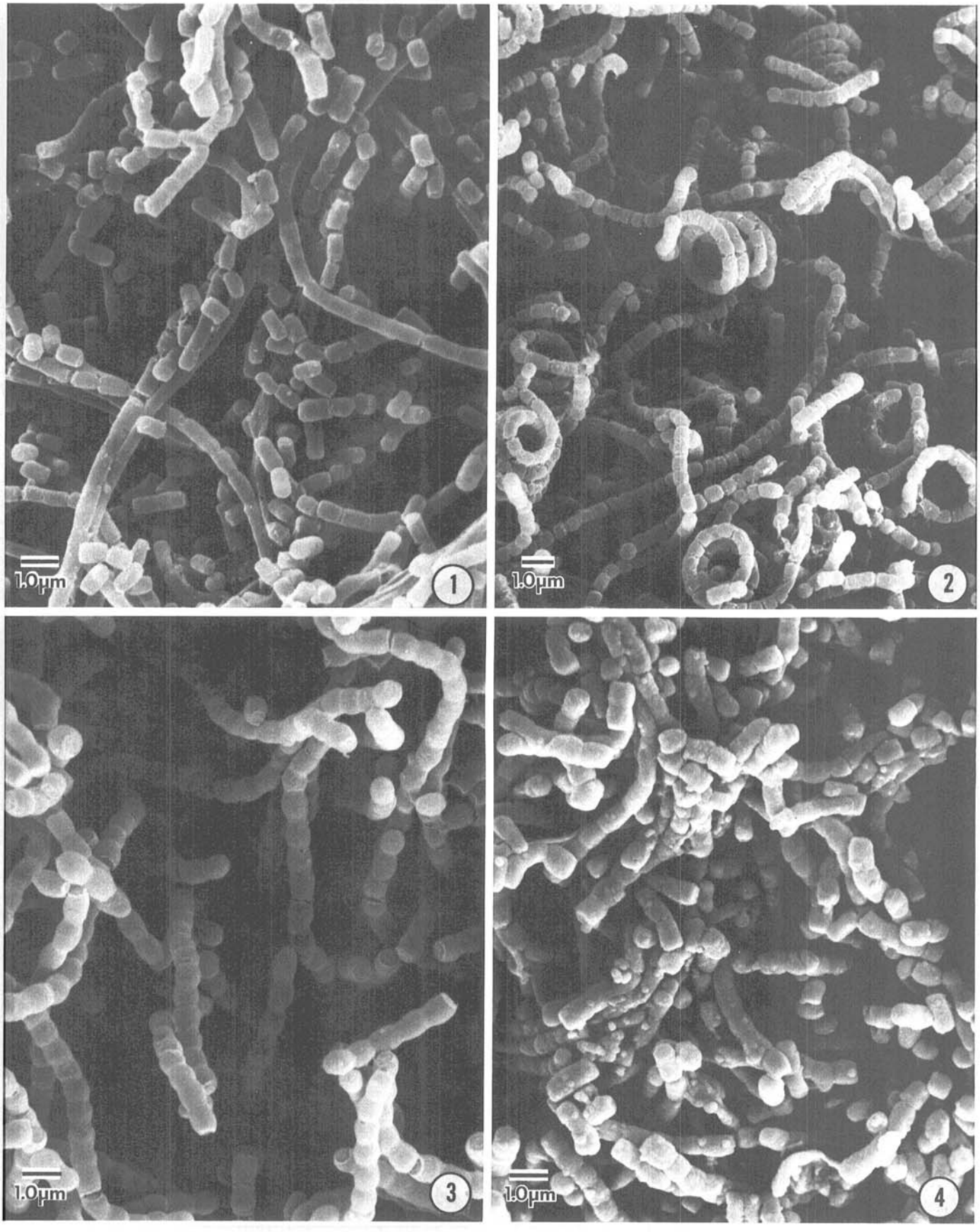
Isolation of streptomycetes. The recovery rate of streptomycetes from scabbed radishes was extremely low. Even with the most successful procedure (the crushed-lesion method) only one in 20 platings produced a viable streptomycete culture. A single radish isolate, R1, was isolated by using the whole-lesion method. The recovery rate of streptomycetes from potato was much higher than from radish. Fifty-five percent of the platings produced viable streptomycete cultures. A single potato isolate, P4, and the R1 radish isolate were used in further tests.

Pathogenicity tests. The final inoculum concentrations (colony-forming units per cubic centimeter of soil) for the pathogenicity trials on radish were 2×10^5 for isolate R1, 5×10^6 for isolate P4, and 10^6 for isolates 3352 and 10246. Twenty-three percent of the

TABLE 1. Growth characteristics of four isolates of *Streptomyces* spp. on several test media

Isolate	Relative growth and color of aerial spores on:						
	WA[a]	PDA[a]	SA[b]	Sx[c]	Litmus milk[a]	Potato plug	TCN[d]
R1	Sparse, white	Heavy, dark gray	Heavy, light gray	Growth	Alkaline, coagulated	Dark brown pigment	Pigment formation
P4	Sparse, white	Heavy, ivory	Moderate, white	Growth	Alkaline, coagulated	Dark brown pigment	Pigment formation
3352	No aerial growth	Heavy, gray	Heavy, dark gray	No growth	Alkaline, not coagulated	No pigment	No pigment
10246	No growth	Moderate, salmon-pink	Moderate, white	No growth	Alkaline, not coagulated	No pigment	No pigment

[a]WA = water agar, PDA = potato-dextrose agar and litmus milk (Difco Laboratories, Detroit, MI 48232).
[b]SA = starch agar. Prepared with 10 gm of soluble starch, 8 gm of nutrient broth (Difco), 15 gm of agar in 1 L of water.
[c]Sx agar prepared according to Schaad (18).
[d]TCN = tyrosine-casein-nitrate medium. Prepared according to Menzies (13).



Figs. 1–4. Spore chains of isolates of *Streptomyces* spp. **1,** Spore chains of radish isolate R1 (*Streptomyces* sp.). **2,** Spiralling spore chains of potato isolate P4 (*Streptomyces* sp.). **3,** Spore chains of ATCC isolate 3352 (*S. scabies*). **4,** Spore chains of ATCC isolate 10246 (*S. scabies*).

radishes grown in R1-infested soil and 55% of radishes grown in P4-infested soil became scabbed. Sixty-five percent of the radishes grown in soil amended with homogenized scabby potato peelings became scabbed. None of the controls became scabbed. The streptomycetes isolated from these radishes (crushed-lesion method) were identical to the ones originally added to the soil. The pathogenicity test was repeated with the reisolated R1 and P4 strains and similar results were obtained. Isolates 3352 and 10246 obtained from the ATCC failed to produce scab lesions on radish in three separate trials.

The final inoculum concentration (colony-forming units per cubic centimeter) for the potato pathogenicity trials were 10^6 for isolates R1, 3352, and 10246. All tubers grown in soil amended with homogenized potato peels became scabbed. None of the controls became scabbed. Sixty percent of the tubers grown in 3352-infested soil and 80% of the tubers grown in 10246-infested soil became scabbed. Tubers grown in soil amended with R1 never became scabbed.

Characterization of streptomycete isolates. The amount and color of aerial and substrate mycelia, as well as reactions on indicator media differed among isolates R1, P4, 3352, and 10246 (Table 1). Scanning electron microscopic observations revealed differences in spore and spore chain morphology between the isolates (the standard deviations for all means is less than 0.05 μm). Spores of the R1 isolate were cylindrical and elongated averaging 0.8 μm × 0.6 μm (Fig. 1). Spores of isolates P4 and 10246 were similar and averaged 0.6 μm × 0.6 μm (Figs. 2 and 4). Spores of isolate 3352 were barrel-shaped and averaged 0.8 μm long × 0.7 μm wide at the center, tapering to 0.6 μm wide at the ends (Fig. 3). Isolate P4 was the only one that exhibited spiralling spore chains on any of the media tested.

Soil moisture experiments. In the 1979 field trial, radishes grown in the relatively wet, undrained plot (soil water potential consistently above −1.5 bar) had significantly less scab ($P = 0.01$) than those grown in the dryer, drained plot (soil water potential consistently lower than −3 bars (Fig. 5)).

In the first 1980 irrigation trial, mean soil water potentials differed significantly ($P = 0.05$) between the three treatments and scab levels were lower in the plots with higher soil water potential (Table 2). In the second 1980 trial, water potential in the intermediate and high soil water potential plots were not significantly different due to high precipitation levels although both differed significantly ($P = 0.05$) from the low moisture plot (Table 2). Scab incidences in the intermediate and high water potential plots were similar and lower than the scab incidence in the low water potential plot.

In the first 1981 irrigation experiment, radishes grown under irrigation had an average of 4% of the roots infected while those receiving no irrigation had 50% of the roots infected, a difference that was significant at $P = 0.01$. For each of the 57 cultivars tested, the incidence of scab was significantly lower in the irrigated plots than in the nonirrigated plots ($P = 0.01$). Soil water potential ranged between −0.1 and −2.3 bars for the irrigated plots (average = −1.1 bars). In the nonirrigated plots, the soil water potential ranged between −1 and −11.2 bars and averages −2.5 bars.

In the second irrigation experiment, radishes irrigated for 3 or 4 wk after planting were significantly less scabbed than those irrigated for 0, 1, or 2 wk after planting (Table 3). Radishes irrigated for 1 wk after planting had the highest incidence of scab while those that had received either no irrigation or 2 wk of irrigation had significantly less scab. The radishes that had received no irrigation were significantly smaller (by weight) than those of any other treatment ($P = 0.01$).

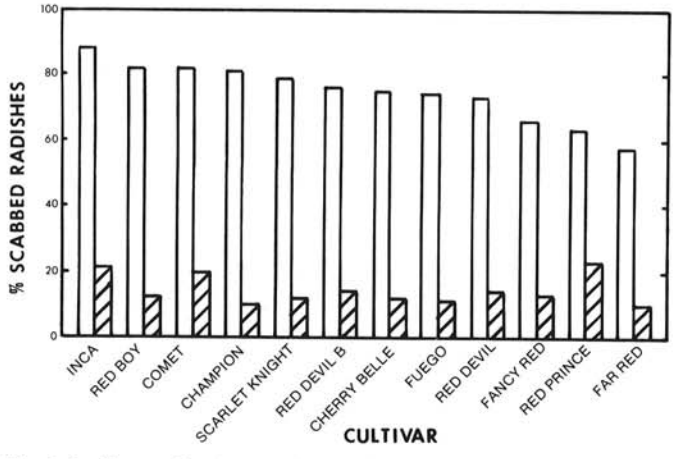


Fig. 5. Incidence of scab on radish cultivars grown at two soil moisture levels. Soil moisture potential in the "wet" plot was greater than −1.5 bar (cross-hatched). Soil moisture potential in the "dry" plot was less than −3.0 bars (open bars).

DISCUSSION

A streptomycete (R1) isolated from lesions of scabbed radishes has been shown to cause the radish scab disease. A streptomycete isolated from potato (P4) was also shown to cause scab on radish. That these morphologically distinct isolates are both pathogenic on radish is consistent with the results of past research (14) which indicated a great variation among streptomycetes causing potato scab. Isolates 3352 and 10246 were shown to be morphologically distinct from each other and from strains R1 and P4. Isolates 3352 and 10246, which are pathogenic in potato, also reacted differently in a number of physiological tests and were not pathogenic on radish. Further study is needed to clarify the interrelationships between pathogenic streptomycetes and their hosts. For instance, P4, which was isolated from potato, was shown to be pathogenic on

TABLE 2. Scab incidence on radishes (Scarlet Knight) grown at different soil water potentials on a Carlisle peat soil in 1980

Treatment (soil water potential)[a]	Scabbed radishes[b] (%)	Lesions/radish (no.)
Trial 1[c]		
Low (−1.8 to −19 bars)	74	3.3
Intermediate (−0.45 to −2.5 bars)	57	2.2
High (−0.3 to −0.58 bars)	24	2.4
Trial 2[d]		
Low (−1.5 to −5.9 bars)	24	2.1
Intermediate (−0.56 to −0.6 bars)	10	1.8
High (−0.4 to −0.58 bars)	12	1.5

[a] Low-moisture plots grown under rain shelters; intermediate-moisture plots received ambient rainfall; high-moisture plots received ambient rainfall and irrigation (2- to 3-day intervals).
[b] Percentage values based on about 200 radishes harvested for each treatment.
[c] Mean water potential levels significantly different at ($P = 0.05$).
[d] Mean low water potential significantly different from others ($P = 0.05$); mean intermediate and high water potentials not significantly different.

TABLE 3. Effects of irrigation timing on incidence of radish scab on cultivar Scarlet Knight in 1981

Treatment (weeks of irrigation after planting)[x]	Scabbed radishes[y] (%)	Mean lesions/per radish (no.)	Mean weight radish (g)[z]
0	30 a	1.7	77 a
1	79 b	2.4	12.0 bc
2	20 a	1.7	11.8 bc
3	1 c	0.7	10.6 b
4	1 c	0.3	13.8 c

[x] Overhead sprinkler irrigation was applied immediately after planting for all treatments and repeated every 2–3 days. Radishes were harvested after 4 wk.
[y] Percentage means of three replicates per treatment; approximately 50 radishes per replicate. Percentages with same letters were not significantly different at $P = 0.01$ (LSD test).
[z] Values with same letters are not significantly different at $P = 0.05$ (LSD test).

radish and R1, which was isolated from radish, was not pathogenic on potato. Even though carrots were reported to be susceptible to scab (5), a number of different carrot cultivars have been grown without becoming scabbed in the fields where we found radish scab to be severe.

The reason for the low recovery rate of the pathogen from radish scab lesions compared to potato scab lesions is not known. There appeared to be no inhibitory effect from radish tissue since the pathogen grew well on media prepared with radish tissue homogenates or around radish extracts placed on a lawn of the growing pathogen (*unpublished*). Streptomycetes are generally considered to be weak pathogens and may exist in low numbers or disappear altogether throughout symptom development (5). This could explain why isolations were more successful with smaller, apparently younger lesions than with larger, apparently older lesions in which secondary organisms may have outgrown the pathogen.

In both preliminary and present experiments involving a large number of radish cultivars, irrigation every 2–3 days over the 4-wk growing period has been shown to significantly reduce the incidence of radish scab. It has been determined that the period of rapid tuber expansion in potatoes is the period of greatest susceptibility to scab and that irrigation during this stage of growth is most effective in controlling the disease (6,9). With radishes, it was found that irrigation for the first 3 wk afforded maximum control of the disease while 2 wk of irrigation also resulted in significantly lower scab. Since radishes are only in the ground for about 4 wk, it is likely that irrigation for 3 wk keeps the soil moisture levels high enough during the remaining week prior to harvest to inhibit infection or symptom development. Also, infections that occur late in the growing period may not manifest symptoms at harvest. The lower incidence of scab observed in radishes receiving no irrigation as opposed to those receiving only 1 wk of irrigation is probably due to the significantly smaller size of the nonirrigated radishes. Since they were grown under a rain shelter for the entire growing period, they were under considerable moisture stress which could account for the stunted condition of the plants.

A better understanding of how and when radishes become infected, and what factors affect lesion development, would aid in determining the best control measures for this disease. At present, irrigation appears to be the most effective control measure available and can be easily incorporated into commercial production. Adjusting the length and timing of the irrigation period may help to avoid an increase in clubroot (caused by *Plasmodiophora brassicae* Wor.), another serious disease of radish which is enhanced by excessively high moisture levels over a prolonged period. In soils where both the scab and clubroot pathogens are present, control can be achieved through the use of clubroot-resistant cultivars (17).

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