This is to certify that the

thesis entitled

ETIOLOGY AND CONTROL OF RADISH SCAB

presented by

David Ross Levick

has been accepted towards fulfillment
of the requirements for

M.S. degree in Plant Pathology

Christine Stephens
Major professor

Date August 7, 1981

ETIOLOGY AND CONTROL OF RADISH SCAB

By

David Levick

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirement
for the degree of

MASTER OF SCIENCE

Department of Botany and Plant Pathology

1981

ABSTRACT

ETIOLOGY AND CONTROL OF RADISH SCAB

By

David Levick

A Streptomyces sp. cultured from scabbed radishes produced typical scab lesions on radishes grown in artificially infested muck soil, and was reisolated from these lesions. A streptomycete isolated from scabbed potato tissue also produced typical scab lesions on radish. These two isolates were compared to two isolates of Streptomyces scabies (Thaxt.) Waksman and Henrici and all four were morphologically and biochemically distinct. None of 29 radish cultivars was highly resistant to scab, however, disease incidence was consistently lower in white cultivars. Neither acidification of the soil with sulfur or $AlSO_4$, nor the addition of minor plant nutrients to soil was effective in reducing scab. A PCNB drench one week after planting significantly reduced scab. A strong negative correlation was found between soil moisture during the first half of the growing period and scab incidence. Irrigated plots had 50% less scab than non-irrigated plots.

to Anton

ACKNOWLEDGEMENTS

I am deeply indebted to Dr. Christine Stephens, Dr. Melvyn Lacy, and Dr. Karen Baker for their patience and guidance, to my wife, Judy, for her constant encouragement, and to my friends for their constructive criticisms. I thank you all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Though radish (Raphinus sativus L.) acreage is relatively small in the United States each year, the high cash value and multiple plantings possible each season make radish an economically important crop. In many areas, radishes are grown on valuable muck soil which necessitates consistently good yields in order to maintain suitable profit margins.

Diseases and insects are among the most important problems for growers. Of the diseases, the more important ones are those that affect the root directly. These include clubroot caused by Plasmodiophora brassicae Woronin; black root caused by Aphanomyces raphani Kendr.; and scab caused by Streptomyces scabies (Thaxt.) Waksman and Henrici.

Since the summer of 1978, various Michigan radish growers have experienced losses due to radish scab. In many fields, infection levels have exceeded 50%, and at one 500 acre muck farm, the severity of the disease necessitated the removal of more than 100 acres from radish production.

Symptoms of radish scab appear after root enlargement begins. At this time small, whitish-gray, scale-like spots, about 1 mm in diameter, develop on the expanding root. Circular lesions develop from these spots, often reaching 1-1.5 cm in diameter at harvest. The edges of the lesions are raised, forming a lip, while the centers are sunken and pitted. These centers appear white in younger lesions but the activity of secondary organisms causes discoloration, softening, or rotting of the exposed tissue. Market expectations make blemished radishes unsalable.

The pathogen, Streptomyces scabies (Thaxt.) Waksman and Henrici, is well known as the cause of a similar disease on potato. Due to the physiologies of radish and potato, and the differences in the cropping systems used for each, scab control strategies may differ for each host. The potato tuber is a storage rhizome with lenticels and stomata which are open to the soil during tuber expansion. These openings are thought to serve as the infection site for S. scabies (17,24). Enlarged radish roots slough off their epidermis while forming a cork phelloderm during expansion. Since radishes do not have lenticels, the mode and time of penetration must differ from that of potato. Radish roots develop just below the soil surface where temperature and moisture content vary greatly. Potatoes develop deeper in the soil where these parameters are relatively stable. The effects of different micro-climates on the pathogen are not known.

Potatoes are grown once each season in any given field, whereas radishes are routinely cropped three and occasionally four times each season. The multiple planting schedule of radishes may allow inoculum build-up through the growing season.

Little work has been done to characterize the radish scab pathogen. Although it is believed to be identical to the pathogen causing potato scab, no comparative studies have been reported.

The objectives of this study were to isolate the organism responsible for radish scab, to compare and contrast it to the potato scab pathogen, and to test control strategies on radish based on previous work with potato scab. Finally, studies were conducted to compare known pathogenic strains of S. scabies to strains isolated from radish and potato on the basis of several morphological and biochemical characters.

LITERATURE REVIEW

Thaxter (69) first isolated the causal agent of common scab of potato in 1890, although the disease had been well known in Europe for many years prior. As early as 1825 (40), recommendations for the control of scab were available. Thaxter (70) named the pathogen Oospora scabies. He believed that it was a fungus but was unsure of his taxonomic designation. Gussow (21) identified the organism as a filamentous, spore forming bacterium which he placed in the genus Actinomyces. Waksman and Henrici (75) divided this genus on the basis of oxygen requirements with the anaerobes retaining the name Actinomyces. The aerobes were further divided on the basis of spore formation. The scab pathogen, being aerobic and forming spores on special aerial filaments, was placed into the new genus Streptomyces.

As systematic methods for identifying actinomycetes became more refined, the taxonomic position of the scab organism fell into doubt. Waksman (72,73,74) characterized S. scabies as tyrosinase-positive and producing curved or spiralled spore chains. Shirling and Gottlieb (65) reported S. scabies as being tyrosinase-negative and forming straight spore chains. Other such discrepancies appear widespread throughout the literature. Bonde and McIntyre (8) discovered two distinct groups of scab producing Streptomyces spp. while Millard and Burr (50) found 24 strains that differed significantly in both morphological and physiological characteristics. Taylor and Decker (67) tested 143 pathogenic

Streptomyces spp. isolates and found a great deal of variation in their biochemical nature. Hughes et al. (28) found different electrophoretic action patterns between different strains.

At present, the taxonomy of S. scabies is unclear. Pridham and Tresner (57) do not consider S. scabies as a legitimate taxon. Gordon (18) suggests that the common scab organisms be placed in the polytypic Streptomyces griseus group. It is evident that the differences between scab-producing Streptomyces isolates are greater than those found within any single species according to the modern classification of the group (57). It is apparent that pathogenicity alone is no longer useful in precise taxonomic determinations and conversely, pathogenicity testing is the only method for identifying a pathogenic strain. In this study, specific streptomycetes will be referred to as pathogenic Streptomyces spp. or Streptomyces scabies with the understanding that the species determination may not be taxonomically legitimate.

S. scabies first gained prominence as a potato scab pathogen that caused economic losses on this important food staple all over the world. The bulk of the literature deals with S. scabies on that host. Few workers have done much more than to acknowledge that the pathogen causes disease on radish (22,29,76). Koranowski et al. (30) reported radish scab occurring in isolated fields in Germany at economically devastating levels.

Much of the work with potato scab deals with environmental factors that affect disease. That soil moisture is closely related to scab severity was first shown experimentally by Sanford in 1923 (59). He found that under very high soil moisture conditions, the numbers of lesions and percentages of infected potatoes were significantly reduced. Staap (66)

reviewed the research on irrigation in relation to disease incidence and found much disagreement as to what soil moisture levels were necessary for reduction in disease. Some of the apparent variability may have been due to the lack of a standardized measurement for soil moisture. Percent soil moisture by weight, which was the most commonly used parameter in these studies, is subject to great variation between different soil types and does not describe the amount of water available to organisms, as does the currently accepted parameter of soil water potential. This parameter may be used for comparative purposes because it is not affected by soil type or structure.

Lapwood et al. and others (31,32,35,36,37,77) found that economical control of potato scab could be achieved on Rathamel clay with soil moisture potentials above -0.4 bars. Lewis (38) reported good control above -0.13 bars. Scab incidence increased as soil moisture potential decreased from -0.13 to -0.8 bars. Davis et al. (12,13) found maximum control above -0.46 bars, increasing severity as the potential decreased to -0.96 bars, and maximum scab below -0.96 bars. No soil moisture studies with potato scab on organic soils have been reported.

Infection of potato tubers occurs through stomata and unsuberized lenticel openings (17,24,34,35) during the period of rapid expansion. Therefore, there is a critical period during which irrigation is effective in influencing the infection process. Lapwood et al. (36) found the first three weeks after tuber initiation to be the most critical period for control by irrigation. Barnes (3) found this critical period to be variable from year to year and Lewis (38) reported no control if irrigation was limited to the first two weeks after tuber initiation. Davis (12,13) found that control by irrigation, though significantly reducing scab

severity, was not generally able to lower disease incidence to levels acceptable for U.S. no. 1 grading.

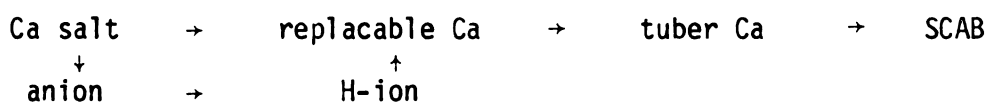
How disease control by means of soil moisture management operates has been the subject of many studies. Sanford (60) hypothesized that high soil moisture reduces the amount of available oxygen in the soil and would thus lead to an inhibition in the growth of S. scabies. Dippenaor (14) found the growth of the pathogen to be six times faster at 25°C than 10°C. This lead him to the conclusion that S. scabies was inhibited by lower soil temperatures caused by high soil moisture. High manganese levels in potato periderm have been related to reduced scab severity and control of scab was obtained through the incorporation of manganese in the soil (42,43,51,52). A lowering of soil oxygen levels through irrigation has been shown to cause an increased uptake of manganese (32,42). This suggested that the apparent control obtained through irrigation was actually due to increased manganese uptake.

In work with the effects of periderm calcium levels on scab incidence, various researchers reported that a decrease in tuber calcium is accompanied by a reduction in scab (13,15,25). An increase in soil moisture resulted in a lowering of tuber calcium, and this has been proposed as the means by which irrigation controls disease.

Lapwood and Adams (32) and Adams and Lapwood (1) proposed bacterial antagonism as the mechanism by which soil moisture affected disease severity. It was shown in these studies that actinomycete population levels around and on susceptible tuber lenticels decreased as soil moisture increased. This was accompanied by an increase in the numbers of saprophytic bacteria at the same locations. It was suggested that under cool, low oxygen levels of wet soil, S. scabies was unable to compete with

other bacteria in the colonization of susceptible tissue.

The addition of sulfur to field soils has been used extensively as a control for potato scab, though the results have been quite variable. Wheeler and Adams (78) obtained significant control with sulfur as well as other compounds which lowered soil pH. Similar work (2,3,4,15,62,68) showed that a drop in pH below 5.3 was possible on mineral soils with the addition of 400 to 2500 pounds per acre of elemental sulfur. This drop in pH, they reported, was responsible for reduced disease. Duff (16) reported inconsistent control of scab by sulfur even with a reduction of pH below 5.3. Limited control with negligible pH effects was obtained using sulfur and calcium sulfate (10). Population levels of actinomycetes were reduced in soil through the addition of sulfur (71), suggesting a direct effect of sulfur on the organisms. Most workers believe that the effects of sulfur are indirectly based on reduction of soil pH. Sulfur, through the acidification of the soil environment, was suggested to cause an increased mobilization of certain cationic plant nutrients such as calcium and potassium (63). Horsfall, Hollis, and Jacobson (25) suggested that calcium affected scab development in potato periderm through the following pathway:



As the hydrogen ion level increases (pH decreases), the amount of replaceable Ca decreases, which in turn reduces the Ca levels in the tuber, ultimately reducing scab. In this system, sulfur would be ineffective under conditions where high levels of replacable Ca already exist, since the subsequent lowering of the pH could not reduce the Ca concentrations to levels suitable for disease control. The work of Gries (20) and Davis

(11) supported this theory as they found an increase in scab as the Ca : K ratio in potato tubers increased. Control was achieved through the use of copper sulfate (52), which is known to inhibit Ca uptake in potato tissue. Copper sulfate is also an effective soil acidifier, but the effect of soil pH was not studied. Doyle (15) was not able to show a relationship between scab and Ca levels but did report effective control through increased irrigation.

Some researchers have reported control of scab through soil treatments with $MnSO_4$ (42,43), while others have found no significant control with $MnSO_4$ (2,3,51). The mode of action was not studied. Gries (19) reported control of scab with $AlSO_4$, while Houghland and Cash (26) were unable to control scab with $AlSO_4$ incorporated into the soil.

Lapwood and Dyson (33) found that scab severity increased with increasing amounts of nitrogen fertilizer in the soil. They assumed that tuber initiation and vigorous growth occurred earlier with the fertilized treatments, and related this to lower soil moisture during the beginning of that particular growing season. Nitrification inhibitory compounds such as N-Serve and N-Forme were found to be effective in reducing scab (54,55,63), while Davis (11) reported no effect with these compounds.

A consistently effective chemical control for common scab has eluded researchers for nearly 100 years. Bolley (7) reported control of scab through potato seed piece treatments and soil drenching with mercuric bichloride. This method never met with widespread approval by scientists and was discarded after work by McMillan (41) who found it ineffective in most tests. Autoxidative products of chlorogenic acid in potato periderms were reported to be a major factor in resistance to common scab (62). These chemicals proved inhibitory to S. scabies in the laboratory but were

never tested in the field. Hooker (23) and Houghland and Cash (26) simultaneously reported the effectiveness of pentachloronitrobenzene (PCNB) in controlling potato scab on mineral and organic soils, respectively. Others (10,48,54,55) confirmed these results and recommended use of PCNB. However, Davis (13) found that PCNB was ineffective when soil moisture potential was allowed to drop below -1.6 bars. This finding led to speculation that improved irrigation and not PCNB was responsible for the control achieved. Houghland and Cash (27) found that high rates of PCNB retarded plant growth. It has been reported that PCNB persists for long periods in the soil (6) and that the roots and hypocotyls of bean plants accumulated PCNB from surrounding soil to levels up to 2 times those in surrounding soils (9). These data, as well as economic considerations, led to the testing of alternative chemicals for control of scab both in the greenhouse and field. Captafol was found to be as effective as PCNB (44,45,46). Daminozide, a systemic growth regulator, controlled scab through foliar applications but was neither effective enough nor cheap enough for large scale use (47).

Nitrate inhibitors such as a urea-formaldehyde mixture were found to be effective in controlling scab (5,54). Schultz et al. (64) and Potter et al. (54) both reported in-row applications of urea-formaldehyde-85 at levels that were economically feasible and still adequate for scab control.

At present, there are no chemicals registered for use on potato or radish specifically for the control of scab. PCNB is registered for control of Rhizoctonia solani Kuhn on potato, but allowable levels are not always effective against scab. It would be difficult to get a registration for PCNB on radish because radishes are in the ground for a

much shorter period than potato, and residue levels of PCNB at harvest would probably be unacceptable. Current recommendations for control of potato scab call for lowering soil pH when possible; maintaining high soil moisture levels; and reducing the amount of free nitrates in the soil.

MATERIALS AND METHODS

All field work was carried out at the Buurma Farm near Gregory, Michigan. The farm was in turf production for a number of years before switching to radish production in 1976. The soil is a homogenous Carlisle muck with a pH of approximately 6-6.5. The three areas used in this study all had a recent history of severe scab. Sites #1 and #2 were tiled and were relatively dry whereas site #3 was untiled and remained relatively wet.

Before each planting, the soil was disced, rolled and treated with a 6-24-24 ammonia based fertilizer. Dyfonate 10G at a rate of 2.24 kg/hectare was placed in the row at planting in 1979 for control of radish maggot. Diazinon 14G was used at the same rate for the 1980 plantings. Seeds were planted with a V-belt hand-pushed planter at 10 seeds per foot and row spacing of 46 cm.

A recording hygrothermograph, recording soil thermograph, tipping bucket rain gauge, and soil tensiometers were set up to measure relative humidity, air and soil temperature, rainfall, and soil moisture levels throughout the growing season.

During the 1979 soil moisture experiments, soil tensiometers (Jet Fill Model 2725; Soil Moisture Equipment Corp., Santa Barbara, CA) were used to measure moisture tensions from two to 25 (typically 5 to 10) centimeters below the soil surface. For the 1980 field trials and greenhouse experiments, a soil moisture curve was generated using a 15 bar

Ceramic Plate Extractor (Model 1500; Soil Moisture Equipment Corp., Santa Barbara, CA). Percent soil moisture was determined at -.12, -.62, -2, -6, and -14 bars using a collective soil sample from site #2 at the Buurma Farm. The percentage was calculated as:

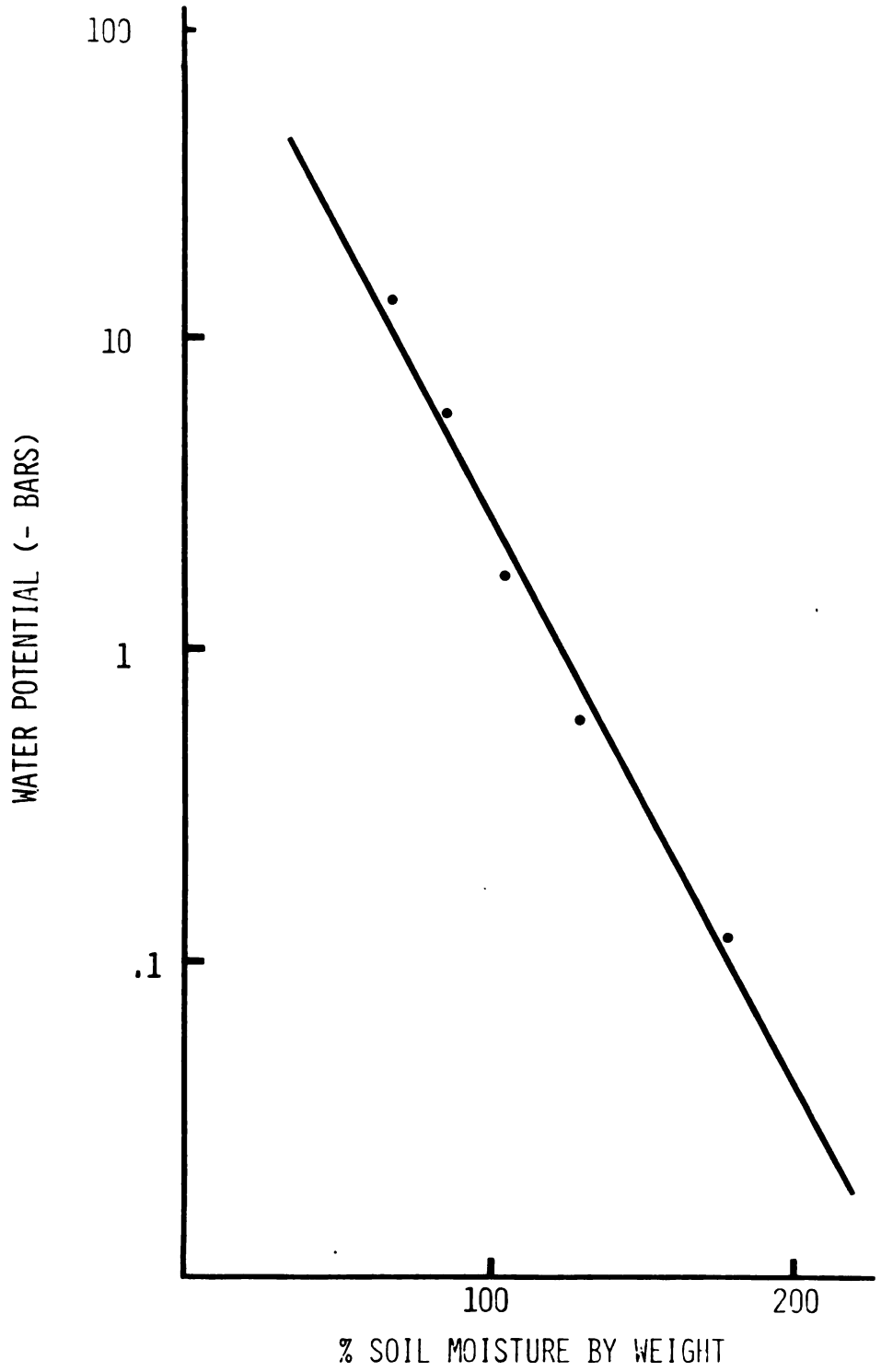
$$\frac{\text{Fresh weight of sample - Dry weight of sample}}{\text{Dry weight of sample}} \times 100$$

where weight was expressed in grams. (Samples were dried at 70°C to avoid oxidation of the muck soil). These values were plotted on semi-log paper with the soil moisture ratio on the X-axis and soil moisture potential (bars) on the Y-axis (Figure 1). The line thus generated served as a standard for further soil moisture measurements. This method was more accurate than direct soil tensiometer readings, especially at potentials below -1 bar where tensiometers become generally unreliable.

Variety Trials

In 1979, two variety trials were carried out to determine if there was resistance to radish scab in commercial cultivars. In the first trial, twenty-two predominantly American cultivars obtained from Northrup King (Minneapolis, MN), Ferry Morse (Mountain View, CA), and Harris Seed Co. (Rochester, NY), were compared to the three cultivars (Red Prince, Scarlet Knight, Icicle Short Top) normally grown at the Buurma farm. Plots were set up in a completely randomized block design at site #1 and #3 (relatively dry and wet, respectively). Fourteen cultivars were planted in six 4.6 meter replicate rows while the remaining eleven were replicated either one or two times depending on the amount of available seed. Seeds were planted on May 15 and roots were harvested on June 18 after 33 growing days. At harvest, the center 3 meters of each plot were hand pulled and the radishes topped, washed, weighed, counted, the number of scab lesions

Figure 1. Relationship between soil water potential and the percentage soil moisture by weight in muck soil at the Buurma Farm. Equation of line $\hat{y} = -0.18 + 191$, $r^2 = .97$.


100
10
1
.1
WATER POTENTIAL (- BARS)
100
200
% SOIL MOISTURE BY WEIGHT

counted, and the percentage of infected radishes determined.

The second variety trial was set up to retest 12 cultivars from the previous planting along with four experimental white cultivars from Harris Seed Co. (Rochester, NY). A single randomized block plot was planted at site #1 with six 4.6 meter replicate rows for each cultivar. Planting took place on August 23 and harvest on September 25 after 33 growing days. The soil preparation, planting and harvesting procedures were identical to those used in the first variety trial.

In 1980, two trials were conducted to test European radish cultivars from resistance to scab. The first was planted July 1 and harvested August 4 after 34 growing days, and the second was planted August 11 and harvested September 16 after 36 growing days. Both trials were set up at site #2 in a randomized block design with six 1.5 meter replicates for each cultivar. Plots were harvested as in the earlier variety trials.

Minor Element Trials

An experiment was designed to determine whether or not the presence of various minor plant nutrients could affect disease incidence or severity. Boron, calcium, copper, magnesium manganese, and zinc salts were applied as drenches, using the sulfate salt of each element at rates recommended to correct nutrient deficiencies (Table 4). For each 4.6 meter replicate, the appropriate salt was dissolved in 2.5 liters of water and applied to each plot immediately after planting. Completely randomized plots were set up at site #1 and #3 with six replicates for each treatment. Row spacing was increased for this experiment to 61 cm to avoid cross-contamination of treatments. The cultivar Red Prince was planted on May 29 and harvested on July 10 after 42 growing days. One

hundred radishes were harvested from the center of each replicate and assessed for disease incidence.

PCNB Trials

Pentachloronitrobenzene (PCNB) was tested for its ability to control radish scab. Drenches of Terraclor 75WP were applied (Olin Chemical Co., Little Rock, AR) at 14 kg ai per hectare immediately after planting seeds of the Red Prince cultivar. Six 4.6 meter replicate rows were planted for both treated and control groups at sites #1 and #3. The experiment began May 29 and was harvested July 10 after 42 growing days. One hundred radishes were harvested from the center of each replicate and disease levels were determined.

The effect of application rate and time of application on control of scab by PCNB were tested. A randomized block plot with six 4.6 meter replicates per treatment was planted at site #2 using the Red Prince cultivar. PCNB was applied at planting and at one, two, or four weeks after planting, at rates of 14 and 28 kg ai/ha. Terraclor 10G (Olin Chemical Co., Little Rock, AR) was incorporated in furrow at planting and Terraclor 75WP was used in a drench for the remaining treatments. Radishes were planted August 23 and harvested October 10 after 47 growing days. One hundred radishes from the center of each replicate were pulled and assessed for disease incidence.

Data from the variety trials, minor element trials, and PCNB trials were analyzed using one-way analyses of variance and Duncan multiple range tests at the 0.05 level of significance. Percentage values were converted by arc-sin transformation before analyses. Correlations were made using linear regression analysis.

pH Reduction Trials

The effects of lowering soil pH with elemental sulfur on disease incidence was tested in single split plot at site #1 and #3. On May 8 ground sulfur was spread by hand over plots measuring 6 x 6 meters at rates of 112, 560, and 1120 kg/hectare. The sulfure was immediately incorporated into the top 15 cm of soil with a rototiller. Control plots receiving no sulfur were also tilled. The pH of each plot was monitored regularly and Red Prince was planted through the plots on June 7, 28 days after incorporation of the sulfur. After 63 growing days, 60 radishes were harvested from each treatment plot and disease levels were determined.

Aluminum sulfate was also tested for its ability to lower soil pH and affect disease incidence. $AlSO_4$ was incorporated into 3 x 12 meter test plots at rates of 2800 and 5600 kg/hectare. A Scott fertilizer spreader was used to distribute the $AlSO_4$ evenly and a rototiller was used to mix it into the top 15 cm of soil. Scarlet Knight and White Icicle radish seeds were planted in alternating rows through each plot three weeks after $AlSO_4$ incorporation. A third plot was rototilled and planted as a control. $AlSO_4$ was incorporated on June 12, radishes were planted July 3, and harvested August 4 after 32 growing days. Harvested radishes were examined for disease levels.

Soil samples removed for pH determinations were mixed at a ratio of 20 ml distilled H_2O per 10 gm soil. These were allowed to sit for 1/2 hour before measuring pH with a Markson Model 4407 ElectroMark Analyser. The average of readings from five samples for each treatment was recorded.

Irrigation Trials

To study the effects of soil moisture on disease development, two blocks consisting of four 6 meter rows of Red Prince were planted with a

30 cm row spacing. Soil tensiometers were set up at each plot to measure soil moisture at a depth of 5 to 10 cm. One hundred and fifty liters of water were added once each week to one plot while the other received only normal rainfall. The experiment began August 23, 1979, and was harvested November 11 after 56 growing days. 320 radishes were pulled from each plot and disease levels were determined.

In the 1980 irrigation trials, three levels of moisture were maintained; low (below -2 bars), normal (-.5 to -2 bars), and high (0 to -0.5 bars). Two 2.4 x 2.4 meter rain shelters were constructed and utilized to block rainfall and maintain dry soil conditions. These were constructed of wood frames and covered with 2 mm clear corrigated fiberglass. Steel pipe (2.54 cm diameter) was used for the legs and the sides remained open for air circulation. The tops of the shelters sat approximately one meter above the soil when anchored in place. The high moisture plots were irrigated using a small gasoline-powered irrigation pump and standard 7.6 cm diameter irrigation pipe and heads. The first irrigation study was set up in a field which had been planted with Scarlet Knight with a row spacing of 15 cm using the Buurma farm's standard planter. The rain shelters were placed randomly in the field but well separated from the irrigation system to avoid water drift. Soil moisture was monitored on a semi-weekly basis by comparing the weight ratios (H_2O/soil) of samples to a standard moisture-potential curve previously generated. The irrigation system was turned on to deliver approximately 2.5 cm of water when soil moisture dropped below 0.5 bars. The trial began July 1 and was harvested August 4 after 33 growing days. All of the radishes from a 1.8 x 1.8 meter area under the center of each rain shelter were harvested and an equivalent number harvested at random from the nontreated and irrigated

plots. These radishes were assessed for disease incidence and severity. The irrigation trial was repeated with a planting on August 11 which was harvested September 26 after 36 growing days.

In order to further determine the relationship of disease incidence to environmental conditions, a 4.6 meter row of Red Prince radish was planted each week for 8 weeks. Rainfall, air temperature, relative humidity, and soil temperature were monitored continuously while soil moisture was monitored biweekly during this period. Each row was harvested approximately four weeks after planting and assessed for disease incidence.

Laboratory Isolations

A number of techniques were utilized in isolating and culturing streptomycetes from infected tissues. Scab-infected potato tubers were surface-sterilized with 10% Clorox for 5 minutes, then washed in distilled water to remove excess bleach. The potatoes were split through the scab lesions and a small amount of tissue lying directly under the lesion was removed. This was placed in a flamed mortar and ground with sterile water under aseptic conditions. One milliliter portions of this slurry were placed in petri dishes and 15 ml of liquid chitin agar (39) with pH adjusted to 10.5 (20 ml of .1 M NaOH added to 180 ml agar) was added to each. The plates were gently swirled and incubated in fluorescent room light at 22°C for 7 to 10 days. Colonies were transferred to water agar (pH 10.5) for further characterization. One streptomycete (P4) was isolated consistently using this technique and was used in subsequent greenhouse and laboratory studies.

A variety of media were used in attempts to isolate *S. scabies* from radish including potato dextrose agar, chitin agar, Czapek's agar,

Czapek's soil extract agar, radish agar, Streptomyces-minimal medium, and others at a pH of either 7.0 or 10.5. In all cases, plates were either contaminated with other bacteria and fungi or else afforded no growth. A number of procedures were tried in the preparation of radish tissue for isolation. Lesions of different size and age were excised from diseased roots with or without prior surface sterilization. Sodium hypochlorite (10% Clorox) or mercuric chloride was used at various concentrations and for various amounts of time. Diseased tissues were removed from different parts of lesions (edges, centers, or underlying tissue) and either plated directly onto solid media, incorporated into liquid agar media, or ground in sterile distilled water, diluted, and then plated in liquid agar media. Plates were incubated in light or dark at either 22°C or 27°C.

Streptomyces spp. were successfully isolated from scab-infected radishes by washing infected roots in running tap water for 5 to 10 minutes. Lesions 5 mm or less in diameter were cut out and crushed in a flamed mortar with 0.1 M phosphate buffer (pH 8.5). The resultant slurry was then diluted serially in sterile distilled water. 0.5 ml aliquots of the 10^0, 10^{-1}, 10^{-2}, and 10^{-3} dilutions were added to petri plates, and 15 to 20 ml of chitin agar were added to each. In this procedure the chitin agar was made using 0.01 M phosphate buffer (pH 8.5) instead of distilled water. Plates were gently swirled and incubated in room light at 22°C for 7 days. Individual colonies were transferred to water agar or PDA for further study.

Only one isolate, R1, was recovered from a scabbed radish by removing a 2 mm diameter lesion without previous washing or surface sterilization of the radish, and plating it directly onto water agar. This isolate was used in further greenhouse and laboratory studies. The extremely low

recovery rate using this technique suggested a possible inhibitory effect of the radish tissue on growth of the pathogen on artificial media.

This possibiilty was explored by first forming wells in chitin agar plates with a 5 mm cork borer. Radish root filtrates were obtained by shaving off the outer layers of 20 healthy radish roots and grinding them in a blender with 50 ml of distilled water. This homogenate was filtered through cheesecloth and No. 4 Whatman filter paper. Plugs were taken from healthy radishes after surface sterilization using a 5 mm cork borer. Portions of the filtrate and plug preparations were autoclaved at 15 lb for 20 minutes. Wells in the chitin agar plates were filled with sterile or non-sterile radish filtrate, radish plugs, or sterile distilled water as a control. Ten plates thus prepared were sprayed with a suspension of the R1 isolate 4 days prior to the filling of the wells while ten more plates were sprayed immediately after the wells were filled. The plates were incubated at 22°C for 10 days at which time they were checked for inhibition of growth.

Two strains of Streptomyces scabies, 3352 and 10246, were obtained from the American Type Culture Collection (Rockville, MD). These were originally isolated from scabbed potatoes.

Characterization of Streptomycete Isolates

Tyrosinase activity of Streptomyces spp. isolates was determined using tyrosine-casein-nitrate agar (49). Characteristics on triphenyl tetrazolium chloride (TTC) media were observed and the ability to utilize starch (Sx media) was determined (61). Reactions on citrate medium (61), phenol red-dextrose medium (Difco), and in litmus milk (Difco) were determined. Potato plugs were cut from firm potatoes and autoclaved at 20 minutes at 15 psi, inoculated with test strains, and then incubated

for one week. The plugs were checked for disintegration of the tissue and pigment formation. Growth characteristics of the Streptomyces spp. were observed on water agar and potato dextrose agar (Difco), glycerol-asparagine agar (56), and starch agar prepared with 20 gm soluble starch, 23 gm nutrient agar (Difco), and 1 liter of H_2O.

For electron microscopy, two or three square centimeter slabs of starch agar or PDA cultures of Streptomyces spp. were fixed in a 1:1 mixture of 2% Osmium tetroxide and 0.2 M phosphate buffer (pH 7.2) for two hours followed by dehydration in ethyl alcohol. Specimens were critical point dried, gold-coated, and examined in a JEOL JSM-35C scanning electron microscope at an accelerating voltage of 15 kV. Secondary electron images were recorded on Polaroid type 665 P/N film.

Greenhouse Trials

A number of methods were utilized in attempts to produce disease in greenhouse grown radishes. The first method consisted of filling 20 cm diameter clay pots with infested soil from the Buurma farm into which four radish seeds were planted. The pots were given full sunlight throughout the experiment. Greenhouse temperatures ranged between 27°C $\pm$ 5°C days and 18°C $\pm$ 5°C nights. Peter's 20-20-20 soluble fertilizer was applied as a drench prior to planting.

In a second trial, plastic pots were used instead of clay, and potted radish seedlings were kept in water baths to maintain soil temperatures of 13°C, 15.5°C, 18°C, and 21°C for the duration of the growing period. A third method, similar to the first, utilized 30 cm diameter pots and artificial fluorescent lighting (1500 lux) for 12 hours per day.

In the winter of 1980, special growing boxes for the greenhouse were constructed of 1.9 cm thick exterior plywood and waterproof Recorcinol

glue, and measured 1.21 meters long x 15 cm wide at the tope x 20 cm deep. The boxes tapered down to 2.5 cm wide at the bottom in a V-shape and each held ca. 0.2 m^2 of soil. A bank of alternating warm white and cool white 40 watt fluorescent lights was suspended 0.6 meters above the top of the boxes giving a light intensity of 22000 lux.

For the following experiments, a single row of radish seeds were planted down the center of each box at 12 seeds per foot after treatment of the soil with Peter's 20-20-20 fertilizer. Fertilizer was applied again two weeks after planting. Radishes were given 16 hours of light per day and the air temperature was maintained at 27°C $\pm$ 5°C days and 21°C $\pm$ 3°C nights. Red Prince was used for all experiments except where noted. All soil was taken from the scab-infested fields at the Buurma farm and stored in large pails for future use. Steamed soil was prepared by steaming in plastic or wooden flats for at least 8 hours at which time the soil temperature had reached 98°C.

Isolates for pathogenicity tests were grown on water agar (pH 10.5) or starch agar as a lawn for ca. three weeks at which time they were scraped (agar included) into a blender and homogenized with distilled water. Thirty 100 x 15 mm agar plates were used for each box. The resultant mixture was adjusted to a pH of 10.5 with 0.05 M NaOH and incorporated into the top 7 cm (.01 m^3) of soil in a growing box. This was allowed to dry for 2 to 3 days before planting. Colony forming unit (CFU) concentrations in the soil were determined from the agar-water homogenate through serial dilution and replating. Controls consisted of both steamed and non-steamed soil treated with sterile agar homogenate.

Scab-infected potato skins were ground in water, adjusted to a pH of 10.5, and incorporated into soil for some pathogenicity tests.

Approximately 800 gm of ground potato skins were mixed with .01 m^3 soil, for each box, but CFU concentrations were not determined.

To ensure dry soil conditions around expanding roots, a sub-irrigation system was installed in the bottom of each box for one of the pathogenicity trials.

Inoculation of radish roots at different stages of development was carried out by using liquid spore suspensions of the R1 strain originally isolated from scab-infected radishes. These were prepared by scraping the aerial mycelia from 30 100 x 15 mm water agar plates and suspending into 125 ml sterile distilled water. Five ml of an 8 x 10^5 CFU/ml suspension were then injected into the soil surrounding enlarging radish roots with a large syringe and an 18 gauge needle. Inoculations were carried out 0, 4, 8, 13, 17, and 22 days after planting using 25 plants each time. This experiment was repeated with inoculations at 6, 11, 16, 21, and 26 days after planting. In both cases the radishes were harvested after 40 growing days.

Direct inoculations were attempted using liquid spore suspensions as previously described. A 22 gauge needle and syringe was used to prick the enlarging radish roots at various depths in the soil while injecting a small amount of inoculum in and around the wound sites. Control plants were either not treated or inoculated using distilled water.

Experiments were carried out in which radish plants were transplanted from infested to non-infested steamed soil and visa versa at 8, 12, 16, 20, and 24 days after planting. Care was taken to wash all soil from the roots before replanting. Diseased and healthy controls were not transplanted. Radishes were harvested 35 days after planting and disease levels were determined.

A fast method for detection of pathogenic Streptomyces spp. was explored in the greenhouse. Radishes were grown in steamed soil and divided into groups of 20. After 21 days, the enlarging roots were carefully uncovered and pricked repeatedly with a small gauge needle. Liquid suspensions of R1, 3352, or 10246 were spread around the wound sites of each radish in a group. The roots were then covered and harvested 14 days later. One group of radishes was inoculated with sterile distilled water while another group was grown in R1-infested soil.

RESULTS

Variety Trials

In the first set of variety trials conducted in 1979, 25 radish cultivars exhibited a wide range in disease frequency (Tables 1, 2). No cultivar tested was highly resistant to scab. At both plot locations Icicle Short Top, a white cultivar, and Scarlet Turnip White Tip, a red/white hybrid, had the lowest scab levels of all those tested. At site #1, disease levels ranged from 46% to 100% while at site #3 disease levels ranged from 3.5% to 64%. There was little consistency between relative disease levels of the cultivars at the two sites; i.e. Red Prince had less disease than most red cultivars at site #1 but had more than most at site #3.

In the second set of 1979 variety trials, one red/white hybrid, five white, and ten red cultivars were compared for resistance to scab. Again, none of the cultivars was highly resistant to disease with scab incidence ranging from 63% to 92% (Table 3). All of the white cultivars had lower disease levels than the red cultivars. In all of the variety trials, the mean numbers of lesions/radish were positively correlated with disease frequency (typically $r^2 = .7$).

Since none of the American varieties tested in 1979 showed resistance to scab, a number of European cultivars were tested in 1980. Radish scab has been reported in only isolated cases in Europe (30), suggesting that varieties grown there may be resistant to the disease. The data from the

Table 1. Scab incidence on radish cultivars grown in drained muck soil on the Buurma farm (site #1).

Cultivar	% Scabbed radishes[a]	Mean lesions/ radish	Mean number radishes/rep[b]	Mean weight/ radish (gm)
Inca	88 A	5.9	78	10.6
Red Boy	82 AB	2.7	97	12.7
Comet,	82 AB	3.0	78	13.8
Champion	81 AB	4.6	95	15.4
Scarlet Knight	79 AB	4.0	96	12.4
Red Devil B	76 AB	2.3	84	12.6
Cherry Belle 6D	74 AB	2.8	91	14.1
Fuego	74 AB	2.2	77	11.6
Red Devil	73 AB	2.7	84	14.3
Fancy Red	67 AB	1.7	79	10.4
Red Prince	63 ABC	2.2	81	11.1
Far Red	58 BC	1.6	73	11.5
Scarlet Turnip White Tip	58 BC	1.7	86	11.3
Icicle Short Top	46 C	1.6	95	15.7
Kutura Hybrid*	100	5.9	91	12.4
Saxa-Treib*	95	9.9	76	13.8
Gaudry*	90	3.6	73	11.2
Carnita*	90	4.0	81	13.7
Parat*	90	3.7	91	13.3
Real*	85	3.6	85	11.6
Exp CHPR/3808*	85	4.7	73	9.7
Saxafire*	83	3.0	68	12.1
Scharo*	80	3.8	70	12.0
Rico*	65	2.7	84	13.1
Exp PRA/3398-3408*	55	1.5	87	10.0

[a]Percentages with same letters not significantly different at P=.05 (Duncan Ranges).

[b]Mean number of radishes harvested from center 3 m of a single replicate.

Cultivars followed by (*) replicated once or twice and not subject to statistical analysis.

Table 2. Scab incidence on radish cultivars grown in nondrained muck soil on the Buurma farm (site #3).

Cultivar	% Scabbed radishes[a]	Mean lesions/ radish	Mean number radishes/rep[b]	Mean weight/ radish (gm)
Red Prince	23	1.8	99	15.6
Inca	21	2.2	81	14.7
Comet	20	1.4	88	15.2
Red Devil	14	1.6	98	16.3
Red Devil B	14	1.6	91	17.5
Fancy Red	13	2.1	87	14.1
Cherry Belle 6D	12	2.4	93	15.4
Red Boy	12	1.8	103	13.4
Scarlet Knight	12	2.0	95	12.5
Fuego	11	1.3	90	13.7
Far Red	10	1.7	79	9.1
Champion	10	1.3	104	23.5
Scarlet Turnip White Tip	6	1.0	107	14.0
Icicle Short Top	3.5	1.3	86	21.4
Real*	64	2.5	90	18.3
Saxafire*	45	2.4	94	17.4
Parat*	43	3.4	102	22.1
Scharo*	42	3.5	69	16.8
Saxa-Treib*	37	1.2	89	21.3
Rico*	26	1.6	80	22.0
Gaudry*	24	1.6	86	14.1
Carnita*	14	2.0	79	15.6
Exp PRA/3398-3408*	11	1.3	79	11.2
Kutura Hybrid*	4	1.3	85	13.1

[a]Percentages not significantly different at P=.05 (Duncan Ranges).

[b]Mean number of radishes harvested from center 3 m of a single replicate.

Cultivars followed by (*) were replicated only once or twice and were not subject to statistical analysis.

Table 3. Scab incidence on radish cultivars grown in drained muck soil on the Buurma farm (site #2).

Cultivar	% Scabbed radishes[a]	Mean lesions/ radish	Mean number radishes/rep[b]	Mean weight/ radish (gm)
Champion	92 A	7.9	99	16.4
Exp PRA/3398-3408	90 AB	6.3	108	13.3
Scarlet Knight	90 ABC	5.1	92	14.5
Red Devil B	89 ABC	4.9	91	18.0
Red Devil	87 ABC	5.1	99	14.1
Fuego	86 ABC	4.3	94	11.6
Scarlet Turnip White Tip	85 ABCD	6.4	96	12.6
Fancy Red	84 ABCD	4.4	92	9.9
Far Red	84 ABCD	4.7	81	6.9
Cherry Belle 6D	83 BCDE	3.7	82	16.1
Red Boy	77 CDE	4.4	104	9.7
XP 3568	72 CDE	2.7	86	16.4
XP 3778	70 DE	3.6	89	14.7
White Icicle	70 DE	3.3	90	13.0
Icicle Short Top	69 E	2.4	95	15.2
XP 3728	63 E	2.4	90	16.2

[a]Percentages with the same letter are not significantly different at P=.05 (Duncan Ranges).

[b]Mean number of radishes harvested from center 3 m of a single replicate.

variety trial, were discarded due to an apparent ambiguity in the labelling of the cultivar lines, while disease levels were so low in the second trial that a meaningful analysis of the data was not possible.

Minor Nutrient Trials

Radishes grown in soil treated with various minor plant nutrients were analyzed for disease incidence. No differences were detected in growth characteristics, harvest size, or harvest weight between treated plants and controls (Table 4). Also, there was no significant difference in disease levels between treated plants and controls. As in the variety trials, scab incidence was substantially lower at site #3 than at site #1.

PCNB Trials

When PCNB was added as a granular formulation at 14 kg/ha ai at planting, no control of radish scab was obtained. At site #1, the treated plots had an average of 64% infection compared with 51% for controls. At site #2 scab incidence was 37% and 33% for diseased and control plots, respectively. In the second PCNB experiment, the fungicide was added at various times and at two concentrations through the growing period. When added as a drench one week after planting at 28 kg/ha ai, PCNB significantly reduced scab incidence (50% in the treated as opposed to 94% in the control) (Table 5) while scab incidence was not significantly reduced on radishes treated with 14 kg/ha ai one week after planting. None of the other treatments differed significantly from the nontreated controls.

pH Reduction Trials

Elemental sulfur was effective in lowering the pH of the muck soil at all concentrations tested. The pH began to drop immediately after application and continued to drop for almost three months after which it began

Table 4. Scab incidence on radishes grown in soil amended with minor nutrient elements.

Treatment	Rate (kg ai/hectare)	Scabbed radishes[a] Site #1	Scabbed radishes[a] Site #3	Mean Lesions/radish Site #1	Mean Lesions/radish Site #3
Boron	1.1	81	40	1.9	5.9
Calcium	22.5	66	34	2.2	2.9
Copper	5.6	60	32	1.8	4.0
Magnesium	22.5	62	21	1.9	1.9
Manganese	9.0	64	29	1.9	2.6
Zinc	3.4	60	44	2.2	4.1
Control	--	51	33	1.9	2.2

[a]Values are the mean number of diseased radishes out of 100 harvested from each of six replicates per site. Values are not significantly different at P=.05 (Duncan Ranges).

Table 5. Effects of PCNB rates, and time of application on disease incidence with the Red Prince cultivar.

Treatment	PCNB (kg/hectare ai)	Scabbed Radishes[a]
In furrow at planting	14	97.0 A
In furrow at planting	28	96.0 AB
Drench; one week after planting	14	77.0 C
Drench; one week after planting	28	50.0 D
Drench; two weeks after planting	14	89.0 ABC
Drench; two weeks after planting	28	87.0 BC
Drench; four weeks after planting	14	94.0 AB
Drench; four weeks after planting	28	93.0 ABC
Control[b]	--	94.0 ABC

[a]Values are the mean number of diseased radishes out of 100 harvested from each of six replicates. Values with same letters not significantly different at P=.05 (Duncan Ranges).

[b]The control radishes received no water other than normal rainfall.

to rise. Radish growth was extremely limited in the plots treated with 1120 kg/ha where the pH dropped to 3.4. Growth was somewhat limited in the other plots treated with sulfur as compared to control plots. Disease levels did not appear to differ significantly between treated and control plots at site #1 or site #3 (Table 6). In general, disease levels were lower at site #3 than at site #1.

It was difficult to determine the effects of soil acidification on disease since the treatment had such an adverse affect on the growth of the radish plants. To alleviate the possible toxic affects of incorporated sulfur, $AlSO_4$ was used in a subsequent experiment. $AlSO_4$ is more soluble than elemental sulfur and should thus cause a faster drop and leveling off of pH. In the plot treated with 5600 kg/ha of $AlSO_4$, the pH dropped from 6.5 to 5.5 in one week. The pH dropped to 5.9 after one week in the plot treated with 2800 kg/ha of $AlSO_4$. In both plots the pH began to rise slowly after one week (Table 7). Neither Red Prince nor White Icicle showed observable differences in harvest size or disease incidence from the control plot.

Irrigation Trials

In the 1979 irrigation study, soil tensiometers were used to measure soil moisture at 7.5 cm depth. The irrigated plot ranged between -0.1 and -1.7 bars while the non-irrigated plot ranged from -1.9 to -3.3 bars. Radishes harvested from the irrigated plot were 20% heavier in fresh weight than those from the non-irrigated plot. Irrigated radishes had 31% less scab and 75% fewer lesions per radish than the non-irrigated radishes (Table 8).

Soil moisture potential was measured more precisely in the 1980 irrigation studies using a soil moisture curve generated from pressure plate

Table 6. Scab incidence on radishes grown in soil amended with elemental sulfur.

Rate[a]	Soil pH at planting	Soil pH at harvest	Total harvested radishes	% Scabbed radishes	Mean lesions/radish	Mean weight/radish (gms)
280 kg S/ha						
Site #1	5.35	5.75	60	22	1.5	15.8
Site #3	5.55	5.9	60	10	1.2	18.8
560 kg S/ha						
Site #1	4.4	4.7	41	29	1.3	15.8
Site #3	5.2	4.45	60	10	2.0	18.7
1120 kg S/ha						
Site #1	4.1	3.7	18	11	1.0	8.3
Site #3	4.6	4.1	60	7	1.9	16.2
Control						
Site #1	6.2	6.25	60	27	1.7	18.3
Site #3	6.3	6.5	60	10	1.2	21.7

[a]Sulfur was incorporated 1 month prior to planting.

Table 7. Scab incidence on Red Prince and White Icicle radishes grown in soil amended with $AlSO_4$.

Rate	Cultivar[a]	Mean pH at planting	Mean pH at harvest	% Scabbed[b] radish	Mean lesion/radish	Mean weight/ radish (gms)
2800 kg/ha	RP	6.04	6.24	34	1.9	10.1
2800 kg/ha	WI			66	2.0	8.0
5600 kg/ha	RP	5.75	6.05	69	2.9	10.9
5600 kg/ha	WI			51	1.7	13.8
Control	RP	6.51	6.70	24	2.3	7.9
Control	WI			36	1.5	8.1

[a]RP = Red Prince, WI = White Icicle.

[b]Percentage values based on 400 radishes harvested for each treatment.

Table 8. Scab incidence on radishes grown in irrigated and nonirrigated muck soils.

Treatment	% Scabbed[a] radishes	Mean number lesions/radish	Mean weight/ radish (gm)
Non-irrigated plot (-1.9 to -3.3 bars)	91	8.29	13.9
Irrigated plot (-0.1 to -0.7 bars)	60	2.05	17.4

[a]Percentage values based on 320 radishes harvested from each plot.

determinations. Also, water was delivered more uniformly and in greater quantities than in the previous experiment due to the irrigation system used. Soil moisture potentials in the irrigated plot ranged between -0.3 and -0.58 bars, the non-irrigated plot from -0.45 to -2.5, and the dry plot from -1.8 to -19.0 bars. The radishes harvested from the irrigated plot had 50% less scab than those grown in dry soil and 33% less than those receiving normal rainfall (Table 9). Radishes from the irrigated plot were again larger and heavier than those from the other plots.

Due to a mechanical breakdown in the irrigation system, it was not possible to maintain high soil moisture levels in the irrigated plots during the second irrigation experiment. Disease levels were similar in the irrigated and normal rainfall plots with 12% and 10% scab, respectively, while in the dry plot the scab incidence was 24% (Table 10).

Radishes were planted weekly from May 23 to July 24. Each planting was harvested approximately one month after planting and assessed for disease levels. A high negative correlation (r^2 = .88) was found between scab incidence for each planting and the amount of rainfall over the first two weeks of each planting (Table 11, Figure 2).

Laboratory Isolations

Pfleger (53), reported isolating *S. scabies* by plating infected tissue on water agar and incubating for 10 days at 75°F. This technique did not lead to successful isolation of any actinomycetes in the present study. In over 800 platings, using a variety of different methods, only one streptomycete (R1) was isolated from radish. At the same time, *Streptomyces* spp. could be isolated from potato 4 out of every 20 plates using the same techniques. This suggested that there may have been some inhibitory compound present in radish tissue that suppressed the growth of

Table 9. Scab incidence on radishes grown under different soil moisture regimes (trial #1).

Treatment	% Scabbed[a] radishes	Mean number lesions/radish	Mean weight/ radish (gm)
Dry plot (-1.8 to -19 bars)	74	3.3	5.0
Normal rainfall plot (-.45 to -2.5 bars)	57	2.2	6.4
Irrigated plot (-0.3 to -0.58 bars)	24	2.4	8.1

[a]Percentage values based on 200 radishes harvested from each plot.

Table 10. Scab incidence on radishes grown under different soil moisture regimes (trial #2).

Treatment[a]	% Scabbed[b] radishes	Mean number lesions/radish	Mean weight/ radish (gm)
Dry plot (-1.5 to -5.9 bars)	24	2.1	4.8
Normal rainfall plot (-0.56 to -0.6 bars)	10	1.9	9.1
Irrigated plot (-0.4 to -0.58 bars)	12	1.5	9.3

[a]Due to a malfunction of the irrigation system, the irrigated and normal plots were at similar moisture levels through most of the growing period.

[b]Percentage values based on 200 radishes harvested from each plot.

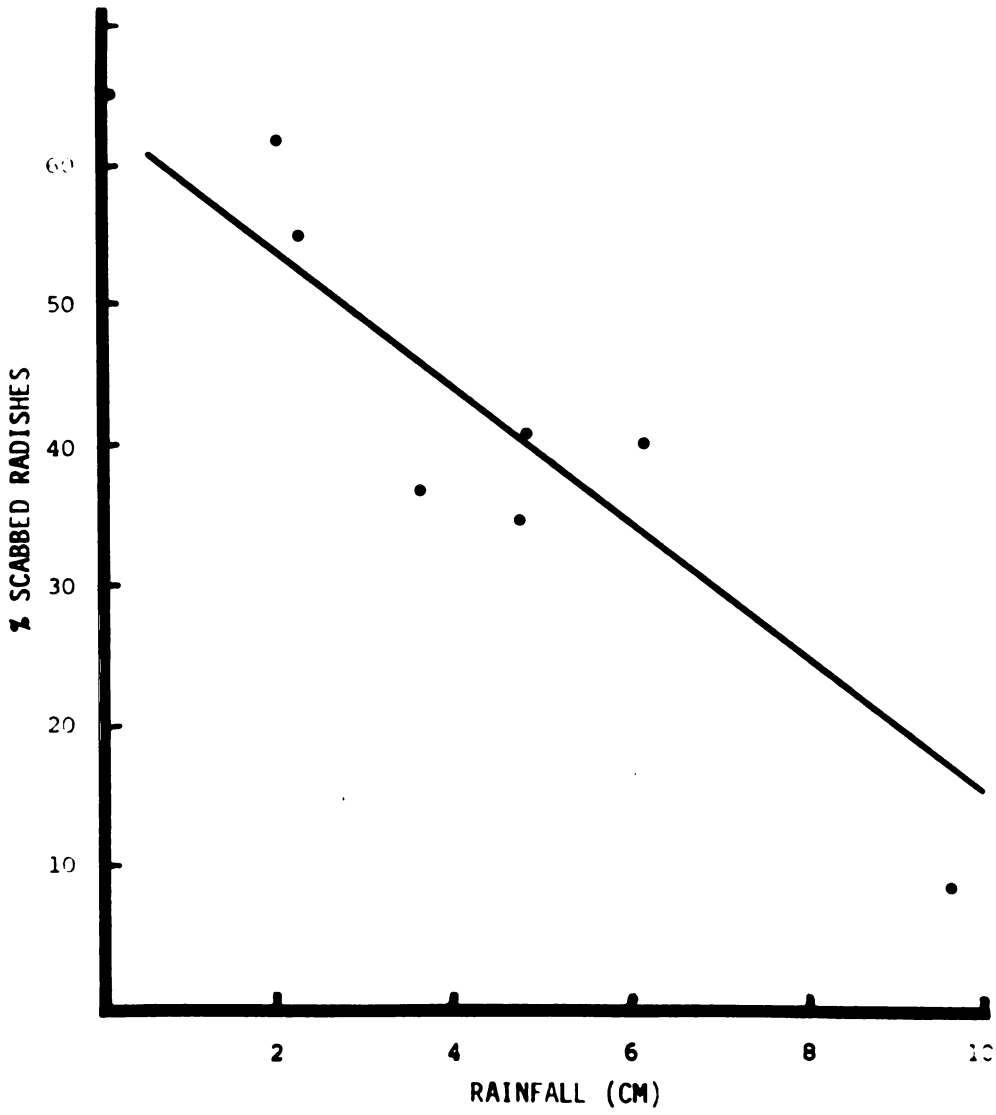
Table 11. The relationship of scab incidence on radishes planted at different times during the growing season with rainfall levels during the first half of each growing period.

Date planted	Date harvested	Rainfall (cm)[a]	% Scabbed radishes[b]
5/23	6/26	4.7	35
5/30	7/1	6.1	42
6/12	7/10	2.2	55
6/19	7/17	0	65
6/26	7/24	2.0	62
7/1	8/4	4.8	43
7/10	8/11	3.6	37
7/24	9/4	9.6	8

[a]Rainfall during the first half of each growing period.

[b]Percentage values based on harvests ranging from 58 to 124 radishes.

Figure 2. Correlation between scab incidence and rainfall during the first half of each growing period. Linear regression significant at P=.01. Equation of line $\hat{y} = -5.8x + 67$, $r^2 = 88$. Data from Table 12.


60
50
40
30
20
10
% SCABBED RADISHES
2
4
6
8
RAINFALL (CM)

the pathogen on artificial media. In all experiments designed to test this possibility, the streptomycete used was able to grow up to all of the wells and in some cases growth seemed to be enhanced. This would indicate that there was no diffusable inhibitory compound in radish tissue responsible for the inability to isolate the pathogen.

Streptomyces spp. were finally isolated from young scab lesions when 0.1 M phosphate buffer (pH 8.5) was used in the grinding and subsequent dilution of tissue before addition to chitin agar which had also been prepared using 0.1 M phosphate buffer (pH 8.5). With this procedure, streptomycetes were isolated in 6 out of every 10 plates.

Characterization of Streptomycete Isolates

A comparison was made between the R1 and P4 streptomycete strains isolated from a naturally infected radish and from a scabby potato peeling, respectively, and strains 3352 and 10246 which were obtained from the ATCC and were isolated originally from scabby potatoes. Eleven other strains of streptomyces were isolated from radish scab lesions but since their pathogenicity could not be determined, they were not included in the present study.

The rate of growth and color of aerial and substrate mycelia differed between the strains tested on various media (Table 12). The reactions of the four isolates on indicator media and in other biochemical tests also varied (Table 13). SEM observations revealed different spore and sporophore morphology for each isolate. The P4 isolate exhibited spiral spore chains while the other three had straight spore chains (Figures 3a, 3c, 4a, 4c). The spores of the R1 isolate appeared relatively long and cylindrical measuring approximately 0.8 µm long and 0.5 µm in diameter (Figure 3b). The spores of the P4 isolate were smooth and approximately

Table 12. Growth characteristics of 4 Streptomyces spp. on various culture media.

Strain	Media[a]			
	WA[b]	PDA[b]	SA[c]	Asparagine Glycerol Agar[d]
R1	sparse aerial growth white	heavy aerial growth dark gray substrate mycelium dark olive	heavy aerial growth light gray not pigment media	heavy aerial growth light gray no pigment in medium
P4	sparse aerial growth white	heavy aerial growth ivory no pigment in medium	slow aerial growth white no pigment in medium	heavy aerial growth gray-pink no pigment in medium
3352	no aerial growth	heavy aerial growth gray substrate mycelium dark olive	heavy aerial growth dark gray brown pigment in medium	heavy aerial growth dark gray no pigment in medium
10246	no growth	light aerial growth salmon-pink substrate mycelium red	light aerial growth white no pigment in medium	no aerial growth no pigment in medium

[a]Cultures grown for 14 days at 22°C in the light.

[b]Difco Laboratories (Detroit, MI).

[c]10 gm Soluble Starch, 23 gm Nutrient agar (Difco) in 1 L H_2O.

[d]Pridham et al. (56).

Table 13. Results of biochemical tests using 4 Streptomyces spp.

Strain	Litmus Milk[a]	Phenol Red-Dextrose[a]	Growth on SX agar[b]	Potato Plug	Growth on TTC[b]	Citrate[b]	TCN[c]
R1	alkaline coagulated	alkaline	+, clearing	dark brown pigments	+	no growth	+
P4	alkaline coagulated	alkaline	+, clearing	dark pigment	-	no growth	+
3352	alkaline not coagulated	alkaline	-, no clearing	no pigment	+	no growth	-
10246	alkaline not coagulated	alkaline	+ clearing	no pigment	+	no growth	-

[a]Difco Laboratories (Detroit, MI).

[b]Schaad (61).

[c]Menzies (49).

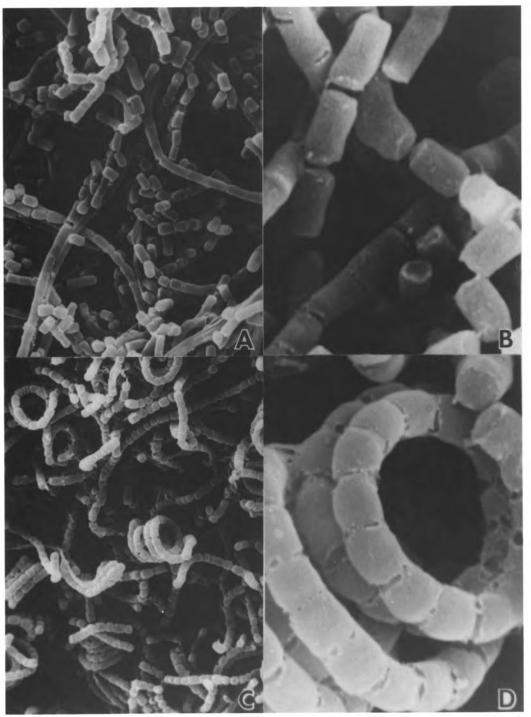
Figure 3. SEM micrographs of spores and spore chains of the R1 and P4 streptomycete isolates.

A. R1 isolate; spore chains. X5600

B. R1 isolate; spores. X23,500

C. P4 isolate; spore chains. X5600

D. P4 isolate; spores. X23,500


A
B
C
D

as long as they were wide, measuring 0.6 x 0.6 μm (Figure 3d). The 3352 strain had barrel-shaped spores which were tapered slightly at each end and measured approximately 0.8 μm long by 0.7 μm in diameter at the center (Figure 4b). The spores of the 10246 strain were similar in size to the P4 isolate but exhibited a marked rough spore wall morphology (Figure 4d).

Greenhouse Trials

No scab was detected on radishes grown in pots with S. scabies infested soil, or in pots with infested soil in temperature controlled water tanks. Supplemental low-intensity artificial lighting (1500 lux) failed to improve root development in these experiments. The radish roots were often small and sometimes did not expand at all.

Radishes grown in soil placed in special boxes under intense artificial (22000 lux) lighting grew quickly and root expansion occurred normally. The R1 isolate was incorporated into soil at concentrations of 2×10^6 and 4×10^6 cfu/cm^3. The P4 isolate was incorporated at 5×10^6/cm^3 soil. Inoculum concentration was not determined for scabby potato peelings. Normal disease symptoms appeared on radishes grown in boxes inoculated with scabby potato peelings and the R1 isolate (2×10^6 cfu/cm^3) through four trials. Scab incidence decreased through the four trials on radishes grown in soil infested with the P4 isolate and R1 (4×10^6 cfu/cm^3) (Table 14). This may have been due to the loss of the starch agar substrate in the soil through the four trials, resulting in a gradual decline in the pathogen population levels. In trials 1 and 2 the control plants grown in non-steamed soil with sterile water agar added did not show disease symptoms. In the third and fourth trials, however, the same control treatments showed low scab incidence while a steamed control

Figure 4. SEM micrographs of spores and spore chains of the 3352 and 10246 streptomycete isolates.

A. 3352 isolate; spore chains. X5600

B. 3352 isolate; spores. X23,500

C. 10246 isolate; spore chains. X5600

D. 10246 isolate; spores. X23,500

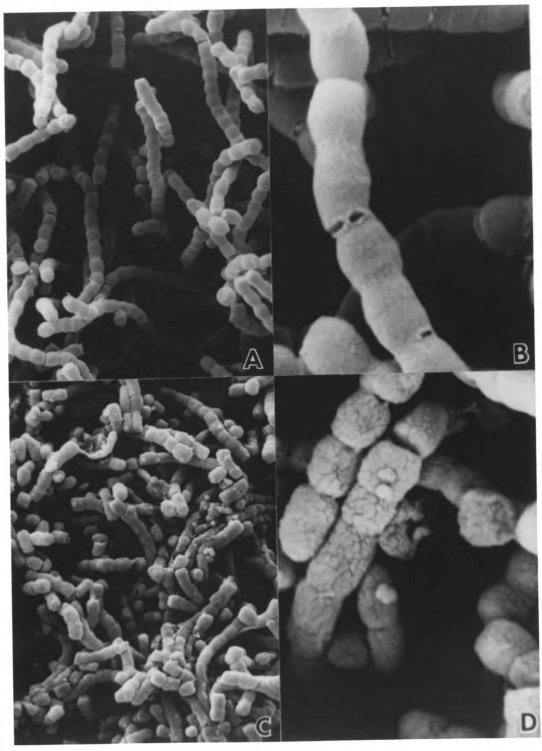

A
B
C
D

Table 14. Scab incidence on radishes grown in soil infested with two Streptomyces spp. and scabbed potato peelings.

Trial	Control	Percent scabbed radishes[a] R1 strain[b] low inoculum	R1 strain[c] high inoculum	P4 strain	Scabby peelings
1	0	16	23	55	65
2	0	60	17	1	96
3	10, 0[d]	29	8	0	56
4	5, 4[d]	19, 24[e]	--	--	80

[a]Percentage values based on approximately 20 radishes harvested from each of two replicates per treatment.

[b]2×10^6 CFU/cm^3 soil.

[c]4×10^6 CFU/cm^3 soil.

[d]Unsteamed and steamed soil controls, respectively.

[e]R1 inoculum added to unsteamed and steamed soil, respectively.

in the fourth trial had 10% scab. This may have been due to uneven heat distribution during soil steaming resulting in the survival of the pathogen and subsequent disease.

In a fifth pathogenicity trial, eleven strains of Streptomyces isolated from diseased radishes in the greenhouse were tested along with the R1, P4, and ATCC strains. No scab was detected on any of the radishes. This may have been due to extremely dry surface soil conditions since sub-irrigation was used in this experiment.

An experiment was designed to test whether radishes were more susceptible to scab during certain periods of growth. Maximum infection occurred when inoculum was added at 13 or 16 days after planting, respectively, for two separate trials (Tables 15, 16). Low numbers of plants harvested, especially in the first trial, may have been due to aphid and white fly damage in the greenhouse.

In a second procedure, radishes were transferred from infested soil to steamed soil and from steamed to infested soil at various times after planting. Scab incidence was virtually the same in radishes transferred from steamed to infested soil at 8 through 24 days after planting (Table 17). Likewise, scab incidence was similar among radishes transferred from infested to steamed soil over the entire range of transplanting times. Scab incidence in the steamed control was 6%, but all of the transplanted radishes (those transferred into and those transferred out of steamed soil) showed disease levels similar to the infested control plants (41%).

In the experiment testing for a direct inoculation procedure (stabbing with a syringe), none of the radishes showed symptoms of scab whereas 58% of the control radishes planted in steamed, R1-inoculated soil did become infected during the same time period.

Table 15. Scab incidence on radishes exposed to the R1 Streptomyces sp. strain at various times after planting.

Days from planting to inoculation	Total harvested	% Scabbed radishes[a]
0	17	0
4	14	27
8	16	12
13	13	46
17	18	3
22	14	14
Noninoculated control	17	0

[a]Percentage values based on 19 to 25 roots harvested for each treatment.

Table 16. Scab incidence on radishes exposed to the R1 Streptomyces sp. strain at various times after planting.

Days from planting to inoculation	Total harvested	% Scabbed radishes[a]
0	21	5
6	19	5
11	20	0
16	22	14
21	22	5
26	24	0
Noninoculated control	21	0

[a]Values based on harvest of from 21 to 25 roots for each treatment.

Table 17. Scab incidence on radishes transplanted from steamed to infested soil, and from infested to steamed soil at various times after planting.

	% Scabbed radishes[a]					controls	
	8[b]	12	16	20	24	infested	steamed
Transfer from steamed to infested soil	27	50	38	42	27	41	6
Transfer from infested to steamed soil	50	55	38	60	44		

[a]Percentage values based on 15 to 20 radishes harvested for each treatment.

[b]Days from seeding to transfer.

DISCUSSION

Through a modified version of Koch's postulates, a Streptomyces sp. has been shown to be a causal agent of radish scab. The R1 strain was isolated from a typical radish scab lesion, cultured, and introduced into steamed muck soil. Radishes grown in the infested soil became infected with scab while those grown in steamed, non-infested soil remained healthy. A streptomycete reisolated from the scabbed radishes was morphologically and biochemically identical to the original R1 strain.

A second Streptomyces sp. (P4) recovered from scabbed potatoes was also found to cause scab on radish. This isolate was found to be morphologically and biochemically distinct from the R1 isolate and from the two ATCC strains studied. Spore and spore chain morphology are considered important characters in species determination of streptomycetes with the former being a relatively stable character and the latter quite variable with different culture media (74). Since all of the isolates were grown on the same medium, the differences observed in spore chain morphology are actual strain differences.

These findings are consistent with earlier reports that many species of Streptomyces are implicated in causing scab on a variety of hosts (50,67). Experimental attempts to test pathogenicity of ATCC strains 3352 and 10246, as well as eleven other isolates, were inconclusive. It is possible that these strains have either lost their virulence or are not pathogenic on radish. In this experiment none of the radishes became

scabbed, including the positive controls. This failure to obtain any scab points out a basic problem with the greenhouse trials in general; namely, the confounding effects of environmental parameters on host and disease development resulting in an inability to obtain consistent results. Factors such as low light intensity, very long photoperiods, high soil temperatures, and very low soil moisture appeared to inhibit root expansion. Poor root development may have been responsible for the low scab levels obtained in some of the experiments. Since low soil moisture was necessary for disease development, a sub-surface irrigation system was installed to supply water to the roots while allowing the surface soil around expanding roots to remain dry. In experiments where no disease was detected, it may have been that soil moisture was so low as to inhibit any biological activity around the roots. Because of the unreliability in obtaining disease in the greenhouse, the results of these studies must be examined cautiously.

Experiments conducted in the greenhouse to determine the period of greatest susceptibilty to scab in developing radish roots showed that radishes inoculated approximately 14 days after planting developed more scab than those inoculated before or after 14 days. These results suggest that radishes may be more susceptible at the two week stage of growth although it was not possible to determine when infection occurred in relation to the addition of inoculum around the roots. If the inoculum remained infectious once introduced, all of the roots should have been equally infected with the exception of those inoculated so late in the growing period that lesions would not be visible at harvest. Since scab incidence was not consistent between treatments, one may assume that the inoculum was infectious for a discrete period of time after introduction.

The greatest scab incidence occurred when the pathogen was introduced two weeks after planting.

However, when radishes were transferred from infested to steamed soil and visa versa at various times after planting, scab incidence was similar for all radishes regardless of when they were transferred. These results conflict with the earlier experiment in which a specific period of greatest susceptibility was indicated. One reason for the consistent scab incidence through the experiment is that plants transferred from infested to steamed soil may have carried inoculum even though care was taken in cleaning away all soil from the roots. Due to limitations in space, it was not possible to use enough plants to generate data that could be statistically analyzed. Further study is needed to more clearly define whether a period of greater susceptibility exists in the development of the radish roots. To do this it is necessary to develop a reliable procedure for infecting radishes under controlled environmental conditions. With such a system, routine pathogenicity tests could be performed and relative pathogenicity of various Streptomyces spp. may be determined.

A knowledge of the infection process would greatly aid in understanding when radish roots become infected. If the pathogen enters through direct penetration of the epidermis or phelloderm of the upper root, then infection could occur at any time during development. If the pathogen enters during the transitional phase when epidermis is being shed and phelloderm tissue is forming, then the period of infection would be limited to a specific developmental stage of the radish. The pathogen may simply depend on natural wounding during root expansion to provide the infection site, in which case radishes would be most susceptible during the latter half of the growing period during rapid expansion.

Although Streptomyces spp. were isolated successfully from potatoes, a great amount of difficulty was experienced in isolating Streptomyces spp., or any other organism, from radish. Since crude radish extracts were tested and found to have no inhibitory effect on the growth of a pathogenic Streptomyces sp. in culture, the possibility of inhibitory compounds being released from cut or crushed tissue must be discussed. Isolations were successful using phosphate buffer (pH 8.1) for crushing of radish tissue and for the preparation of the chitin agar media. Before using buffer, approximately 1 in 200 plates yielded a streptomycete. With buffer, the rate of recovery increased to approximately 6 plates in 10. The difficulty in isolation may have been due to sensitivity of the organism to changes in pH which might occur when infected tissue is cut or crushed. It is also possible that the pathogen occurs in only localized regions of a lesion or may be viable only in lesions of specific size or age. The necessity for using very fresh material in isolations also added to the difficulty experienced.

A number of control strategies were examined for their ability to reduce the incidence and severity of scab on radish. Varietal resistance would be the simplest and perhaps most cost effective method of control. However, a comparison of cultivars planted in different fields revealed little consistency in relative disease levels. Red Prince was ranked 11th in percent scabbed roots at site #1 while at site #3 it ranked 1st (Tables 1, 2). Kutura hybrid had 100% scabbed roots at site #1 but only 4% at site #3. The white icicle cultivars had consistently lower scab levels than red globe cultivars through all of the trials. Though these may afford a source of resistance for future breeding programs, there is little market demand for white varieties so their use as disease resistant

replacements for red varieties is limited.

Several European and Asian radish cultivars found to be highly resistant to clubroot caused by Plasmodiophora brassicae (58) may also be resistant to scab. There have been few reports of heavy scab outbreaks from Europe and the Orient even though the presence of the scab pathogen on potato has been well documented in these areas. Due to problems with the European variety trials in this study, it was not possible to determine whether or not there is resistance in these cultivars and further testing is necessary.

If resistant cultivars are found, there are complicating factors which could limit their use. These new cultivars may be more susceptible to insect or other disease problems to which present cultivars are resistant. Horticultural characteristics such as an unfavorable taste, size, shape, or color will limit consumer acceptance, while increased length of the growing period, improper size of the tops at harvest, or poor storability will limit grower acceptance.

After testing 29 cultivars, it is apparent that no high degree of resistance exists, at least in the United States. Under severe disease conditions where some cultivars were nearly 100% infected, the most resistant cultivar had over 46% of the roots infected. Though this level of disease is unacceptable under present commercial production standards, the use of less susceptible varieties could play a role in an integrated control program.

When manganese is present in the soil at near phytotoxic levels, potato scab has rarely been a problem, but it has been shown previously that reduced scab incidence is not always correlated with increased levels of manganese in potato tuber periderm (52). This suggests that the

manganese is affecting the pathogen directly. In the present study, manganese had no affect on scab incidence. However, the rate used was that recommended for offsetting natural deficiencies while in some of the previous studies (51,52) much higher rates were used which may account for the different results.

Control of scab with copper has been correlated with the accumulation of copper in the periderm of potato tubers (52). Increase in scab incidence has been correlated with increased calcium levels in the soil and potato periderm (25). In both cases, the effect of the element was based on the physiology of the potato, its ability to accumulate these ions, and the metabolic pathways in which they were utilized. In the present study, the inability of copper or calcium to affect scab incidence on radish may have been due to the physiology of the radish plant which may not accumulate, metabolize, or store these ions in the same way as does potato.

Further testing with minor nutrients should be carried out using high rates rather than corrective rates. The predominantly organic constituents of muck soil may tie up nutrient cations more than mineral soils which have been used in most previous studies. The amount of the nutrients available to radishes in this study may have been much lower due to this phenomenon.

PCNB has been shown to be effective in controlling potato scab (55). There are several problems, however, associated with its use. In most of the field trials where PCNB reduced scab incidence, the rates used (50 to 1000 kg/ha) were well above the maximum rates registered for use on potato (28 kg/ha for control of Rhizoctonia solani). Furthermore, Davis et al. (13) found that PCNB controlled scab only when the soil moisture was above

-1.62 bars and had no effect on scab below that level.

The results of the present study indicate that PCNB was effective in controlling radish scab when added as a drench one week after planting, although it is possible that soil moisture played a role in the observed scab reduction. An increase in soil moisture at a particularly susceptible period of growth (in this case due to the PCNB drench), may have resulted in the decrease in scab incidence. If this was the case, one would expect to find no difference in disease incidence between radishes treated with different rates of PCNB. The results, however, indicated a significant difference in percent scab between radishes treated with different rates of PCNB. PCNB then appears to be at least partially responsible for the observed reduction of scab on radish.

Registration for the use of PCNB on radish seems unlikely. Producers of the chemical would probably find it uneconomical to expand the chemical label for such a small market. PCNB is currently on the R.P.A.R. list as a cancer suspect so it is unlikely that the federal government would approve its use on a directly consumable root crop. Unlike potato, radishes are in the ground for only four weeks which might lead to some residue problems with PCNB. The actual effectiveness of the chemical must also be considered. In the present study PCNB gave significant control with 50% of the treated roots infected as compared with 94% for the controls. Although PCNB by itself may not give satisfactory control under severe disease conditions from a commercial aspect, it might play a role as one of a number of chemical and cultural control strategies.

Sulfur has been used as a soil acidifier for many years and its effectiveness in controlling scab has been demonstrated (78). In the present study, both elemental sulfur and $AlSO_4$ failed to control radish

scab. Radishes grown in soil treated with elemental sulfur appeared stunted and grew slowly. This may have been the result of direct toxicity of the sulfur or toxicity due to very low soil pH. The pH of soil treated with elemental sulfur dropped for six weeks after incorporation and then began to rise steadily. The pH of plots treated with $AlSO_4$ dropped for one week after incorporation and then began to rise. The differential effects of the two treatments may be attributed to the different solubilities of the sulfur compounds used. In both cases, however, the buffering capacity of the muck soil was great enough to overcome the effects of sulfur after relatively short periods of time. For this reason, as well as possible phytotoxicity, sulfur applications may not be a feasible method for controlling radish scab on neutral to high pH organic soils. Also, strains of Streptomyces spp. have been found which are pathogenic on potatoes grown in soils with pH values below 5.0 (8). Under such circumstances, soil acidification would not be effective in controlling scab.

Irrigation appears to be the most promising strategy for the control of radish scab. In several field experiments, radishes grown in soil maintained above -.58 bars had at least 50% less scab than those grown in soil below -1.5 bars. Under the moisture regimes tested, irrigated radishes grew as well as non-irrigated ones and had greater harvest weights. Since radishes are packed by weight, this would increase the market value of the crop.

A correlation was observed between rainfall during the first half of the growing period and scab incidence. Futhermore, in both the variety trials and minor nutrient trials, radishes grown in the relatively dry, tiled plot had a higher scab incidence than those grown in the wetter, untiled fields.

These results agree with previous research (36) and irrigation has been used successfully in controlling potato scab on a commercial basis for many years.

Potatoes are most susceptible during rapid tuber expansion and irrigation for a 4 to 6 week period is required to control the disease (36). There does not appear to be a specific period of susceptibility in radish, although increased soil moisture during the first half of the growing period is more highly correlated with scab reduction than soil moisture during the second half of growth period. Maintaining high soil moisture through irrigation for approximately a two week period could be difficult under present cropping procedures. Irrigation has generally been used only to ensure proper tilth for planting. In order to irrigate each planting for two weeks, more irrigation equipment and the labor to move it might be required. Besides economic considerations, there are other problems which might arise from high soil moisture over prolonged periods of time. Clubroot, black root, and damping-off, none of which are serious problems under dry soil conditions, may become more prevalent. Also, slight injuries on the enlarging roots are more likely to be invaded by soft rot organisms under moist conditions.

In order to increase the effectiveness of irrigation in controlling scab on radish, further study is needed to "fine tune" the system. More detailed field studies of irrigation timing and levels of soil moisture necessary for control, are needed. A decrease in the time and amount of irrigation needed, as well as a better understanding of the role of other environmental parameters (temperature, pH, etc.) may lead to a practical means of control through manipulation of soil moisture.

It is still not known how increased soil moisture reduces disease

incidence. It has been reported that increased soil moisture inhibits the infection process on potato since the period of greatest susceptibility to infection and greatest control by irrigation are the same. This inhibition may be related to a direct effect of soil moisture on the activity of the pathogen or to morphological or physiological effects of moisture on the host which in turn inhibit infection. Given that increased soil moisture reduces scab incidence on radish and potato, and due to the different structures and physiologies of the two hosts, it seems more likely that soil moisture is directly inhibiting the infection process.

Recently, workers have proposed that increased soil moisture allows saprophytic microorganisms to compete with the pathogen for infection sites on potato (1), thus reducing infection. In this respect, control is achieved through the manipulation of the soil environment to promote one organism over another. It may be possible to promote this competition through means other than irrigation. The addition of soil amendments which favor certain saprophytes or the additon of certain microorganisms to the soil might be as effective as irrigation, yet economically more feasible in radish production.

It is apparent that no single control measure studied thus far will afford consistent control of radish scab to levels suitable for commercial production. A combination of different strategies such as the use of resistant varieties, PCNB, and irrigation, could reduce scab for economical control. This integrated approach to disease control is important in light of the fact that more than one species of Streptomyces might be involved in disease development. With a single control method, the danger of the pathogen developing resistance is great. With a multiple control

strategy approach, this possibility is greatly reduced. Further research is needed to improve upon the controls already identified and to find new approaches to the control of radish scab.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. ADAMS, M.J., and O.H. LAPWOOD. 1978. Studies on the lenticel development, surface microflora and infection by common scab (Streptomyces scabies) of potato tubers growing in wet and dry soil. Ann. App. Biol. 90:335-343.

2. BARNES, E.D. 1971. Common scab of the potato: the effects of irrigation, manganese sulphate, and sulfur treatments for common scab of the potato on the mineral composition of plant material and soil extracts. Res. Exp. Rec. Min. Agric. N.I. 20:53-58.

3. BARNES, E.D. 1971. The effects of irrigation, manganese sulphate, and sulfur applications on common scab of potato. Res. Exp. Rec. Min. Agric. N.I. 20:35-44.

4. BARNES, E.D., and CHESTNUTT, D.M.B. 1966. Some aspects of the control of common scab in potatoes. Res. Exp. Rec. Min. Agric. N.I. 15:35-42.

5. BARTZ, J.F., and K.C. BERGER. 1858. Urea formaldehyde concentrate-85, a promising control for potato scab. Agr. and Food Chemistry 6:675-677.

6. BECK, J., and K.E. HANSEN. 1974. The degredation of quintozene, pentachloronitrobenzene, hexachlorobenzene and pentachloroaniline in soil. Pestic. Sci. 5:41-48.

7. BOLLEY, H.L. 1893. Prevention of Potato Scab. North Dakota Agr. Exp. Sta. Bull. 9:27-33.

8. BONDE, M.R., and G.A. McINTYRE. 1968. Isolation and biology of a Streptomyces sp. causing potato scab in soils below pH 5.0. Am. Pot. J. 45:273-278.

9. BRISTOW, P.R., J. KATAN, and J.L. LOCKWOOD. 1973. Control of Rhizoctonia solani by pentachloronitrobenzene accumulated from soil by bean plants. Phytopath. 63:808-813.

10. DAVIS, J.R., J.G. GARNER, and R. H. CALLIHAN. 1974. Effects of gypsum, sulfur, terraclor, and terraclor super-x for potato scab control. Am. Pot. J. 51:2. 35-43.

11. DAVIS, J.R., R.E. McDALE, and R. H. CALLIHAN. 1976. Fertilizer effects on common scab of potato and the relation of calcium and phosphate-phosphorus. Phytopath. 66:1236.

12. DAVIS, J.R., G.M. McMASTER, R.H. CALLIHAN, J.G. GARNER, and R.E. McDALE. 1974. The relationship of irrigation timing and soil treatments to control of potato scab. Phytopath. 64:1404-1410.

13. DAVIS, J.R., G.M. McMASTER, R.H. CALLIHAN, F.H. NISSLEY, and J.J. PAVEK. 1976. Influence of soil moisture and fungicide treatments on common scab and mineral content of potatoes. Phytopath. 66:228-233.

14. DIPPENAOR, B.J. 1933. Environmental and control studies of the common scab disease of potato, caused by Actinomyces scabies (Thaxt.). Gussow. Sci. Bull. Dept. Agr. Union of South Africa. No. 136, 78 p.

15. DOYLE, J.J., and A.A. MacLEAN. 1960. Relationships between Ca:K ratio, pH, and prevalence of potato scab. Can. J. Plant Sci. 40:616-619.

16. DUFF, G.H., and C.G. WELCH. 1927. Sulphur as a control agent for common scab of potato. Phytopath. 17:297-314.

17. FELLOWS, H. 1926. Relation of growth in the potato tuber to the potato scab disease. J. Agr. Res. 32:757-768.

18. GORDON, R., and A. C. Horan. 1968. A piecemeal description of Streptomyces griseus (Kransky) Waksman and Henrici. J. Gen. Microbiol. 50:223-233.

19. GRIES, G.A. 1951. Inhibition of the potato scab organism by soluble aluminum ions. Phytopath 41:15 (Abstr.).

20. GRIES, G.A., J.G. HORSFALL, and H.G.M. JACOBSON. 1944. The balance of calcium and potassium in relation to clubroot of cabbage and potato scab. Phytopath 34:1001 (Abstr.).

21. GUSSOW, H.T. 1914. The systematic position of the organism of the common potato scab. Science 39:431-433.

22. HOOKER, W.J. 1949. Parasitic action of Streptomyces scabies on roots of seedlings. Phytopath. 39:442-462.

23. HOOKER, W.J. 1954. Pentachloronitrobenzene soil treatment for potato scab control. Plant Dis. Rep. 40:190-192.

24. HOOKER, W.J. and O.T. PAGE. 1960. Relation of potato tuber growth and skin maturity to infection by common scab, Streptomyces scabies. Amer. Pot. J. 37:414-423.

25. HORSFALL, J.G., J.P. HOLLIS, and H.G.M. JACOBSON. 1954. Calcium and potato scab. Phytopath. 44:19-24.

26. HOUGHLAND, G.V.C., and L.C. CASH. 1954. Effectiveness of certain soil fungicides in the control of potato scab. Plant Dis. Rep. 38:777-780.

27. HOUGHLAND, G.V.C., and L.C. CASH. 1957. Carry-over effects of PCNB applied to the soil for control of potato scab. Am. Pot. J. 34:85-88.

28. HUGHES, C., R.C. McCRUM, and F.E. MONZER. 1971. Protein and esterase electropheretic patterns of Streptomyces spp. isolated from Maine potatoes. Am. Pot. J. 48:206-213.

29. KENKNIGHT, G. 1941. Studies in soil actinomycetes in relation to potato scab and its control. Mich. State Coll. Agr. Exp. Sta. Tech. Bull. 178.

30. KORONOWSKI, V.P., and MaBFELLER, D. 1972. Eine Aktinomykose an Radieschen (Raphanus sativus L., var. radicula Pers.). Nachrichienbl. Deutsch. Pflanzenschutzd. (Braunschweig) 24. 1972, 152-154.

31. LAPWOOD, D.H., and M.J. ADAMS. 1973. The effect of a few days of rain on the distribution of common scab on young potato tubers. Ann. Appl. Biol. 73:277-283.

32. LAPWOOD, D.H., and M.J. ADAMS. 1975. Mechanisms of control of common scab by irrigation. pages 123-129 in: G.W. Bruehl, ed. Biology and Control of Soil-borne Plant Pathogens. Am. Phytopath. Soc., St. Paul, MN.

33. LAPWOOD, D.H., and P.W. DYSON. 1966. An effect of nitrogen on the formation of potato tubers and the incidence of common scab. Pl. Path. 15:9-14.

34. LAPWOOD, D.H., and T.F. HERRING. 1968. Infection of potato tubers by common scab during brief periods when soil is drying. Eur. Pot. J. 11:177-187.

35. LAPWOOD, D.H., and B.G. LEWIS. 1967. Observations on the timing of irrigation and the incidence of potato common scab. Pl. Path. 16:131-135.

36. LAPWOOD, D.H., L.W. WELLINGS, and J.H. HAWKINS. 1973. Irrigation as a practical means to control potato common scab: final experiment and conclusions. Pl. Path. 22:35-41.

37. LAPWOOD, D.H., L.W. WELLINGS, and W.R. ROSSER. 1970. The control of common scab of potatoes by irrigation. Ann. Appl. Biol. 66:397-405.

38. LEWIS, B.G. 1970. Effects of water potential on the infection of potato tubers by Streptomyces scabies in soil. Ann. Appl. Biol. 66:83-88.

39. LINGAPPA, Y., and J.L. LOCKWOOD. 1962. Chitin media for selective isolation and culture of actinomycetes. Phytopath. 52:317-323.

40. LOUDON, J.C. 1825. An Encyclopedia of Agriculture. 1st edition. Longman, Hurst, Rees, Orine, Brown and Green, London.

41. MacMILLAN, H.G. 1922. Potato seed-treatments in western states. Phytopath. 12:39.

42. McGREGOR, A.J., and G.S.C. WILSON. 1964. The effect of applications of manganese sulphate to a neutral soil upon yield of tubers and the incidence of common scab of potatoes. Plant Soil 20:59-64.

43. McGREGOR, A.J., and G.S.C. WILSON. 1966. The influence of manganese on the development of potato scab. Plant Soil 25:3-16.

44. McINTOSH, A.H. 1973. Glasshouse tests of chemicals for control of potato common scab. Ann. Appl. Biol. 73:189-196.

45. McINTOSH, A.H. 1976. Glasshouse tests of quinones, polyhydroxybenzenes and related compounds against potato common scab. Ann. Appl. Biol. 83:239-244.

46. McINTOSH, A.H. 1977. Field trials of chemicals for control of common scab by soil treatment. Potato Res. 20:225-229.

47. McINTOSH, A.H., and G.L. BATEMAN. 1979. Effects of foliar sprays of daminozide on the incidence of potato common scab. Ann. Appl. Biol. 92:29-38.

48. MENZIES, J.D. 1957. Dosage rates and application methods with PCNB for control of potato scab and Rhizoctonia. Am. Pot. J. 34:219-226.

49. MENZIES, J.D., and C.E. DADE. 1959. A selective indicator medium for isolating Streptomyces scabies from potato tubers on soil. Phytopath. 49:457-458.

50. MILLARD, W.A., and S. BURR. 1926. A study of twenty-four strains of actinomycetes and their relation to types of common scab of potato. Ann. Appl. Biol. 13:580-644.

51. MORTVEDT, J.J., M.H. FLEISCHFRESSER, K.C. BERGER, and H.M. DARLING. 1961. The relation of soluble manganese to the incidence of common scab in potatoes. Am. Pot. J. 38:95-100.

52. MORTVEDT, J.J., K.C. BERGER, and H.M. DARLING. 1963. Effect of manganese and copper on the growth of Streptomyces scabies and the incidence of potato scab. Am. Pot. J. 40:96-102.

53. PFLEGER, F. 1975. Unpublished research report on scabby radishes. University of Minnesota (Vegetable pathologist).

54. POTTER, H.S., W.J. HOOKER, W. CARGO, and G.T. STACHWICK. 1958. Pentachloronitrobenzene and urea-formaldehyde for potato scab control in Michigan. Plant Dis. Reptr. 43:633-637.

55. POTTER, H.S., H.L. WARREN, and W.J. HOOKER. 1961. Soil fungicides for potato scab control. Quart. Bull. Mich. Agr. Exp. Sta. 43-3:532.

56. PRIDHAM, T.G., and A.J. LYONS, JR. 1961. Streptomyces albus (Rossi-Doria) Waksman et. Henrici: Taxonomic study of strains labeled Streptomyces albus. J. Bact. 8.81:431-441.

57. PRIDHAM, T.G., and H.D. TRESNER. 1974. In "Bergey Manual of Determinative Bacteriology", 8th edition. Williams-Wilkins Co., Baltimore. U.S.A. 1268 pp.

58. ROWE, R.C. 1980. Evaluation of radish cultivars for resistance to clubroot (Plasmodiophora brassicae) race 6 for midwestern United States. Pl. Dis. 64:462-464.

59. SANFORD, G.B. 1923. The relation of soil moisture to the development of common scab of potato. Phytopath. 13:231-236.

60. SANFORD, G.B. 1926. Some factors affecting the pathogenicity of Actinomycetes scabies. Phytopath. 14:525-547.

61. SCHAAD, N.W. 1980. Laboratory Guide for identification of plant pathogenic bacteria. American Phytopathological Society, St. Paul, MN.

62. SCHAAL, L.A., and G. JOHNSON. 1955. The inhibitory effect of phenolic compounds on the growth of Streptomyces scabies as related to the mechanism of scab resistance. Phytopath. 45:626-628.

63. SCHROEDER, R.A., and W.A. ALBRECHT. 19 . Plant nutrition and the hydrogen ion. II. Potato Scab. Soil Sci. 53:481-490.

64. SCHULTZ, T.H., K.C. BERGER, H.M. DARLING, and M.H. FLEISCHFRESSER. 1961. Urea formaldehyde concentrate-85 for scab control in potatoes. Am. Pot. J. 38:85-8.

65. SHIRLING, E.B., and D. GOTTLIEB. 1968. Cooperative description of type cultures of Streptomyces. III. Additional species descriptions from first and second studies. Intern. J. of Syst. Bact. 18:279-392.

66. STAPP, C. 1956. Streptomykose der kartoffein (kartoffelschorf). In Appel, O. and H. Richter, Bakterielle Krankheiten. Handbuch der Pflanzenkrankheiten. 2:494-534.

67. TAYLOR, C.F. and P. DECKER. 1947. A correlation between pathogenicity and cultural characteristics in the genus Actinomyces. Phytopath. 37:49-58.

68. TERMAN, G.L., F.H. STEINMETZ, and A. HAWKINS. 1943. Effect of certain soil conditions and the treatments upon potato yields and the development and control of potato scab. Maine Agr. Exp. Sta. Bull. 463.

69. THAXTER, R. 1890. The Potato Scab. Conn. Agr. Exp. Sta. Bull. Vol. 105. (pp).

70. THAXTER, R. 1891. Potato Scab. 15th Ann. Rep. Conn. Agr. Exp. Sta. 153-160.

71. VLITOS, A.J., and W.J. HOOKER. 1951. The influence of sulfur on populations of Streptomyces scabies and other streptomycetes in peat soil. Am. J. Bot. 38:678-683.

72. WAKSMAN, S.A. 1957. In "Bergey's Manual of Determinative Bacteriology". 7th edition. Williams and Wilkins Co., Baltimore, U.S.A. 1094 pp.

73. WAKSMAN, S.A. 1961. The Actinomycetes. Vol. 2. Classification, identification and descriptions of genera and species. The Williams and Wilkins Co., Baltimore.

74. WAKSMAN, S.A. 1967. The Actinomycetes. A summary of current knowledge. The Ronald Press Co., New York.

75. WAKSMAN, S.A., and A.T. HENRICI. 1943. The nomenclature and classification of the actinomycetes. J. Bacteriol. 46:337-341.

76. WALKER, J.C. 1952. Diseases of vegetable crops. McGraw-Hill Book Company, Inc., New York.

77. WELLINGS, L.W., and D.H. LAPWOOD. 1971. Control of common scab by use of irrigation. Pot. Res. 14:128-137.

78. WHEELER, H.J., and G.E. ADAMS. 1897. On the use of flowers of sulfur and sulfate of ammonia as preventive of the potato scab in contaminated soils. 10th Ann. Rept. Rhode Island Exp. Sta.