PROBLEMS INVOLVED IN EVALUATING SWEET POTATOES FOR THEIR REACTION TO SOIL ROT

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INTRODUCTION

The current sweet potato breeding program at the Oklahoma Agricultural Experiment Station, in which the Department of Botany and Plant Pathology is cooperating with the Department of Horticulture, has as one of its objectives the identification and incorporation of disease resistance into superior sweet potato lines. Stem rot (*Fusarium oxysporum* f. *batatas* (Wr.) Snyder and Hansen) and root-knot (*Meloidogyne incognita* var. *acrita* Chitwood) are two examples of sweet potato diseases which have been dealt with in the breeding program. Resistance to these diseases has been combined with other desirable characters to develop new sweet potato varieties.

In recent years, particularly since drier conditions have prevailed, soil rot (*Streptomyces ipomoea* (Person and Martin) Waks. and Henrici) has come to be of increasing importance in the culture of sweet potatoes in Oklahoma. Work was begun to evaluate presently available sweet potato lines for their reaction to soil rot. This information should be of value to the plant breeder in developing sweet potato varieties resistant to this disease.

A preliminary survey of presently available facts on soil rot and its causal organism revealed that comparatively little is known concerning either the disease or its causal agent. This is probably in part due to the great amount of confusion associated with the early attempts to determine the causal organism involved.

The initial objective of the present investigation was to devise a laboratory or greenhouse technique for evaluating the reaction of sweet potato lines to soil rot. Because of problems encountered in isolating and maintaining the causal organism in culture, the work has, of necessity, been more restricted to investigations dealing with techniques involved in handling the organism than to investigations of varietal reaction to the disease.

REVIEW OF LITERATURE

The first published record of soil rot or pox of sweet potatoes was in 1890 by Halsted (9) who coined the common name "soil rot" and stated that the causal agent was a previously undescribed fungus, Acrocystis batatas Ell. and Hals. Twenty-four years later, Taubenhaus (24) reported that after two years of investigation he was unable to find A. batatas associated with this disease. Elliott (7) in 1916 published the results of a rather extensive investigation on the disease and concluded that the causal agent was a slime mold which he designated as Cystospora batata Ell. This work includes descriptions of the life cycle of the slime mold and illustrations of the pathological histology involved in the disease. He considered Halsted's illustrations of Acrocystis batatas to represent stages in the life history of the slime mold rather than the fungus with which Halsted thought he was dealing. In spite of the fact that Elliott was setting up a new genus and species of the slime mold he did not present a valid description of either so the binomial Cystospora batata has no taxonomic status.

Taubenhaus (25) in 1918 confirmed the work of Elliott and agreed that Halsted was in error in designating Acrocystis batatas as the causal agent of soil rot. Neither Taubenhaus nor Elliott was able to find the organism Halsted had described and questioned its existence. During the course of his work Taubenhaus very frequently obtained a new species of Actinomyces from soil rot lesions. This organism was

described as Actinomyces poolensis Taub. and was considered to be a secondary invader of lesions induced by Cystospora batata.

In 1921, attempts by Manns and Adams (13) to confirm the work of Elliott and of Taubenhaus resulted in complete failure. They were unable to detect the presence of a slime mold in the mature lesions on fleshy roots. It was their opinion that what Elliott had interpreted as cysts of the slime mold he described were simply products of metabolism and remains of cellular debris. The following year, as a result of their failure to demonstrate the presence of a slime mold, Manns and Adams (14) stated definitely that the cause of soil rot was not Cystospora batata as was reported by Elliott and by Taubenhaus.

Manns and Adams (15) in 1924 reported further cytological investigations. By removing the cover glasses from pox material which had been stained earlier with Flemming's triple stain and then restaining with Ziehl-Neelson's carbol-fuchsin, they found evidence, in every case, of an actinomycete. The next year they reported (16) further definite evidence of an actinomycete as the causal organism of soil rot. The organism which they found resembled Actinomyces poolensis Taub.

Adams (2) in 1929 made a series of comparisons between A. poolensis and the actinomycete which he had isolated from pox lesions and found the two to be entirely different. A. poolensis proved to be nonpathogenic to sweet potatoes while the undescribed actinomycete he had isolated was found capable of producing typical soil rot lesions. Cultural studies of the latter organism revealed it to be separate and distinct from A. poolensis. The organism was not, however, named

or described as a new species, but for convenience was referred to as Actinomyces Pox.

Person and Martin (19) in 1940 reported a detailed study of the soil rot problem in Louisiana. It was stated that the organism isolated and determined by them to be pathogenic was a previously undescribed actinomycete. It was described as a new species, Actinomyces ipomoea Person and W. J. Martin.

In 1943 the actinomycetes were reclassified by Waksman and Henrici (27). According to the new classification, the genus Actinomyces, in the family Actinomycetaceae, was restricted to the anaerobic, pathogenic species. The genus Streptomyces and the family Streptomycetaceae were created to include all aerobic species producing conidia on aerial hyphae. Since the organism described by Person and Martin as Actinomyces ipomoea produces conidia in chains from aerial mycelium, the organism is now referred to as Streptomyces ipomoea (Person and Martin) Waks. and Henrici.

Adams (1) showed that penetration by the pathogenic actinomycete with which he worked was through lateral roots or root tips and that infection occurred more rapidly at 33°C. than at room temperature. Poole (18) found that low moisture content of the soil contributed to the severity of the disease. He reported that there was no infection at either saturation or 15 per cent moisture, based on the water-holding capacity of the soil. At 10 per cent of the water-holding capacity there was slight infection and very severe infection at 2 to 5 per cent.

To control soil rot Person (20) reported that reducing the soil

pH to 5.0 or less was effective. By applying sulfur at the rate of 500 to 800 pounds per acre to silt loam soils it was possible to lower the pH to about 5.0 and maintain it at that level for four to six years. The use of resistant varieties offers a potential means of control but little work has been reported on this subject. Taubenhaus (25) reported that considerable differences seemed to exist in the resistance of varieties of sweet potatoes to soil rot. Of the limited number of lines tested he gave a tentative classification of their resistance based on the number of roots infected. Watson (29) reported the varietal reaction of 27 lines of sweet potatoes determined on the basis of whether or not mature roots showed symptoms. Certain varieties were found free of disease while others had up to 28 per cent of the roots infected.

SYMPTOMATOLOGY

To evaluate properly the resistance of sweet potatoes to soil rot, a thorough familiarity with the symptoms of the disease is essential. Symptoms of soil rot are quite distinct from those of any other sweet potato disease. Following is a summary of these symptoms as they have been observed.

On heavily infested soil or when susceptible varieties are grown, the above ground portions of the plant present a stunted, chlorotic appearance and, under conditions for optimum disease development, death of the plant may occur. This reduction in growth is a result of damage done to the small fibrous or feeder roots. Generally speaking, an entire lateral root can become infected and completely destroyed. On the other hand, a root may exhibit several lesions, each separated from the other by healthy tissue.

On the mature, fleshy root the typical symptom is a dark brown to black, pitted, coriacious lesion measuring up to an inch or more in diameter. Several lesions may coalesce to form a scabbed area several inches across. The surface of a young lesion, at first unbroken, later cracks, dries and becomes hard and brittle with a jagged irregular margin (Fig. 1). The mature root is sometimes badly misshapened by a lesion which has girdled the entire root producing a somewhat dumbell-shaped potato.

Fig. 1. Allgold sweet potatoes showing typical soil rot lesions.

MATERIALS AND METHODS

Each isolate used in this work was obtained from one of five different sources: (1) mature infected potatoes, (2) young fleshy roots, (3) Dr. W. J. Martin, Plant Pathology Department, Louisiana State University, (4) the American Type Culture Collection, or (5) infested soil from the Horticultural Vegetable Research Station at Bixby, Oklahoma.

Several media were used in attempts to isolate actinomycetes from infected roots. Czapek's agar, potato-dextrose agar, sweet potato-dextrose agar, and Jones' modification of Ashby's mannitol agar (11) were first used in isolating from infected roots. Later it was found that soil-extract agar was superior to any of the above mentioned media in that it did not readily support the growth of filamentous fungi. Soil-extract agar was prepared by extracting the water soluble portion of 50 g of soil in 1 liter of distilled water and solidifying with 17 g of agar.

The method used in isolating actinomycetes from infected roots was as follows. A very small portion of the surface of a lesion was cut away and a portion of the remaining necrotic tissue, together with a small portion of surrounding tissue was removed and placed in a 15% solution of Clorox for 15 - 25 seconds. The tissue was then macerated in a few drops of sterile water in a petri dish and covered with melted soil-extract agar. Rotating the dish aided in dispersing the macerated tissue throughout the medium. The plates

were incubated at 32°C. for 3 - 7 days.

Streptomyces spp. were isolated from infested soil according to the method of Allen (3). This method involved the preparation of a series of dilutions from 10 g of soil suspended in 90 ml of water. One ml amounts of each of the dilutions, 1:10, 1:50, and 1:100, were used in separate petri dishes. Czapek's agar was then mixed with each of the dilutions and the plates were incubated at 32°C. for 3 - 5 days.

All isolates were maintained either on Czapek's medium or under sterile mineral oil on potato-dextrose agar until it was found that isolates were not stable when kept on an agar medium. All isolates were then maintained in tubes of sterile soil. It should be pointed out that while an agar medium did not serve to maintain isolates for any length of time, an agar medium was satisfactory for increasing the organism to obtain quantities sufficient for inoculation purposes.

Laboratory testing of the pathogenicity of isolates was done in one of two ways. During earlier attempts at pathogenicity trials, the method of Person and Martin (19) was employed. This consisted of surface sterilizing pieces of sweet potato stems with one or two nodes and placing them on sterile water agar in 150 x 20 mm petri dishes. After sufficient rootlet formation, each stem piece was transferred to a 100 x 15 mm petri dish containing water agar. A small piece of inoculum to be tested was then placed on each rootlet and the plates were incubated at 32°C. for 4 - 7 days. Pathogenic cultures were reported by Person and Martin to produce a browning of the tissue in contact with the inoculum.

The second method used later consisted essentially of a modi-

fication of Hooker's (10) method as used with scab of white potato. Vine cuttings of a susceptible line of sweet potatoes were grown in 175 x 20 mm test tubes containing soil-extract agar which had been seeded with an isolate in question. After placing a rooted cutting in a tube, the inoculum which was first mixed with an equal amount of distilled water was added to the tube. The amount of inoculum used per tube was one-half the growth scraped from an agar slant in an eight-ounce glass prescription bottle. The tube was then filled with melted 1.7% soil-extract agar. If the isolates were pathogenic, a browning of the tips of the larger roots or complete browning of the smaller lateral rootlets would occur. This method proved to be a more satisfactory one than that of Person and Martin. Selected isolates were further tested for pathogenicity by growing cuttings in inoculated sand, soil, or vermiculite.

In an attempt to evaluate sweet potato lines for resistance or susceptibility to soil rot, cuttings were grown under greenhouse conditions in benches containing infested soil. The soil temperature was maintained at approximately 27°C. while the air temperature varied from a low of 70°F. at night to a high of 105°F. during the daylight hours.

Material for histological examination was killed and fixed in formalin-acetic acid-alcohol solution, using a dioxan dehydrating series (22). Sections which had been cut from embedded root tissue were stained with Heidenhain's iron-alum hematoxylin and mounted in Canada balsam for observation.

RESULTS

A. Isolation Trials

During the course of the present investigation, 73 cultures, isolated from mature, infected potatoes, were found to be in the genus Streptomyces while three were apparently in the genus Micromonospora. The genus Micromonospora differs from the genus Streptomyces in that conidia are produced singly on the conidiophores whereas in Streptomyces the conidia are produced in chains. Since the conidia of the genus Streptomyces are easily separated, a member of the genus could easily be mistaken as being a member of Micromonospora. Lesions from young infected sweet potato roots yielded only isolates of the genus Streptomyces. Isolations from infested soil also yielded only Streptomyces spp. Thirty isolates were secured from young infected roots while sixteen were taken from infested soil. A total of nine isolates were obtained from Dr. W. J. Martin.

Although no attempt was made to classify all Streptomyces isolates to species, it was believed that, on the basis of color and colony characteristics, many cultures obtained from the same source were duplicates.

With experience, it was noted that most isolates of Streptomyces seemed to thrive well on a synthetic medium such as Czapek's agar. This medium was then used to maintain all cultures until it was noted that pathogenic isolates capable of producing typical soil rot lesions

soon lost their pathogenic properties after three or four transfers when grown on this medium. At one time during the course of this work all pathogenic isolates were lost as a result of keeping them on Czapek's agar. It should be pointed out that in addition to the loss of pathogenicity on this medium, the growth rate of these isolates was reduced to the extent that, upon subculturing, all grew very sparsely or not at all. To avoid a repetition of this loss of isolates, all pathogenic isolates were subsequently maintained in tubes of sterile soil as is recommended by Waksman (28).

Three pathogenic isolates of S. ipomoea were kept in sterile soil for a period of four months at the end of which time they were re-isolated from the soil and tested for pathogenicity by growing susceptible sweet potato cuttings in inoculated soil-extract agar. The same three isolates were also maintained on slants of Czapek's agar and were tested at the same time. The isolates which had been stored in sterile soil were capable of producing the typical browning and dying back of the rootlets. Cuttings grown in the medium containing the isolates which had been maintained on Czapek's agar did not vary significantly from the cuttings grown in tubes which contained no inoculum.

It was noted during the isolating and culturing of pathogenic isolates that distinct differences existed between the pathogenic isolates used in this work and Streptomyces ipomoea as described in Bergey's Manual of Determinative Bacteriology (4). The first differences observed were in the color of the colonies when the pathogenic isolates were grown on Czapek's medium. Since Waksman (28)

reported that pigmentation represents one of the most variable properties among the actinomycetes, physiological tests were run in an attempt to determine if any physiological differences existed between S. ipomoea as described in Bergey's Manual and the pathogenic isolates used in this work (Table I). Standard methods were used in the performance of all the tests. Environmental conditions were varied in culturing the isolates to rule out the possibility of environmental factors altering the reaction of the isolates to the tests. It was found that all pathogenic isolates did not reduce nitrates to nitrites nor did they liquify gelatin. Repeated tests on all pathogenic isolates used in this work revealed that the differences noted were stable properties of the isolates

TABLE I

COMPARISON OF STREPTOMYCES IPOMOEA
ISOLATES FROM DIFFERENT SOURCES

Isolate and Source	Color[1] on synthetic agar	Color[1] on potato plug	Production of nitrites from nitrates	Liquify gelatin
S. ipomoea as described in Bergey's Manual	olive yellow	light brown	+	+
American type culture collection	lilac gray	fuscous black	+	-
Culture from Louisiana	cartridge buff	chamois	-	-
Okla. No. 4-8 from infected sweet potato	cartridge buff	chamois	-	-
Okla. No. 4-15 from infected sweet potato	cartridge buff	chamois	-	-
Okla. No. X3 from infected sweet potato	cartridge buff	chamois	-	-

[1]All colors from Ridgway (21) except those used in describing S. ipomoea in Bergey's Manual.

B. Pathogenicity Trials

Attempts to confirm the pathogenicity of an isolate from a mature infected root after the isolate had been screened in the laboratory were unsuccessful. Of 76 isolates obtained from lesions on mature roots, the one most promising isolate was selected on the basis of the Person and Martin laboratory test for pathogenicity and was increased on potato-dextrose agar. The method used for testing was essentially that of Dykstra (6) as used successfully by him in testing the reaction of white potato seedlings to scab. The inoculum including the agar medium was chopped in a blendor and mixed with vermiculite at the rate of one eight-ounce glass prescription bottle slant per pound of vermiculite. Before adding the inoculum, a complete fertilizer was thoroughly mixed with the moistened vermiculite at the rate of 1 pound of complete fertilizer to 20 pounds of vermiculite. Sixteen rooted vine cuttings of each of 24 sweet potato lines were then planted in the infested vermiculite. Six-inch pots were used with 4 cuttings in each pot. This totaled 4 replicates of each sweet potato line. After 9 weeks the roots of the plants were examined for soil rot lesions after carefully washing the vermiculite away from the roots. In all cases the roots were clean and healthy with no soil rot symptoms whatsoever.

Since all isolates obtained from mature infected roots were apparently nonpathogenic, additional isolates were obtained from freshly dug, young, infected, fleshy roots grown in infested soil at Bixby. Of 30 isolates obtained from this source, 5 were selected for further pathogenicity trials on the basis of their ability to

produce a positive reaction to the Person and Martin test for pathogenicity. Although the reactions to this latter test were not as distinct as might be desired, they were decidedly more pronounced than were those reactions obtained where isolates from mature roots were used. The method used was to plant rooted vine cuttings in pots of sand, soil, or vermiculite which had been seeded with the isolate in question. The inoculum was increased on slants of Czapek's agar in eight-ounce glass prescription bottles and was added to the sand, soil, or vermiculite in the amount of one bottle per six-inch pot. In the preparation of the inoculum, the entire contents of the bottle including the agar medium were placed in a blendor and chopped. Only a single isolate was used per pot. The sweet potato lines Allgold, B 6455, and G 50-30-2 were used in this test because, on the basis of field trials, Allgold was thought to be susceptible to soil rot whereas the latter two were thought to possess some degree of resistance. Three cuttings, one of each line, were used per pot. The identity of the above three lines is as follows:

Allgold - an open-pollinated seedling of Okla. parent 10 which originated in turn from a cross between a selfed seedling of Creole and an open-pollinated seedling of Triumph.

B 6455 - a seedling from the cross B 6184 x B 6251. From C. F. Steinbauer, U.S.D.A. at Beltsville, Md.

G 50-30-2 - a Georgia seedling.

After nine weeks in the greenhouse, the plants were harvested and the roots examined for symptoms of soil rot. All five isolates used were found to be equally pathogenic on each of the three lines of sweet potatoes. Where root enlargement had occurred there were

typical soil rot lesions while the smaller rootlets exhibited the blackened and rotted areas associated with the disease.

It should be noted that lesions were more extensive and pronounced on the roots grown in the sand medium than on the roots grown in soil or vermiculite. Sand also proved to be superior to soil or vermiculite because it was easily washed from the roots without excessive damage to the smaller rootlets.

All isolates obtained from infested soil were screened for pathogenicity by the Person and Martin method. No evidence of pathogenicity was found in any of the isolates.

The above pathogenicity trials indicate that the isolation of pathogenic isolates of S. ipomoea is best accomplished from freshly dug, young, fleshy roots. While it is possible that pathogenic isolates could also be isolated from mature infected roots and from infested soil, all isolates from these two sources showed no evidence of pathogenicity. This experience was confirmed in a personal communication from Dr. W. J. Martin who stated that it was difficult or impossible to isolate S. ipomoea from lesions on mature roots.

Because the method of Person and Martin produced such an indistinct reaction in the screening of isolates for pathogenicity, a method was devised using a modification of Hooker's work with S. scabies (10). As described in the Materials and Methods section, this method consisted of growing sweet potato cuttings in tubes of inoculated soil-extract agar. The use of soil-extract agar enabled one to observe the roots at any time without disturbing the plant. Nonpathogenic isolates obtained from lesions on mature roots were used as controls

during four trials in screening fresh isolates. Pathogenicity was indicated by a distinct browning of the roots. Approximately 75% of the isolates obtained from young roots were shown to be pathogenic by this method. Roots in tubes with nonpathogenic isolates remained clean and sound as did those in tubes to which no organism had been added (Fig. 2).

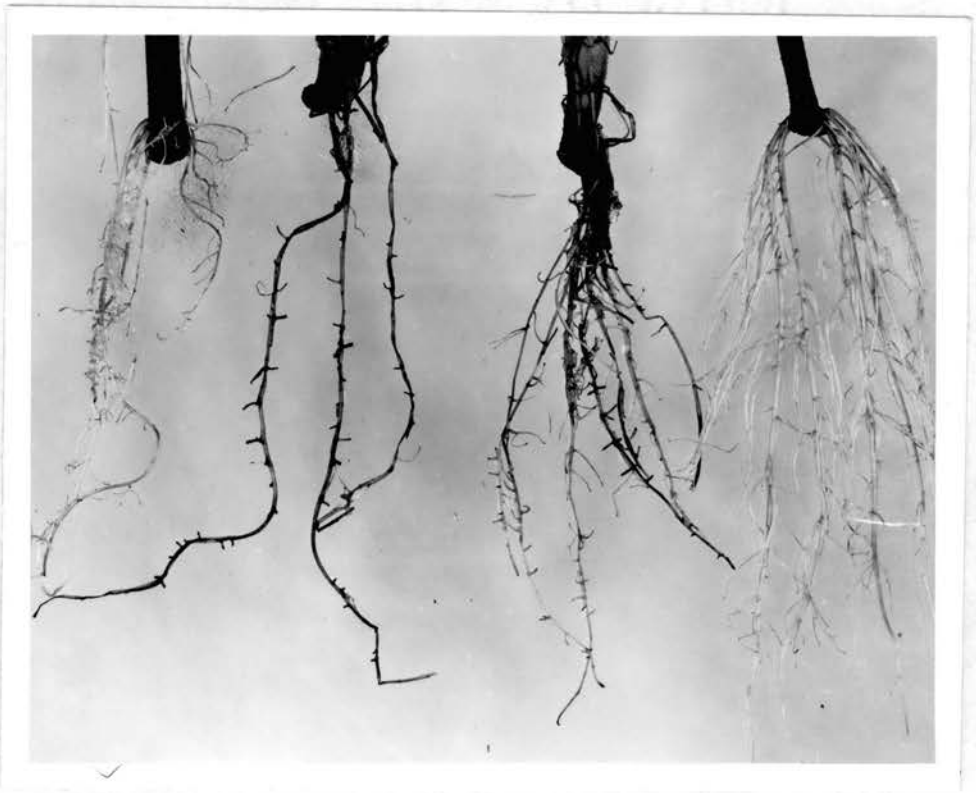

Fig. 2. Sweet potato cuttings of the line P 108 grown in soil-extract agar. The two middle cuttings were grown in agar seeded with a pathogenic isolate of S. ipomoea.

C. Resistance and Susceptibility

Since one of the original objectives of the present investigation was to devise a laboratory or greenhouse method of evaluating the reaction of sweet potato lines to soil rot, and since the just mentioned difficulties in isolating and maintaining the pathogen were encountered, it was decided to proceed with naturally infested soil as the test medium rather than delay until such time as a more satisfactorily controlled technique might be devised.

On two occasions infested soil from the Bixby Station was obtained and placed in benches in the greenhouse. Because of the likelihood of root knot nematode in this soil the first lot was fumigated with the nematocide Dorlone which is a mixture of ethylene dibromide and technical dichloropropenes.

Thirteen different sweet potato lines were grown in both lots of this soil. Rooted vine cuttings were set and allowed to grow for 4 to 6 weeks at the end of which time they were carefully removed, washed and the roots observed for soil rot symptoms. During the period of each experiment frequent observations were made on the relative state of growth of these plants. Each sweet potato line was tested 3 different times in the above manner. In each test in each lot of soil every line was set in 4 replicates of 6 plants each. Placement of the replicates in the bench was at random. The temperature of the soil was maintained at approximately 27°C. with a thermostatically controlled, buried, electric soil cable. The range of greenhouse air temperatures during these tests was approximately 21° to 40°C. Since other investigators had reported that soil rot

develops best under conditions of low soil moisture, the soil in the bench was watered only sufficiently to keep the plants from wilting.

The results from these tests are summarized in Table II. The reaction of each of the lines was sufficiently consistent throughout the series of tests so that the results as presented are representative of any one or all trials. Grading was done on entire root systems on the basis of severity of infection. Healthy plants with vigorous root systems with few or no lesions were considered resistant. Lines considered susceptible had plants that were stunted and the root systems were considerably reduced with remaining roots being heavily infected. Lines with plants showing good top growth and roots with many lesions but with little death or rotting of roots were considered intermediate or tolerant in reaction.

To date there has not been sufficient experience to assess properly the significance of these results on immature plants and their reaction to plants grown to maturity in the field. In those few cases where field results are available it will be noted that there is, for the most part, agreement between greenhouse and field results.

Apparently the fumigant did not appreciably reduce the S. ipomoea population but the nematode population in the unfumigated soil increased to the extent of being a problem after 4 sets of cuttings had been grown in the soil.

TABLE II

REACTION OF 13 SWEET POTATO LINES TO SOIL ROT WHEN GROWN IN INFESTED SOIL

Sweet Potato Line	Reaction[1] as determined in: Greenhouse	Field
Georgia Red	S	R
Okla. 51	R	R
Allgold	I	I
G 50-30-2	I	R
Nemagold	R	R
P 108	S	-
P 97	S	-
Unit No. I Porto Rico	I	I
Orlis	I	-
E 7	R	-
B 6455	I	R
Okla. 29	I	-
T 31-4	R	-

[1] R = Resistant; S = Susceptible; I = Intermediate; a dash indicates no data available.

D. Pathological Histology

In order to determine the effect of the pathogen on the host tissue and to investigate the possibility of the existence of morphological or cytological differences between resistant and susceptible varieties, a series of prepared slides were made. In the examination of the prepared slides, three methods of staining were used in an attempt to find a staining technique that would demonstrate the hyphae in the host tissue. Although Ziehl-Neelson's carbol-fuchsin and safranin-fast green revealed the cellular details, it was only with the use of Heidenhain's iron-alum hematoxylin that the presence of the hyphae could be demonstrated.

Examination of the prepared sections revealed that the pathogen had penetrated throughout the smaller fibrous roots whereas in the larger roots, which had undergone some degree of secondary thickening, the inner limits of a lesion were in the cortical tissues.

A general host response noted was a slight hypertrophy. This was followed by dissolution of cell walls and a complete breakdown of invaded tissues. In earlier stages of the disease a definite enlargement of cell walls was also evident. Lutman (12), in his work on the Irish potato scab, reported that, because of the mycelial growth, cell walls are split by the dissolution of the pectin in the middle lamella and that split walls always appear thicker than intact ones owing to the intrusion of the Streptomyces filaments. It is possible that this could also serve as an explanation of the apparent enlargement of cell walls in the sweet potato.

Cytological differences between resistant and susceptible sweet potato varieties might well exist in the periderm of the fleshy roots. Preliminary investigations indicated varietal differences in the phellem layer of the periderm. By staining freehand sections of the epidermis of sweet potatoes with acetocarmine, it was noted that the number of nucleated cells was greater in the phellem layer of those potatoes thought to be resistant to soil rot than in the apparently susceptible lines. Cooper et al (5) in their work with the Irish potato found that the more susceptible varieties had fewer nucleated cells in the phellem layer than did the resistant varieties. They postulated that the increased number of dead cells on the epidermis rendered the potato more susceptible to infection. Because of early difficulties involved in obtaining and maintaining pathogenic isolates of S. ipomoea in the present investigation, there was not opportunity to pursue further this and other aspects of pathological histology.

DISCUSSION

Although the initial objective of this work was to devise a suitable method for the rapid evaluation of the reaction of sweet potato lines to soil rot, problems encountered during the study tended to restrict the scope of investigations to the isolation, maintenance and pathogenicity of Streptomyces isolates. Perhaps the most perplexing problem was that of obtaining Streptomyces isolates capable of producing typical soil rot symptoms on sweet potatoes. Almost one-half of the time allotted to this study was consumed before it was found that the only source of pathogenic isolates was freshly dug, young, infected, fleshy roots. Isolation of the pathogen from any other source is apparently extremely difficult.

Another problem of considerable consequence encountered in this study was the variability found in the pathogenic isolates used in this work. This problem might well have been anticipated because the actinomycetes in general are noted for their variability. The most variable properties noted in the isolates used in this work were the rate of growth, ability to produce aerial mycelium, and pathogenicity. The decline and sometimes the eventual loss of one or more of these properties was most rapid when an agar medium was used in subculturing and keeping isolates. Erikson (8) reported that saprophytic soil actinomycetes also lose their capacity for producing aerial mycelium when grown on an agar medium.

The fact that actinomycetes are such a variable group might

also account for the differences noted when the pathogenic isolates used in this work were compared with Streptomyces ipomoea as described in Bergey's Manual. Then too, since S. ipomoea is so closely related taxonomically to Streptomyces scabies, the causal organism of scab of white potatoes, it is reasonable to assume that the two organisms would have similar cultural characteristics. Schaal (23) found that isolates of S. scabies obtained from different geographical areas differed markedly in their cultural and pathogenic characteristics. Although no evidence of physiological specialization in S. ipomoea was observed in this study, Thomas (26) reported the isolation of six physiological races of S. scabies which differed in their cultural characteristics. Millard and Burr (17) reported that of 24 strains of Streptomyces isolated from scabbed white potatoes, 11 were found to be pathogenic on white potatoes and that different isolates produced different types of lesions. They concluded that the different isolates each constituted a separate species. If there is any correlation between S. scabies and S. ipomoea, it is not unreasonable to suggest the existence of variants of S. ipomoea or even the existence of different species capable of causing soil rot.

Preliminary investigations on the nature of resistance and susceptibility indicate a need for further study. While investigations made in this study were not conclusive, there were enough differences noted in the periderm of the roots that, with further work, it is possible that the evaluation of the reaction of sweet potato lines to soil rot could be made on differences found in the periderm. A further line of work with reference to the symptomatology of the disease is the correlation between symptoms on young plants and the

symptoms on mature plants. Although some degree of consistency was found when symptoms on young root systems as compared with symptoms on mature roots were used in evaluating the reaction of sweet potatoes to soil rot, enough differences were noted to warrant further study. Infection is thought to occur at an early date. However, if infection can occur at any time during the growing season, the use of young plants, as was done in this work, in evaluating the reaction of sweet potatoes to soil rot would be of doubtful value.

SUMMARY

Investigations were begun on soil rot of sweet potatoes with the ultimate objective being the development of a suitable test for the rapid evaluation of the reaction of sweet potato lines to soil rot. Problems encountered in the isolation and handling of the organism tended to restrict the investigation to methods of isolating and handling the causal organism.

Isolates of Streptomyces pathogenic to sweet potato could not be recovered from lesions on mature potatoes or from known infested soil. The pathogen was recovered only from lesions on freshly dug, young, fleshy sweet potato roots.

Comparison of the pathogenic isolates obtained in this investigation with Streptomyces ipomoea as described in Bergey's Manual revealed that distinct differences existed between the two. Differences in colony color as well as certain physiological differences were noted. While there is not sufficient evidence at hand from the present investigation to assess the full significance of these differences, it is suggested that the local Streptomyces isolates may represent a morphologic or physiologic variant of the species.

Subculturing of the pathogenic isolates on an agar medium resulted in rapid noticeable variations which included a decline in the rate of growth, a decline and sometimes the eventual loss of the ability to produce aerial mycelium, and a loss of pathogenicity. It

was found that isolates could be maintained in a stable condition by keeping them in tubes of sterile soil.

Considerable differences in the reaction of sweet potato lines to soil rot were noted when cuttings were grown in infested soil in the greenhouse. With further work on this problem, the differences noted should be of value in evaluating sweet potato lines for resistance or susceptibility to soil rot.

Histological examination revealed a general host response to the organism was a slight hypertrophy and an enlargement of the cell walls. This was followed by a complete breakdown of the invaded tissues.

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