A SURVEY OF METHODS FOR EVALUATING SWEET POTATO LINES FOR THEIR REACTION TO SOIL ROT

BY

LESTER WILLIAM CARLSON

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Carroll College

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Thesis Approved:

F. Ben Stubbs
Thesis Adviser

Dean of the Graduate School

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INTRODUCTION

The extensive sweet potato breeding program of the Department of Horticulture at the Oklahoma Agricultural Experiment Station has made available many thousand sweet potato seedlings to be evaluated for their relative value as potential new sweet potato varieties. One of the objectives of this program is to produce sweet potato lines that, in addition to other desirable characters, are resistant to as many diseases as possible. Investigations have revealed that there are available in sweet potato breeding lines sources of resistance to several diseases including wilt (5, 6) (causal agent, Fusarium oxysporum f. batatas (Wr.) Snyder and Hansen), root knot (7) (causal agent, Meloidogyne incognita acrita Chitwood), and soil rot (19) (causal agent, Streptomyces ipomoea (Person and Martin) Waks. and Henrici). It is the latter disease with which the present investigation is concerned.

A proper evaluation of resistance or susceptibility to a given disease requires that there be an adequate knowledge of the host, the pathogen, and host-pathogen reactions in a disease relationship.

Present knowledge of soil rot of sweet potatoes is such that evaluation of varietal reaction can be done only in infested soil in the field. This is necessarily time consuming and is not consistently reliable. A better knowledge of several factors involved in this disease should lead

to a more reliable technique for evaluating sweet potato lines for their reaction to soil rot.

This investigation has been primarily concerned with the evaluation of several possible techniques for determining the reaction of sweet potato lines to soil rot. This evaluation has necessarily involved certain studies of the pathogen, the host and the disease.

REVIEW OF LITERATURE

Until the work of Person and Martin (22) in 1940, the causal organism of soil rot remained in doubt. The causal agent had been described by Halsted (10) as a previously undescribed fungus Acrocystis batatas Ell. and Hals. and by Elliott (8) as a new slime mold, Cystospora batata Ell. Manns and Adams (15, 16, 17, 18) and Adams (1, 2), in a series of papers presented evidence that an actinomycete was the organism responsible for soil rot. The work of these latter investigators still left some doubt as to the exact identity of the causal agent since they did not present a formal description of the actinomycete with which they worked. Their organism was simply designated as Actinomyces pox, as stated by them, for convenience.

Person and Martin (22) reinvestigated the soil rot organism and after studies of morphology, physiology and pathogenicity designated the causal agent as Actinomyces ipomoea Person and Martin.

Waksman and Henrici (29) reclassified the actinomycetes and separated a new genus, Streptomyces, from what had previously been Actinomyces. This new genus was characterized as being aerobic, having aerial mycelium, and with conidia in spiral chains. The sweet potato soil rot organism was found to have characters which would cause it to be placed in the new genus. Presently the soil rot organism is designated as Streptomyces ipomoea (Person and Martin) Waks. and Henrici.

The fact that the actinomycetes in general are quite variable in morphology and physiology has been noted by many investigators. Because of this, Waksman (28) suggested that the group be classified into "group species" with limits set on the amount and type of variation. Variation in pathogenicity in the actinomycetes has been reported by Millard and Burr (20) in their work with common scab (causal agent, Streptomyces scabies (Thaxter) Waks. and Henrici) of white potato. Leach et al (14) and Schaal (25) reported the existence of physiologic forms of S. scabies. While the same phenomenon no doubt exists in S. ipomoea, there is at present only a limited amount of evidence (26) that this is so.

Usually the recommended method for control of soil rot of sweet potato is to effect a reduction of soil pH through application of sulfur to infested soils (11, 18, 21). This method is not generally used because of possible deleterious effects from low soil pH on crops used in rotation with sweet potato and because of cost. Resistance, undoubtedly, would be the best and most practical means of control.

The fact that there are sources of resistance to soil rot in sweet potatoes has been demonstrated by Watson (30), by unpublished work at the Oklahoma Agricultural Experiment Station and by Martin (19). One of the most difficult aspects of breeding for this resistance is the ready identification of it. Martin pointed out, after several years of attempts to develop a laboratory or greenhouse technique, that he still had to resort to field tests to evaluate resistance or susceptibility. He stated further that the resistance available in even the more resistant varieties such as Heartogold and Acadian is not of a particularly high type. However, a higher type of resistance was found in several

Louisiana seedlings and U. S. Department of Agriculture Plant Introduction selections. Based on observation, Martin suggested that resistance in Heartogold and Acadian is at least partially due to the ability of these varieties to produce many roots which grow rapidly and thus escape the effects of the early rootlet rot phase of soil rot.

MATERIALS AND METHODS

Cultures of Streptomyces ipomoea used in the present investigation were obtained from Dr. W. J. Martin, Department of Plant Pathology, Louisiana State University; the American Type Culture Collection; or were isolated from infected sweet potato roots grown in infested soil from the Horticulture Vegetable Research Station at Bixby, Oklahoma. Some of the cultures from this latter source had been isolated by Weast (31); others were isolated by the author.

Weast had tried several media for isolation and found soil-extract agar as satisfactory as any medium. Water agar (1.7%) was found to be as useful as soil-extract agar in the present investigation.

Actinomycete isolates were obtained from small, unbroken, root lesions. These latter were surface sterilized for approximately 60 seconds in a 10% Clorox solution, rinsed in sterile distilled water, and macerated in warm, sterile water agar. After incubation for 4 days at 32° C, individual colonies were picked and transferred to slants of Czapek's agar. As soon as good growth resulted (14 days), Streptomyces isolates were transferred to sterile soil-sand (1.5 parts soil to 1 part sand) mixture. The purpose in transferring immediately to soil-sand was an attempt to maintain the cultures in as stable a state as possible.

Several different methods for testing pathogenicity of Streptomyces isolates were tried and these tests will be found elaborated under culture studies in the section on results.

Inoculum for all tests requiring it was prepared by scraping the 14 day-old growth from a slant of Czapek's agar in an 8 oz. prescription bottle. The resulting mycelium and spores, suspended in 200 ml of sterile distilled water, were chopped in a blendor and used without further dilution.

RESULTS

A. Culture Studies

Authentic cultures of Streptomyces ipomoea were essential for other phases of this investigation; consequently, it was important that each of the cultures at hand be properly identified. This involved studies of colony morphology and color, physiological tests, and pathogenicity tests. After a certain amount of experience, S. ipomoea could be identified with a high degree of certainty on the basis of colony morphology and color alone.

There were a total of 54 Streptomyces cultures available. Of these, 47 had been isolated by the author from plant material grown in the Bixby soil, 2 were from Louisiana, 3 from Weast's earlier work in Oklahoma, and 2 from the American Type Culture Collection. According to Bergey (3), colonies of Streptomyces ipomoea on a synthetic medium should be moderately wrinkled, superficial, and olive yellow in color. Any isolates that did not closely resemble this description were not used in later pathogenicity trials.

Key physiological characters of S. ipomoea are ability to liquify gelatin, reduce nitrates to nitrites, and inability to produce soluble pigment on a potato plug. All of the isolates later demonstrated to be pathogenic exhibited these characters; non-pathogenic isolates had none or only some of these characters. The Louisiana isolates and the Okla-

homa isolates used by Weast proved to be identical in physiological characters to those isolated by the author. The American Type Culture Collection isolates labeled S. ipomoea differed in all respects from the other cultures. It is suggested that the American Type Culture Collection isolates may have mutated or in some other way been altered in culture so that they no longer represent typical S. ipomoea. Representative S. ipomoea isolates are compared with respect to key characters in Table I.

Weast (31) presented evidence, on the basis of these key characters, that the Oklahoma isolates of S. ipomoea with which he worked possibly were different or variant forms of the species. The present investigator has been unable to confirm this work.

There were 54 isolates of S. ipomoea available but only 25 were tested for pathogenicity and of these only 18 were found to be pathogenic. The reason that all 25 selected as S. ipomoea were not pathogenic is attributed to the fact that many of the isolates were selected early in this investigation before skill had been achieved in choosing typical cultures on the basis of color and morphology.

Several different methods to determine pathogenicity of cultures were tried and all but one gave unsatisfactory or unreliable results. Among the methods tried was one suggested by Person and Martin (22) which involves the use of infested agar blocks placed on roots of rooted cuttings under essentially aseptic conditions. Another method tried was that used by Weast (31) which involved growing of rooted cuttings in soil-extract agar seeded with the isolate in question. In addition, rooted cuttings whose roots had been dipped into a suspension of inoculum were

TABLE I

COMPARISON OF KEY CHARACTERS OF DIFFERENT *STREPTOMYCES IPOMOEA* ISOLATES

Culture	Color[1] on Czapek's agar	Soluble pigment on potato plug	Nitrates reduced	Gelatin liquified
S. ipomoea by Bergey's Manual	olive yellow	none	yes	yes
American Type Culture Collection No. 11747	whitish gray	dark soluble pigment	no	no
Okla. No. 58-40	olive yellow	none	yes	yes
La. isolate, Sept., 1958	olive yellow	none	yes	yes
Weast isolate 4-15	olive yellow	none	yes	yes
Okla. No. 58-29	olive yellow	none	yes	yes
American Type Culture Collection No. 10896	whitish gray	dark soluble pigment	no	no

[1]All colors from Ridgway (24) except those used in describing *S. ipomoea* in Bergey's Manual.

grown in vermiculite or soil. The response with any of these techniques was a discoloration of the roots. The extent of discoloration from test to test, using the same technique, was so variable as to make results questionable. Similar discoloration frequently resulted on roots inoculated with cultures known to be non-pathogenic and even on uninoculated controls.

A technique adapted from one used by Lawrence (13) in work with Streptomyces scabies on detached white potato tubers proved quite satisfactory. As adapted, this technique consisted of using small (3-6 mm in diameter), freshly dug, soil rot susceptible sweet potato roots which were cut into sections approximately 6 cm long, washed in sterile distilled water, and dipped momentarily into a chopped mycelium-spore suspension of the isolate in question. These pieces of root were then embedded in sterilized vermiculite in 145 x 20 mm Petri dishes and incubated at 32° C for 5-7 days. Prior to autoclaving the volume of vermiculite necessary to fill one of these dishes was moistened with 100 ml of distilled water.

Consistently good results were obtained through the use of this technique of testing isolates for pathogenicity. Pin-point lesions were evident 3 days after inoculation with a pathogenic culture. Five days after inoculation lesions were quite evident. The soil rot organism was easily reisolated from unbroken young lesions on these roots.

This test made it possible to show that with repeated transfers of pathogenic cultures on a synthetic medium there resulted a gradual loss of pathogenicity. This was evident with as few as 4 transfers. The effect on pathogenicity of repeated transfers on Czapek's agar is illus-

trated in Fig. 1. The best way to eliminate this problem was found to be periodic reisolation of the desired culture from the soil-sand stock culture. Cultures have been reisolated from 2-year-old soil-sand stock culture without evident loss of pathogenicity. Waksman (27) has stated that cultures of actinomyetes that have degenerated with repeated transfers on a synthetic medium can be restored to their original state when re-established in sterile soil-sand or an glycerol nutrient agar. This method for restoring pathogenicity in the present work was not evaluated.

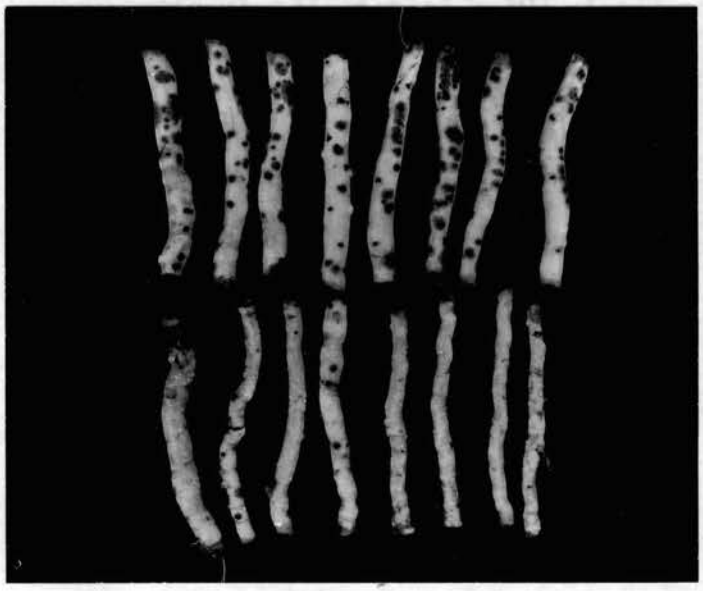

Fig. 1. Sweet potato roots of a susceptible line, P-97, showing relative amounts of infection when inoculated with a pathogenic isolate, 58-40 (upper row), and a similar isolate, 58-M-1 (lower row), after four transfers on Czapek's agar.

B. Tests of Methods for Evaluating Resistance and Susceptibility

The principal objective of this investigation was to provide a relatively simple, reliable method for evaluating the reaction of sweet potato seedlings or lines to soil rot. Methods for obtaining and maintaining pathogenic isolates of the soil rot pathogen have been presented in the previous section. The search for a method for evaluating varietal reaction in sweet potato necessitated the use of lines or varieties that have a known reaction to soil rot. The following varieties or lines were selected as representing a range of reactions from resistant to susceptible: Acadian, Nemagold, Allgold, 4-126 (an Oklahoma seedling), P-97 (an Oklahoma parent line), and Unit No. 1 Porto Rico.

Three different types of methods for evaluating varietal reaction were investigated. These were methods dealing with a response of the sweet potato plant in intimate association with S. ipomoea, a method for determining the relative amount of chlorogenic acid present in roots, and methods for determining possible morphological differences associated with resistance or susceptibility.

Weast (31) had used a method for evaluating varietal reaction in which rooted cuttings were grown in naturally infested soil in the greenhouse. The basis for evaluation in this test was the relative amount of root rot or discoloration and top growth. He obtained good agreement between greenhouse and field results where the latter were available. The greenhouse testing method outlined by Weast was used in the present investigation to test the 6 standard sweet potato lines indicated earlier. Greenhouse air temperatures ranged from 20°-35° C, while soil temperature was about 27° C. Water was supplied only as needed to keep plants from

wilting as low soil moisture has been reported to enhance soil rot development. The plants were removed after 6 weeks and evaluated for their reaction to soil rot. Differences between lines with respect to severity of soil rot were observed, but these differences were not considered sufficiently distinct and consistent to be of real value. This testing procedure was repeated 3 different times with similar results.

Another test method tried was one adapted from work reported by Pieringer (23) in which white potato seedlings were tested against common scab. This method involved the growing of rooted sweet potato cuttings in a vermiculite-soil mixture to which a suspension of the soil rot organism had been added. The suspension was first thoroughly mixed with vermiculite. The resulting vermiculite-inoculum was then mixed 1 to 1 with sterilized soil. Sweet potato plants were left to grow in this mixture for 2 months at air temperatures ranging from 20° to 40° C. When harvested, the root systems of all lines of sweet potato used in this test were extremely discolored. While there were observable differences between varieties with respect to the severity of discoloration, these differences were too subtle to make this a useful test.

After observation, roots of plants were rinsed in distilled water, and the plants were repotted in sterilized soil. Two months later the roots were again examined to determine how much, if any, the disease had spread to new roots. There was no evidence that soil rot had spread from the infected to new roots.

Rooted sweet potato cuttings were potted in a mixture of naturally infested soil and sterile vermiculite in another test. Results here were essentially the same as those from the tests in which naturally infested soil was used alone.

Another method tested involved the use of rooted cuttings dipped into a mycelium-spore suspension of S. ipomoea. These were then potted in sterile, slightly moistened vermiculite and grown for 2 weeks at room temperature (28°-32° C). Evaluation of root systems for relative amounts of soil rot was not possible because all root systems of inoculated and control plants were rather uniformly discolored.

Because rooted cuttings had failed to give clear cut differential varietal reactions when inoculated with S. ipomoea, the modified Lawrence technique, which had already been shown to give good results in pathogenicity tests, was tried to see if varieties might respond differentially in this test. Roots 3-6 mm in diameter obtained from plants of the 6 standard varieties grown in the field were inoculated and incubated for 1 week. Lesion counts on each root piece were made at this time. This test was repeated twice, each time with 8 roots of each variety and with each of 3 different S. ipomoea isolates. Results from 1 of these tests with 1 isolate are presented in Table II. There was considerable variation from root to root within a variety with respect to the number of lesions, but it was still possible to demonstrate a statistical difference between lines in the number of lesions. However, these differences could not be correlated with varietal differences as determined from field experience. Because of this and the fact that considerable numbers of roots per test would have been necessary to compensate for the variation noted above, this test was considered unsatisfactory for differentiating soil rot reactions in sweet potato.

In the tests just reported it has been noted that 3 different isolates of S. ipomoea were used. In the results there was a suggestion of

TABLE II

RESPONSE AS MEASURED IN NUMBER OF LESIONS ON SIX SWEET POTATO LINES INOCULATED WITH ISOLATE OKLA. NO. 58-40 OF STREPTOMYCES IPOMOEA

Sweet Potato Line	Root Number 1	2	3	4	5	6	7	8	Total
Unit No. 1 Porto Rico	5[1]	7	10	3	5	3	5	4	42
Acadian	9	10	6	6	2	7	8	5	53
4-126	5	3	3	5	16	11	11	18	72
Nemagold	10	21	17	25	7	13	11	10	114
P-97	13	7	12	8	19	43	28	24	154
Allgold	26	40	15	19	23	31	27	25	206

[1]Lesions per root.

possible differences between isolates with respect to their pathogenicity, but because of the great amount of variation in lesion numbers between root replicates, it was not possible to obtain convincing evidence of these differences.

In another test using the modified Lawrence technique mature roots, 3-6 cm in diameter, were substituted for the smaller diameter roots used previously. Three roots of each of the 6 sweet potato lines were tried against S. ipomoea isolate Okla. No. 58-40. Lesions resulting on these roots were too numerous to count. Evaluations were based on overall appearance of the roots. In an attempt to avoid prejudice due to familiarity, 3 persons in addition to the author evaluated these roots for relative severity of soil rot. Results of these ratings are presented in Table III. There was rather general agreement in the rank of each of the varieties. These ratings generally fit with observed field reactions of these varieties.

Six standard sweet potato lines along with 9 lines whose soil rot reaction was unknown to this investigator were inoculated to test this method further. Three mature roots of each line were used. Again, results from the 6 standard lines were found to be in agreement with observed field reactions. However, results with the 9 unknowns did not agree at all with evidence from field data. While, admittedly, the field data may not in all cases be accurate, the fact that there was no agreement between the 2 sets of data would suggest that the laboratory test is not a good one for the purpose intended.

Johnson and Schaal (12) presented evidence that resistance or susceptibility of white potato to common scab was correlated with the relative amount of chlorogenic acid or related phenolic compounds present in

TABLE III

EVALUATION OF INFECTED, MATURE SWEET POTATO ROOTS BY FOUR OBSERVORS

	Sweet potato lines rated by			
Rating[1]	A[2]	B	C	D
1	Acadian	Acadian	Acadian	Nemagold
2	Nemagold	P-97	Nemagold	Acadian
3	P-97	Nemagold	P-97	Allgold
4	Allgold	Allgold	Allgold	P-97
5	4-126	4-126	Unit #1 Porto Rico	Unit #1 Porto Rico
6	Unit #1 Porto Rico	Unit #1 Porto Rico	4-126	4-126

[1] 1 = best; 6 = worst.

[2] A = author; B = non-familiar observor; C = familiar observor; D = non-familiar observor.

the outer cell layers of tubers. Chlorogenic acid was detected by macerating pieces of tuber peelings in a 2% aqueous ferric chloride solution. The intensity of the resulting green coloration indicated the relative amount of chlorogenic acid present in the tissues. The more intense the color, the more chlorogenic acid there was present. Resistance was associated with higher amounts of chlorogenic acid. Other investigators have demonstrated chlorogenic acid in other tissues of the white potato and in tissues of other plant species.

Rootlets and pieces from the outer portion of larger sweet potato roots from each of the 6 standard varieties were tested for chlorogenic acid according to the method outlined above. As detected by this test, differences in chlorogenic acid content of the several lines were too subtle and varied too much from test to test to be of possible value in separating the lines according to their relative susceptibility to soil rot.

Cooper et al (4) discovered that resistance and susceptibility to common scab in white potato were associated with the relative number of non-nucleated cell layers in the periderm of tubers. Resistant lines had no, or few, non-nucleated layers while susceptible lines had several such layers.

Sweet potato lines were evaluated in the present work for resistance or susceptibility to soil rot by comparing layers of non-nucleated cells in the periderm of roots. Free-hand sections of root periderm were stained with aceto-carmine and the layers of non-nucleated periderm counted.

Data on the non-nucleated periderm from mature roots of the 6 standard sweet potato lines used in this work are presented in Table IV.

TABLE IV

MEAN LAYERS OF NON-NUCLEATED PERIDERM ON ROOTS OF SIX SWEET POTATO LINES WITH STATISTICAL SIGNIFICANCE AS SHOWN BY DUNCAN'S MULTIPLE RANGE TEST

Lines	Mean layers of non-nucleated periderm for each indicated root			
	1	2	3	Mean
Nemagold	3.00[1]	2.75	3.00	2.92
Acadian	3.75	3.75	3.75	3.75
Allgold	5.25	3.25	4.00	4.17
4-126	4.50	4.50	5.00	4.67
P-97	5.75	5.50	5.00	5.42
Unit #1 Porto Rico	7.25	7.00	6.50	6.92

[1] Each figure is a mean based on four observations.

Statistical Significance:

Nemagold	Acadian	Allgold	4-126	P-97	Unit #1 Porto Rico
2.92	3.75	4.17	4.67	5.42	6.92

Note: Any two means underscored by the same line are not significantly different at the 5 per cent level. Any two means not underscored by the same line are significantly different at the 5 per cent level.

Counts were made on each root at each of 4 locations and 3 roots of each line were used. The maximum variation observed in layers of non-nucleated periderm on any one root was one layer. There was statistical significance between lines with respect to the relative number of layers of non-nucleated periderm. The more resistant a line was the fewer the non-nucleated periderm layers; the more susceptible a line, the more non-nucleated periderm layers. These data correlate very well with observations from the field. Differences in the periderm also were present in roots 5-10 mm in diameter. Differences between 2 sweet potato lines relative to cell layers of non-nucleated periderm are illustrated in Fig. 2.

The validity of this periderm test for identifying resistance or susceptibility to soil rot in sweet potato was tested further with 23 additional sweet potato lines plus the 6 standard lines. Counts of layers of non-nucleated periderm were again made from stained sections of mature roots. The range in mean layers of non-nucleated periderm layers was from 1.25 in Heartogold to 9.5 in L 3-77. Each line was rated as resistant, intermediate or susceptible in its expected reaction to soil rot according to the number of non-nucleated cell layers found. These ratings compared with ratings based on field data are presented in Table V. It will be noted that there is a very high degree of correlation in the sweet potato lines tested between ratings based on non-nucleated periderm and ratings based on data obtained from these lines planted in infested field soil.

The scale of ratings based on non-nucleated periderm presented in Table V is somewhat arbitrary but is based partly on the statistical data presented in Table IV and partly on observed field reactions in relation to periderm. There is not a clear cut break in non-nucleated

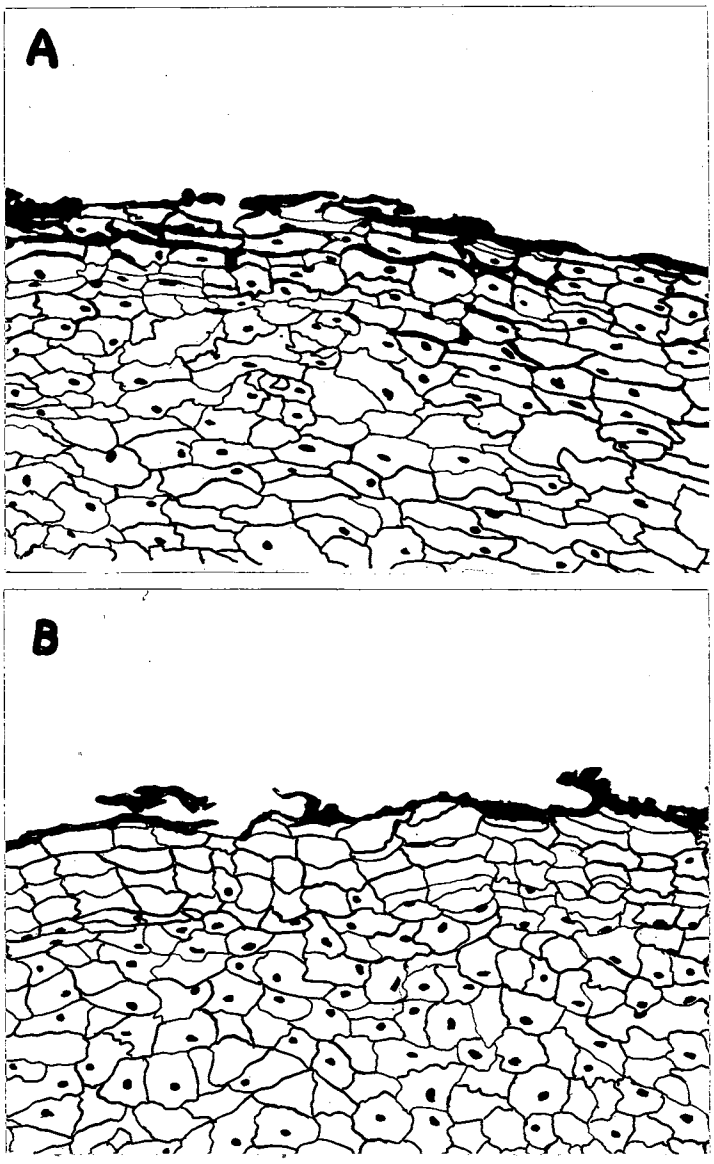

Fig. 2. Comparison of two lines of sweet potatoes with reference to non-nucleated layers of periderm (using small roots 5 mm in diameter). A. Acadian - a resistant line. B. P-97 - a susceptible line.

TABLE V

EVALUATION OF SWEET POTATO LINES WITH REFERENCE TO NON-NUCLEATED PERIDERM AND FIELD DATA

Sweet Potato Line	Non-nucleated[1] Periderm Data	Field Data	Sweet Potato Line	Non-nucleated Periderm Data	Field Data
Heartogold	R[2]	R	P-97	S	S
Acadian	R	R	P-101	I	I
Nemagold	R	R	P-108	S	S
Allgold	I	I	P-131	I	I
Unit #1 Porto Rico	S	S	P-139	R	R
Redgold	I	I	4-107	I	R
Georgia Red	I	I	4-126	I	S
Okla. 51	S	I	5-26	I	I
Okla. 53	S	S	5-66	R	R
Okla. 54	S	S	5-84	R	R
Okla. 61	I	I	5-122	S	S
L 3-77	S	S	5-146	I	I
P-15	I	I	5-154	R	I
P-81	I	I	5-196	I	I
P-89	R	I			

[1]Rating according to layers of non-nucleated periderm: R = 0-2.99; I = 3.00-4.99; S = 5.00 or over.

[2]R = resistant; I = intermediate; S = susceptible.

periderm layers between resistance and susceptibility. Rather, there is a gradation from resistance to susceptibility which makes it difficult to establish absolute groups. It will be noted in Table V that where the 2 sets of data have not received the same rating that the difference is between intermediate and resistant or between intermediate and susceptible. It should be pointed out that the field data are not necessarily in all cases true representations of the reaction of a given sweet potato line. There are many factors which may not permit the accurate evaluation of these lines in field trials. Further, in presently available sweet potato lines there does not appear to be a high degree of resistance. On the basis of the foregoing, it would seem that a reasonably accurate measure of resistance or susceptibility to soil rot in sweet potato is the number of non-nucleated layers in the periderm of mature roots.

It had been observed in these studies that some of the more resistant lines apparently produced more roots and longer individual roots than did susceptible lines. Martin (19) also observed this phenomenon in the relatively resistant variety Heartogold. In an attempt to demonstrate quantitatively how consistently this ability was associated with resistance in the present work, 10 cuttings each of 12 different sweet potato lines were rooted for 1 week in water. Roots on each cutting were then counted and measured. The average number of roots per cutting was multiplied by the average root length to give a single value. The product was designated as a value representing relative root development for the line. The results of this test are compared with data on the reaction of these lines as observed in the field in Table VI. Differences in root growth between 2 sweet potato lines are illustrated in

TABLE VI

ROOT DEVELOPMENT IN EACH OF TWELVE SWEET POTATO LINES AS RELATED TO SOIL ROT REACTION IN FIELD TESTS

Lines	Average Number of Roots	Average Length per Root	Relative Root Development	Field Rating
Acadian	18.00	5.00	90.00	R[1]
Nemagold	18.88	4.11	77.60	R
P-81	16.00	4.40	70.40	I
4-126	22.00	3.10	68.20	S
4-107	8.20	6.00	49.20	R
Okla. 61	11.67	3.83	44.70	I
Redgold	8.10	3.75	30.38	I
Allgold	4.50	6.10	27.45	I
P-97	11.00	2.30	25.30	S
Okla. 51	7.80	2.50	19.50	I
P-108	5.90	2.35	13.87	S
Unit #1 Porto Rico	8.40	1.50	12.60	S

[1]R = resistant; I = intermediate; S = susceptible.

Fig. 3. A resistant compared with a susceptible line may have either a greater number of roots per plant or the average root length may be greater or both may occur. Generally the more resistant lines have an appreciably higher root development value than do susceptible lines. Higher root development values do not in all cases coincide with resistant field ratings. This can be explained by the fact that the field ratings are for the most part based on readings from mature roots and mature root resistance is not necessarily correlated with rootlet resistance.

There is evidence, then, that the more roots a given sweet potato line produces and the more rapidly they grow the more likely the line is to be resistant to soil rot. These abilities probably allow more roots to escape infection and thus there is less rootlet rot than is likely to occur with a susceptible line such as Unit No. 1 Porto Rico.

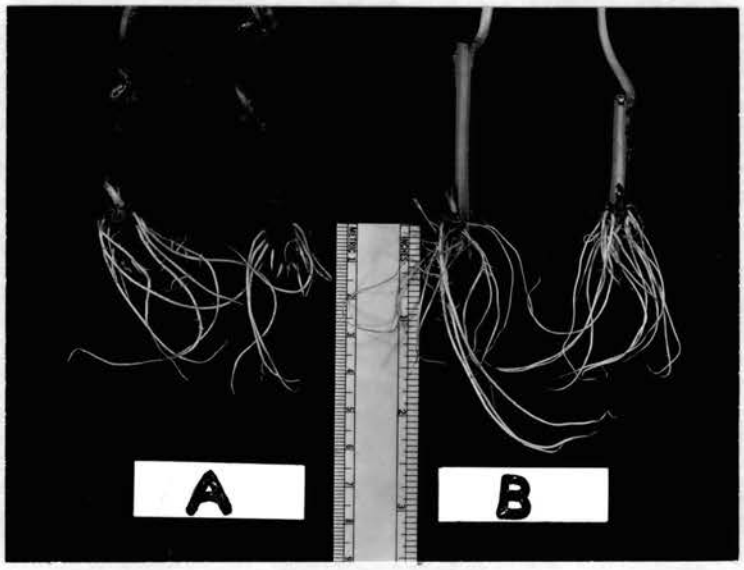

Fig. 3. Comparison of root systems of two sweet potato varieties. Note that with Nemagold (B) there are more and longer roots than with All-gold (A).

C. Pathological Histology

Knowledge of the time and place of infection by S. ipomoea could be important in understanding the nature of resistance. If infection takes place only in areas of non-nucleated periderm this could mean that resistance might be due to the absence of non-nucleated periderm. If the time necessary for infection were relatively long this could mean that rapidly developing roots might not be in contact with the causal organism for a sufficient time for infection to occur.

Detached roots (4-6 mm in diameter) of P-97 (susceptible) and Acadian (resistant) were inoculated with S. ipomoea isolate 58-40 using the modified Lawrence technique in an attempt to answer some of the questions relative to where infection occurs and how long it might take. One root from each sweet potato line was removed from the inoculum at 12 hour intervals following inoculation, killed and fixed in Craf III solution. This process was repeated 7 times or until 84 hours had elapsed. Each fixed root was cut into small sections (3-4 mm long), dehydrated in a dioxan-butyl alcohol series, embedded in paraffin, and sectioned at 10 u. Sections were mounted, stained in iron-alum hematoxylin, mounted in synthetic resin and examined.

There was evidence in this material that infection had occured in less than 12 hours, but it was only through careful observation that it could be detected. Mycelium tended to collect on the outside of the root and appeared as a mass of heavily staining tissue. Mycelium was observed intracellularly only where there were non-nucleated cells in the periderm (Fig. 4). No difference was detected between P-97 and Acadian with respect to the time or place of infection.

Fig. 4. Section of non-nucleated periderm cells showing hyphae within a cell.

There was evidence (Fig. 5), in other prepared sections, that infection tended to sever the rootlets. This is good evidence for occurrence of infection in living tissue but the writer has never observed mycelium in apparently living cells.

Because of time limitations further studies of pathological histology were not possible.

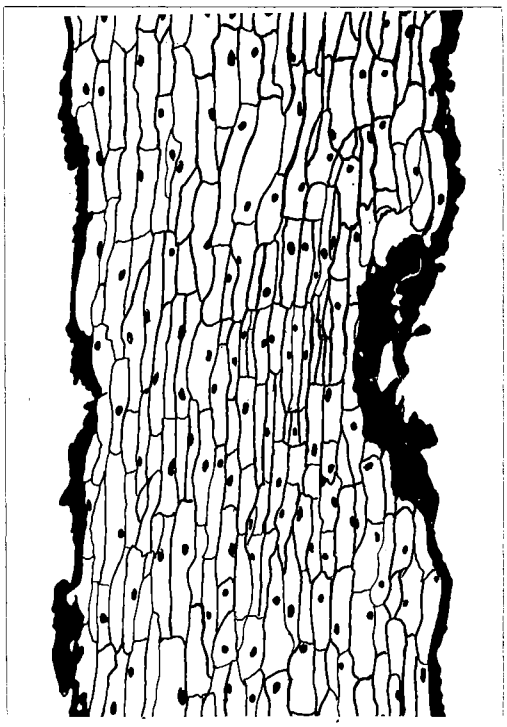

Fig. 5. Longitudinal section through a lesion on a young root of Nemagold.

DISCUSSION

The main objective of the present work has been to develop a technique by which sweet potato lines could be evaluated for their reaction to soil rot in the laboratory or in the greenhouse. It was essential in these studies that pathogenic cultures of the causal agent be readily available. The first major problem encountered was that of determining pathogenicity of the isolates. Several methods of testing for pathogenicity as measured by rootlet rot or discoloration were tried but these were found to be relatively of little value because of the difficulty of distinguishing rootlet discoloration caused by factors other than S. ipomoea. Results with these tests were also too inconsistent to be useful.

A satisfactory technique for testing pathogenicity of S. ipomoea isolates was developed as a part of the present investigation. This consisted essentially of inoculating detached roots (3-6 mm in diameter) and incubating them for 5-7 days in moist vermiculite. Pathogenic isolates in such a test produced distinct lesions which are readily identified. This test, used in conjunction with colony morphology and physiological tests, should positively identify pathogenic isolates of S. ipomoea as the fungus is recognized at this time.

While physiologic races of S. ipomoea may exist, there was no evidence of this from the present investigation. Variability in pathogen-

icity of isolates of S. ipomoea was observed but the differences between isolates were not of an order sufficient as to distinguish races. Observed differences in pathogenicity were probably due to cultural conditions. There was evidence that as cultures aged on a synthetic medium and were subsequently transferred there was a loss of certain morphological and physiological properties. These phenomena have also been noted by Waksman (28) and Weast (31). Erikson (9) observed the loss of potential to produce aerial mycelium in certain actinomycetes. It seems reasonable to assume that factors for pathogenicity might also be altered or lost with repeated transfer of isolates.

The most perplexing problem encountered in the present work was the ready identification of resistance or susceptibility to soil rot in sweet potato lines. A variety of testing techniques was tried in an attempt to indentify varietal reaction. For the most part these tests were dependent upon rootlet rot or discoloration and in every case differences between lines were too subtle to be of any real value for distinguishing differences.

Two techniques for evaluating sweet potato lines for their resistance or susceptibility to soil rot were developed and appear to be reliable. The facts have been presented to show that resistance is associated with relative number of non-nucleated cell layers in the periderm of sweet potato roots. The fewer of these layers, the more resistant the line; the more of these layers the more susceptible is the line. These layers were found to be more readily distinguishable in fresh, mature roots. While readings could be made from stored roots, nucleated cells in the periderm of these tend to become more vacuolate and the nuclei more difficult to see distinctly, thus making it diffi-

cult to distinguish nucleated from non-nucleated layers. Exactly how many non-nucleated layers it takes to make a line susceptible can not, at present, be explained. Non-nucleated periderm and resistance or susceptibility may simply be chance correlations. It is suggested that one way in which non-nucleated periderm may function in making a variety susceptible or resistant is to allow S. ipomoea to become established where there are several layers, or to make it difficult for S. ipomoea to become established where there are fewer layers.

The second technique which evidence shows may be of use in identifying resistance or susceptibility is the measurement of the rapidity with which an abundant root system is produced. Exactly how an abundant, rapidly growing root system makes a plant more resistant can not be explained except to suggest that with these qualities the plant may be better able to survive after loss of some rootlets. Martin (19) suggests that an abundant root system allows a plant to escape the early effects of rootlet rot. It should be pointed out that apparent resistance to the rootlet rot phase of soil rot does not necessarily mean that older or mature roots may not be severely attacked.

It is believed that through the use of the non-nucleated periderm test and the measuring of root development an accurate evaluation of the reaction of a sweet potato variety to soil rot can be obtained. The one test will indicate how mature roots might be expected to react and the other how young roots might react.

SUMMARY

Two techniques for the evaluation of the reaction of sweet potato lines to soil rot have been developed. Essentially these methods involve the determination of the number of layers of non-nucleated periderm on mature roots and the rate at which young plants produce a root system. Resistance to soil rot was found to be associated with fewer non-nucleated periderm layers and an abundant, rapidly growing root system. Results from these tests correlated well with field data.

It was found that any inoculation technique which was dependent on any phase of rootlet rot for results was of relatively little value in distinguishing varietal reactions or as a pathogenicity test.

A reliable technique for determining pathogenicity of isolates of Streptomyces ipomoea was demonstrated and involved the use of inoculated thickened sweet potato roots incubated 5-7 days in moist vermiculite.

No evidence of physiologic specialization in S. ipomoea was found.

Studies of pathological histology revealed that infection by S. ipomoea can occur anywhere on the root surface and that in the case of thickened roots, 12 hours or less is sufficient for infection.

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VITA

Lester William Carlson

Candidate for the degree of

Master of Science

Thesis: A SURVEY OF METHODS FOR EVALUATING SWEET POTATO LINES FOR THEIR REACTION TO SOIL ROT

Major Field: Botany and Plant Pathology

Biographical:

Personal data: Born in Town of Warren, Waushara County, Wisconsin, September 12, 1933.

Education: Attended elementary school near Lannon, Wisconsin; graduated from Menomonee Falls High School in 1951; attended Wisconsin State College at Stevens Point, 1951-53; received Bachelor of Science degree from Carroll College in May, 1955; entered Oklahoma State University of Agriculture and Applied Sciences in fall of 1957; completed requirements for the Master of Science degree in August, 1959.

Professional Experience: Laboratory assistant, Biology Department, Carroll College, 1954-55; U. S. Army, 1955-57; graduate research assistant Department of Botany and Plant Pathology, Oklahoma State University, 1957-59.

Member of The American Phytopathological Society, member of Phi Kappa Phi, and associate member of Sigma XI.

Date of Final Examination: June, 1959.