A STUDY OF RESISTANCE TO THE SWEET POTATO WILT PATHOGEN, *FUSARIUM OXYSPORUM* SCHLECHT F. *BATATIS* (WR.) SNYDER & HANSEN, AND OF HISTOLOGICAL ASPECTS OF THE HOST-PATHOGEN COMPLEX

by

John Milton Wells
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APPROVAL SHEET

Title of Thesis: A Study of Resistance to the Sweet Potato Wilt Pathogen, Fusarium oxysporum Schlecht f. batatis (Wr.) Snyder & Hansen, and of Histological Aspects of the Host-pathogen Complex.

Name of Candidate: John Milton Wells
Master of Science, 1963

Thesis and Abstract Approved:

James G. Kantzes

James G. Kantzes
Associate Professor of Plant Pathology
Department of Botany

Date approved: July 23, 1963

ABSTRACT

Title of Thesis: A Study of Resistance to the Sweet Potato Wilt Pathogen, Fusarium oxysporum Schlecht f. batatis (Wr.) Snyder & Hansen, and of Histological Aspects of the Host-pathogen Complex

John Milton Wells, Master of Science, 1963

Thesis directed by: Dr. James G. Kantzes
Associate Professor of Plant Pathology

Research on Fusarium wilt of sweet potato, a vascular disease caused by Fusarium oxysporum Schlecht f. batatis (Wr.) Snyder & Hansen, was undertaken to determine the susceptibility of various sweet potato lines to Maryland isolates of the pathogen under field and greenhouse conditions. Highly resistant lines would be useful as sources of resistance to Fusarium wilt in sweet potato breeding programs.

In 2 years of field and greenhouse trials, 94 different lines of sweet potato were inoculated with a composite spore and mycelial suspension of 5 Maryland isolates of F. oxysporum f. batatis. Results indicated that the following lines were highly resistant to the pathogen: the foreign plant introductions P.I. 153655 ("Tinian"), P.I. 153906, P.I. 153907, and P.I. 251602; the variety Pelican Processor; and the breeding selections B-6842 from the United States Department of Agriculture Plant Industry Station at Beltsville, Maryland, and T-7 from the Georgia Coastal Plain Agricultural Experiment Station at Tifton, Georgia.

Greenhouse experiments showed that the host range of Fusarium oxysporum f. batatis should include an additional species of Morning Glory, Ipomoea pandurata (L.) G. F. W. Mey. Furthermore, no symptoms

of infection were obtained on various crop plants commonly grown in rotation on land used for sweet potato culture.

Physiological studies in the laboratory and greenhouse indicated that no significant levels of fungitoxic substances were present in either uninoculated or inoculated "Tinian" plants. Nor could a fungal metabolite be detected, under the existing experimental conditions, which was toxic to a susceptible variety of sweet potato (Porto Rico) but not to the resistant "Tinian".

A study was made of the basis for resistance of the foreign plant introduction "Tinian" (P.I. 153655). Histological examinations of serial stem sections of the susceptible sweet potato variety Porto Rico and of the resistant foreign plant introduction "Tinian" were made from plants collected at 3-day intervals following inoculation with spores of the pathogen. It was found that "Tinian" responded to infection by the production of tyloses in advance of the fungus. Twelve days after inoculation, 75 - 88% of the vessels which were 22 - 32 mm above the invasion site at the base of the plant were completely filled with tyloses. This compared to only 0 - 3% in the uninoculated control plants. Furthermore, no mycelia or spores could be detected in this region but were present in 25 - 50% of the vessels within 11 mm of the invasion site. In the variety Porto Rico the occurrence of tyloses in the inoculated plants was not significantly greater than in the uninoculated controls, except near the invasion site where after 12 days 3 - 6% of the vessels contained small tyloses. The pathogen was not limited, as in "Tinian", to the immediate invasion site. This suggests that the production of tyloses in "Tinian may represent an important defense mechanism against Fusarium wilt.

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INTRODUCTION

Fusarium wilt is one of the most serious field diseases of sweet potato, Ipomoea batatis (L.) Lam. The disease is also known as stem rot and it has been known to destroy more than 50% of the plants in some infected fields (4). The first indication of Fusarium wilt in the plant is chlorosis of the leaves which is frequently followed by their defoliation. The stems split open and expose noticeably discolored vascular bundles. Death of the plant follows.

Stem rot of sweet potato was first reported and described by Halsted in New Jersey in 1890 (10). It had been commonly observed by growers, however, in the fields of New Jersey as early as the 1860's. It is prevalent in all sweet potato growing areas in this country and is thought to have originated in the northern range of the sweet potato belt, an area which includes New Jersey, Maryland, Delaware, West Virginia, Kentucky, Ohio, and Illinois. It was first reported in Maryland by J. B. S. Norton in 1910 (16).

The causal organism is a soil-inhabiting member of the Fungi Imperfecti, Fusarium oxysporum Schlecht f. batatis (Wr.) Snyder & Hansen. Halsted (11), in error, attributed the disease to Nectria ipomoea Hals., the same organism that caused stem rot of eggplant. In 1913 Harter and Field (13,14) correctly identified the pathogen as one that had earlier been described by Wollenweber (38), Fusarium batatatis Wr. and F. hyperoxysporum. The complicated and confusing species concept of the genus Fusarium was simplified in 1940 by Snyder and Hansen (32), who recommended that the number of species be reduced to those having reliably consistent morpho-

logical characteristics. Furthermore, the biotypes within a species were to be classified as formae.

Fusarium oxysporum f. batatis was originally considered to be pathogenic only to sweet potato in nature. However, subsequent work has demonstrated that its host range may include other plant species. The pathogen has been shown to produce symptoms on a species of Morning Glory, Ipomoea hederaceae (L.) Jacq. (16), and to colonize many unrelated hosts (17).

Adequate control of Fusarium wilt has not been satisfactorily achieved by hill selection of disease-free planting stock (6,15,23), nor by the use of fungicidal dips for root and sprout treatments (8,27,29). Growers sometimes resort to escape rather than control by the practice of planting only symptom-free sprouts or vine cuttings on frequently rotated fields. The most effective control, however, is in the use of resistant varieties. In Maryland, extensive breeding programs are conducted from which horticulturally-promising selections are screened for resistance to Fusarium wilt and other diseases (36). Highly resistant foreign plant introductions are frequently used in these programs as sources of disease resistance.

The favored method for determining the resistance of sweet potato plant material to Fusarium wilt is by the infection, under greenhouse conditions, of rooted vine cuttings with spores (34), or by infection under field conditions of sprouts inoculated with a liquid culture of the pathogen.

The purpose of this investigation was, first, to test under field conditions prevalent in Maryland selected lines of sweet potatoes for resistance to Fusarium wilt; second, to test the pathogenicity of Maryland

isolates of Fusarium oxysporum f. batatis on plants commonly found growing in rotation on fields used for sweet potato culture in Maryland; and third, to detect and study any physiological or anatomical differences that might exist between a resistant and a susceptible variety with respect to their response to invasion by the pathogen.

These studies were intended to increase our understanding of the nature of resistance to wilt and to establish some basis for developing a more efficient technique for screening resistant plant material which could be utilized in breeding programs.

MATERIALS AND METHODS

Source of plant materials.--A collection of 94 different lines of sweet potato was tested during the summers of 1961 and 1962 for resistance to Fusarium wilt. The tests were conducted at the University of Maryland Vegetable Research Farm, Salisbury, Maryland, and at the greenhouses of the University of Maryland at College Park, Maryland. Roots and rooted sprouts for the tests were obtained from the Plant Industry Station of the United States Department of Agriculture (USDA) at Beltsville, Maryland and from the University of Maryland Vegetable Research Farm.

The collection included a) 18 "name" varieties:

1. Allgold
2. Apache
3. Australian Canner
4. Carogold
5. Centennial
6. Goldrush
7. Jersey Orange
8. Maryland Golden
9. Nancy Hall
10. Nemagold
11. Nugget
12. Oklamar
13. Pelican Processor
14. Porto Rico
15. Spanish
16. Sunnyside
17. Triumph
18. Whitestar

b) 26 foreign introductions, designated by their USDA Plant Introduction (P.I.) codes:

19. 153905
20. 153906
21. 153907
22. 153655
23. 239690
24. 239692
25. 240936
26. 242104
27. 246409
28. 246410
29. 246477
30. 246478
31. 248731
32. 248732
33. 250120
34. 215602
35. 259165
36. 259480
37. 259481
38. 259482
39. 259484
40. 259485
41. 259486
42. 259487
43. 260613
44. 746128

and c) 50 new seedling selections designated by letter for station of origin (see foot-note), and code number:

45. B-98
46. B-1474
47. B-3102
48. B-3851
49. B-4091
50. B-4252
51. B-4282
52. B-4991
53. B-5999
54. B-6031
55. B-6211
56. B-6261
57. B-6293
58. B-6401
59. B-6511
60. B-6521
61. B-6631
62. B-6708
63. B-6716
64. B-6751
65. B-6811
66. B-6842
67. B-6891
68. B-6915
69. B-6916
70. B-6921
71. B-6941
72. B-6942
73. B-7661
74. B-7671
75. C-2051-D
76. HM-550
77. E-8672
78. L-489
79. L-717
80. L-867
81. L-892
82. L-939
83. L-956
84. L-966
85. M-2821
86. M-0362
87. M-728
88. M-974
89. M-94154
90. NC-162
91. NC-188
92. NC-198
93. T-7
94. V-2158

B - USDA Plant Industry Station, Beltsville, Maryland
C - California Agric. Expt. Station, University of California, Berkeley, California.
HM- USDA Plant Industry Station, Meridian, Mississippi.
E - South Carolina Agric. Expt. Station, Edisto, S. C.
L - Louisiana Agric. Expt. Station, Baton Rouge, La.
M - Mississippi Agric. Expt. Station, State College, Miss.
NC- North Carolina Agric. Expt. Station, Raleigh, N. C.
T - Georgia Coastal Plain Agric. Expt. Station, Tifton, Ga.
V - Virginia Truck Experimental Station, Norfolk, Va.

Seed for a study of the host range of Fusarium oxysporum f. batatis was obtained from commercial sources and from the Seed Laboratory of the University of Maryland. Strawberry plants were from a nursey.

Two species of Morning Glory, which are common weeds in sweet potato fields in Maryland, were also included in the host range study. Rooted young vines of Ipomoea hederaceae (L.) Jacq. (Wild Morning Glory) were collected from outdoor sand propagation beds at the University of Maryland Vegetable Research Farm, Salisbury, Maryland, where they were growing as volunteers. Ipomea pandurata (L.) G. F. W. Mey. (Wild Potato Vine) was propagated in the greenhouse from seed collected in a sweet potato field near Snow Hill, Maryland.

Source of inoculum.--Five virulent strains of Fusarium oxysporum f. batatis were obtained from Dr. C. E. Steinbauer, horticulturist, at the USDA station in Beltsville, Maryland. They were part of a collection of Maryland isolates which had been maintained in stock culture for more than 10 years, and had the USDA designations F-1371, F-1376, 733b-1, 7313a-1, and 7287e-1.

A culture of Fusarium oxysporum f. lycopersici (Sacc.) Snyder & Hansen, a wilt pathogen of tomato plants, was used in some physiological studies. This was obtained from Dr. R. E. Webb, plant pathologist, at the USDA station in Beltsville.

Cultures of Fusarium oxysporum f. batatis were also obtained directly from infected plants by isolation techniques. Collections of the infected plants were made in a sweet potato field near Snow Hill, Maryland.

Isolation of the pathogen.--Stems of the diseased plants were surface-sterilized in a 20% commercial Clorox solution (5% sodium hypochlorite) for 5 minutes and rinsed in sterile distilled water.

Cross-sections were aseptically sliced 1/8 cm thick, placed on 2% Difco potato-dextrose-agar (PDA) plates, and incubated at 27° C.

In the course of 2 days hyaline mycelia radiated out from the tissue onto the agar from where terminal growth was cut away with a transfer needle and transferred to PDA slants in test tubes. The tubes were incubated at 27° C for 2 weeks and refrigerated for storage.

Culture of inoculum.--All cultures of Fusarium oxysporum f. batatis were maintained under refrigeration on PDA slants and transferred to fresh slants every 6 months.

Inoculum was prepared by inoculation of a modification of Steinbauer's liquid synthetic medium (18): sucrose 10 g, magnesium sulfate 5 g, monopotassium (dihydrogen) phosphate 1 g, and ammonium nitrate 1 g per liter of distilled water. One liter of solution was prepared in a two-liter Ehrlenmeyer flask, autoclaved for 15 minutes, and inoculated with each of the 5 USDA isolates of Fusarium oxysporum f. batatis. The composite was then agitated continuously at room temperature for 2 weeks.

Five liters of composite inoculum were prepared for field resistance tests involving the inoculation of approximately 3000 plants. Prior to use, the inoculum was blended for 30 seconds in a Waring Blendor and diluted 50% with water.

For smaller-scaled greenhouse tests involving less than 100 plants, spores and mycelia from one liter of composite inoculum were harvested by filtration through Whatman No. 1 paper in a Buchner funnel and were immediately resuspended in 200 ml of water.

Spore suspensions were frequently used as inoculum for physiological studies involving only a few plants. They were prepared by adding 5 ml of distilled water to a PDA slant of the pathogen and vigorously agitating

the tube for 30 seconds. The larger mycelial strands were allowed to settle out for 5 minutes and the supernatant spore suspension was then collected.

Culture and inoculation of plants.--Sweet potato sprouts approximately one month old were used for field resistance studies. Sprouts were obtained from storage roots which had been set in outdoor fumigated sand propagation beds or in steam-sterilized sand beds in the greenhouse.

Hanna's method of inoculation (12) was employed with modifications. The rooted basal ends of the sprouts were crushed with a sharp blow from a wooden mallet; the wounded root systems were then immersed briefly in the inoculum and kept moist with wet paper towels until planting.

Within 5 hours after inoculation all sprouts had been planted with a tractor-driven mechanical transplanter. Care was taken to plant the uninoculated controls first and to prevent their contamination with inoculum. When planting, an aliquot of starter solution was injected into the soil next to the roots of each sprout.

Weather conditions during the summer months of both years were seasonably warm. The precipitation of the summer of 1961 was average, but the summer of 1962 was unusually dry until the end of the season when rain did occur.

Greenhouse inoculation tests were carried out with rooted vine cuttings (35). A continuous source of disease-free vine cuttings of the susceptible variety Porto Rico and of the resistant foreign introduction "Tinian" (P.I. 153655) was maintained in the greenhouse for this purpose, and also for various physiological and histological studies which were conducted on these plants.

Twenty-four plants of each variety were planted a foot apart in steam-sterilized sandy-loam beds, and their growth was trained onto vertical

bamboo trellises. Overhead lights provided 10 hours of daily illumination throughout the winter months, and the plants were periodically treated with liquid fertilizer and with insecticide sprays. Greenhouse temperatures during the winter months fluctuated between 60 and 80° F., and during the summer between 70 and 95° F.

For testing, the vines were cut 4 to 5 nodes from the apex, the basal leaves of the cutting were trimmed away, and the cuttings rooted in steam-sterilized sand beds for 2 weeks.

Inoculations of the rooted cuttings were accomplished by their immersion into a spore and mycelial suspension. First the cuttings were uprooted, wounded by trimming off the callused basal end, and then placed in a 125 ml Ehrlenmeyer flask containing 10 ml of the prepared inoculum diluted to 100 ml. The cuttings were maintained in the flasks throughout the experiment. The water was replenished as evaporation from the flask and as transpiration of the plants required. Symptoms generally began appearing within 7 days after inoculation.

Inoculations for the host range studies were made on seedlings approximately one month old. The seedlings were uprooted from the germinating beds, their root systems dipped into the inoculum, and then were transplanted to a steam-sterilized greenhouse sand bed.

One year old strawberry plants were similarly inoculated and planted.

Field plot design.--Each field resistance test was replicated four times, and included an uninoculated control plot. Ten individual plants represented each sweet potato line in each replicate, and all lines were randomized in the plots (Figure 4).

Physiological techniques.--A comparative physiological study was conducted with the susceptible variety Porto Rico and the resistant "Tinian". Two general problems were investigated, each entailing special

methods.

1) Methods used for studying the growth of the pathogen on available host nutrients and for the detection of fungitoxic substances in resistant plant tissues:

a) Healthy stem sections, were aseptically cut open lengthwise and inoculated at one end with a small block of seeded agar from a stock culture of Fusarium oxysporum f. batatis. The inoculated sections were then placed upon 4 layers of moist filter paper in a sterile petri dish and incubated at 27° C for 2 weeks. Observations on the extent of mycelial growth on the exposed tissues were recorded.

b) A crude plant agar-extract was prepared by expressing with a hydraulic press at room temperature 50 ml of juice from sweet potato root and stem tissues. Dilutions of the extract, ranging from 1% to 40%, were made with distilled water in 2% agar, and then each poured into petri plates. The plates were autoclaved for 15 minutes at 17 lbs pressure. After cooling and gelation, a small block of seeded agar, cut from a Fusarium oxysporium f. batatis stock culture, was placed at the center of each plate, and observations on mycelial growth recorded after a 3-day incubation period at 27° C.
Growth was measured as "mean colony diameter" which was derived by averaging 2 right-angle measurements of colony diameter.

c) The effect of sap from both healthy and infected resistant plant tissue on spore germination was observed by severing off the terminal end of a vine and allowing drops of the freely-exuded sap to fall within the ceramic rings of a sterile "Perma-slide" micro-test slide. One drop of a spore suspension in 1% glucose was added to one drop of sap. Germination data was taken after 16 hours of incubation at 27° C.

d) An extract of healthy and infected resistant tissue was prepared by slivering lengthwise the lower 10 cm of a rooted vine cutting, and immersing the lacerated tissue in 10 ml of distilled water for one hour with frequent agitation. The preparation was sterilized by ultrafiltration with a Millipore membrane filter and aseptically transferred to sterile test tubes in 2 ml aliquots. The tubes were inoculated with 0.5 ml of a spore suspension in 5% glucose (final glucose concentration of 1%), and germination data recorded after 16 hours of incubation at 27° C.

2) Methods used for studying the effects of culture filtrates of the pathogen on resistant and susceptible plants:

a) A culture filtrate of Fusarium oxysporum f. batatis grown for 2 weeks on Steinbauer's modified liquid artificial medium was collected by preliminary filtration through a Whatman No. 1 filter on a Buchner funnel. A final filtration was made through a Millipore membrane filter so as to remove all spores

and mycelial fragments. The filtrate was then diluted 50% with distilled water, and poured into 125 ml Ehrlenmeyer flasks into which the roots of the test plants were immersed for a three-week observation period. Controls were included in which plants were similarly immersed in the following: a culture filtrate of Fusarium oxysporum f. lycopersici, a two-week old uninoculated artificial media, and distilled water.

b) The study was repeated with a filtrate obtained from the growth of Fusarium oxysporum f. batatis on a media prepared from sweet potato plant tissue. Seventy-five grams of root and stem tissue in 400 ml of distilled water was macerated for 5 minutes in a Waring Blendor. The macerate was then diluted to one liter with water, enriched with 5 gms of glucose, and autoclaved. Standard procedures were used for inoculation and for collection of the filtrate. An uninoculated media control and a distilled water control were included.

Histological materials.--A comparative histological study was made of the susceptible variety Porto Rico and of the resistant foreign introduction 'Tinian'. Stems of rooted vine cuttings were collected at three-day intervals following their inoculation, then killed and fixed in formalin-acetic-alcohol (FAA) for at least 24 hours before sectioning. Longitudinal sections 50 microns thick and 1 cm long were cut with a freezing microtome connected to a carbon dioxide supply (24). Sections were then mounted on glass slides and stained with a few drops of water soluble

cotton blue in lacto-phenol.

Paraffin-embedded cross sections were also prepared by standard tertiary butyl alcohol dehydration, paraffin infiltration, and microtoming technique. A staining procedure was used which was differential for fungus and for host and which involved a 30-minute thionin stain, a light green counterstain with an "Orange G" and erythrosin mixture (25).

Scoring systems for estimation of disease severity.--Data regarding the responses of the inoculated sweet potato plants in the field tests was recorded throughout the growing season.

Immediately following the planting, a check was made of the exact number of plants in each plot. At monthly intervals during the growing season records were taken of the number of surviving plants in each plot, and at the end of the summer a final count was taken. In addition, at harvest time, each surviving plant was cut open at the stem near the soil line so as to determine if there were any symptoms of vascular discoloration.

Finally, the sweet potato lines were scored according to their overall response to inoculation. Of the ten plants of each line originally inoculated, a value of 10 was assigned to each surviving plant which was free of vascular discoloration, and a value of 5 was assigned to each surviving plant which was discolored. Using these values, a total mean score was obtained for each line from the four replications.

The final mean value obtained for each line ranged between 0 to 100 and is referred to as the "resistance index" of that line. A resistance index of 100 represents high resistance to Fusarium wilt, and an index of 50 or below represents high susceptibility.

Quantitative histological observations required a method for

estimating a) the extent of infection and b) the extent of the resulting histological response by the plant. Barratt and Horsfall's scoring method (19) was adapted and used to determine approximate percentage of vessels in the vascular system of the stem which had been invaded by the pathogen (i.e. which showed the presence of mycelia), and the approximate percentage of the vascular system in which tyloses could be observed:

Score	% vessels with tyloses, (or) % vessels with mycelia
1	0 - 3
2	3 - 6
3	6 - 12
4	12 - 25
5	25 - 50
6	50 - 75
7	75 - 88
8	88 - 94
9	94 - 97
10	97 - 100

RESULTS AND DISCUSSION

Field and greenhouse resistance tests.--The sweet potato lines that were tested are itemized in Table 1 in their order of highest resistance index.

The foreign plant introduction "Tinian" (P.I. 153655) consistently demonstrated the highest degree of resistance. In all tests conducted it indexed higher than 97 (Figure 5). Other highly resistant lines which had resistance indexes above 90 included the plant introductions 153906, 153907, 251602, 259481, 259485, and 259487; the variety Pelican Processor; and the seedling selections B-6842 and T-7. These could be utilized by plant breeders as sources of resistance to the sweet potato wilt pathogen in breeding programs.

Lines which indexed between 80 and 90 were also considered resistant and included the varieties Goldrush, Whitestar, and Nugget, and a number of foreign introductions and seedling selections. Among the highly susceptible varieties were Nancy Hall, C-2051-D, Australian Canner, Porto Rico, and Maryland Golden.

In the sweet potato lines that were included in more than one of the three resistance tests, there was good correlation in the results. Variations which did occur, such as in those of the variety Nugget where the indexes for the 3 tests were 82, 76, and 100 (see Table 1, item 28), were probably due to differences in the environmental conditions affecting host or pathogen which prevailed during each of the tests.

Variances which occurred within the replications of some lines in a

Table 1.--Resistance indexes of 94 lines of sweet potato inoculated with Fusarium oxysporum f. batatis in 2 field resistance tests and one greenhouse resistance test.

Sweet potato line	Field Test 1961	Field Test 1962	Greenhouse test
1. 153655 ("Tinian")	97 [1]	100	100
2. B-6842	--	97	--
3. 153906	--	96	--
4. 251602	96	--	85
5. 259480	95	--	95
6. 153907	--	92	--
7. 259485	92	--	80
8. T-7	--	90	--
9. Pelican Processor	--	90	--
10. 259487	90	--	90
11. 746128	92	--	65
12. 242104	92	--	65
13. 259482	88	--	60 [2]
14. 259484	88	--	60 [2]
15. M-974	88	50 [2]	100
16. 259480	87	--	65
17. B-3102	87	--	65
18. Goldrush	78	85	90
19. M-94154	--	85	--
20. B-6261	--	85	--
21. Whitestar	--	84	--
22. B-4991	--	84	--
23. 250120	84	--	--
24. NC-188	--	83	--
25. M-2821	83	--	60
26. 248731	83	--	85
27. 259486	82	--	80
28. Nugget	82	76	100
29. B-6811	--	81	--
30. B-7671	--	80	--
31. B-6521	--	80	--
32. 240936	80	--	80
33. B-7661	--	79	--
34. NC-198	--	79	--
35. L-892	--	79	--
36. 153905	--	79	--
37. 246410	78	--	75
38. B-6708	--	75	--
39. B-6941	--	75	--
40. 24677	73	--	--
41. B-6716	73	--	90
42. 246490	72	--	85
43. Carogold	--	71	--
44. 246478	70	--	60
45. Nemagold	70	65	70
46. 248731	67	--	55
47. NC-162	--	65	--
48. Allgold	--	65	--
49. 260613	65	--	60
50. Centennial	65	58	85
51. B-6031	--	64	80
52. M-0362	--	63	100
53. Oklamar	--	62	--
54. B-6915	--	61	--
55. L-489	40	60	--
56. E-8672	78	56	80
57. B-6751	--	56	--
58. Jersey Orange	55	55	--
59. HM-550	--	55	--
60. L-966	--	55	--
61. B-6921	--	55	75
62. B-5999	--	55 [2]	--
63. 239692	52	--	--
64. M-728	--	50	--
65. B-6916	--	50 [2]	90
66. Triumph	--	49	85
67. B-6842	--	49	--
68. L-939	--	46	60
69. B-98	45	--	70
70. Apache	--	41	55
71. B-6631	--	39	--
72. B-4282	--	39	--

1. Interpretation of index values: 100 = highest resistance; 50 and below = high susceptibility.
2. A comparable degree of infection also occurred in the uninoculated controls in this test.

Ta

Green-house test	Sweet potato line	Field Test 1961	Field Test 1962	Green-house test
1. 85	73. Spanish	--	38	--
2. 60	74. L-867	--	36	--
3. --	75. Sunnyside	--	35	--
4. --	76. V-2158	--	34	--
5. --	77. L-717	--	28	--
6. --	78. L-956	--	28	--
7. 70	79. Maryland Golden	35	26	30
8. 80	80. B-6211	--	23	--
9. --	81. B-3851	22	21	20
1(30	82. Porto Rico	32	20	50
1 --	83. B-1471	20	19	30
1 --	84. Australian Canner	--	16	--
1 --	85. B-6293	--	13	--
1 --	86. 259165	13	--	25
1 45	87. 239692	13	--	20
1 --	88. B-6511	--	10	--
1 --	89. B-6401	--	9	--
1 --	90. B-4091	8	--	10
1 --	91. C-2051-D	--	3	--
2 --	92. B-6942	--	3	--
2 --	93. Nancy Hall	--	3	--
2 --	94. B-4252	2	--	0
2 --				
2 --				

test may be attributed to the fact that some of the plant material used may have already been infected. This appears to be true especially with those sweet potato lines where infection occurred in the uninoculated controls.

Another possible source of infection in the uninoculated control plots in the field tests is that of isolated cases of infection from pathogen already present in the soil.

Host range studies.--Maryland isolates of Fusarium oxysporum f. batatis caused no visible symptoms on the commercially cultivated plants which had been inoculated for these tests. Attempts were made to isolate the pathogen from these plants but without success. Hendrix and Nielson (17) reported, however, that certain cultivated crops harbor the pathogen in a symptomless parasitical relationship. These workers also reported that the common Morning Glory, Ipomoea hederaceae, was susceptible to Fusarium oxysporum f. batatis and that typical disease symptoms were produced.

The results of the present host range studies confirmed the susceptibility of Ipomoea hederaceae. Also, an additional Morning Glory species, Ipomoeae pandurata, was found to be susceptible (Figure 8) and can thus be added to the known hosts of the pathogen (Table 2).

It may be concluded that these Morning Glory species, and possibly some crop plants, perpetuate the pathogen in soils used for sweet potato culture. This, therefore, may account for the failure of the practice of crop rotation to be an adequate control measure for Fusarium wilt of sweet potatoes.

Physiological tests.--An interesting feature of the results of the field resistance tests was the wide range of responses existing between the most resistant varieties and the most susceptible. In general,

Table 2.--Effects of inoculation with Maryland isolates of Fusarium oxysporum f. batatis on plants commonly found in rotation on land used for sweet potato culture, and on 2 species of Morning Glory commonly weeds in these areas.

Species	Disease Symptoms
1. Cucumber (Cucumis sativus L.), variety Ashley	--
2. Cantaloupe (C. Melo L.), variety Early May	--
3. Lima bean (Phaseolus limensis Macfad.), variety Fordhook 242	--
4. Snap bean (P. vulgaris L.), variety Black Valentine	--
5. Carrot (Daucus carota L. var. sativa DC.), variety Red Core Chantilly	--
6. Beet (Beta vulgaris L.), variety Detroit Dark Red	--
7. Eggplant (Solanum melongena L. var. esculentum, Nees.), variety Black Beauty	--
8. Pepper (Capsicum frutescens L. var. grossum, Bailey), variety California Wonder	--
9. Pumpkin (Cucurbita pepo L.), variety Connecticut Field	--
10. Squash (C. pepo L. var. condesa, Bailey), variety Yellow Early Prolific	--
11. Watermelon (Citrullus vulgaris Schrad.), variety New Hampshire Midget	--
12. Tomato (Lycopersicon esculentum Mill.), variety Beefsteak	--
13. Soybean (Glycine max L.)	--
14. Field corn (Zea mays L.)	--
15. Barley (Hordeum vulgare L.)	--
16. Oats (Avena fatua L.)	--
17. Wheat (Triticum aestivum L.)	--
18. Rye (Lolium perenne L.)	--
19. Strawberry (Fragaria chiloensis L. var. ananassa), varieties Empire, Midland, and Sparkle	+
20. Wild Morning Glory (Ipomoea hederaceae L. Jacq.)	+
21. Wild Potato Vine (I. pandurata L. G.F.W. Mey.)	

several patterns of response can be described.

The most severe reaction was that of the highly susceptible plant. Within seven days after inoculation, vascular discoloration of the root and of the stem at the soil line could be detected (Figure 9). As the discoloration progressed to the apex of the plant, the epidermal-cortex tissues of the stem ruptured, the stem acquired a noticeably blue color due to the internal discoloration, and the leaves became chlorotic, wilted, and soon abscissed. Death of the plant followed 2 to 3 weeks after inoculation (Figure 10).

Less susceptible plants, that is, those which possessed a degree of tolerance to Fusarium wilt, underwent a prolonged period of symptoms and decline for as long as several months before death. Characteristic of the condition was a weakened and stunted plant with rosetting of the leaves. Vascular discoloration and chlorosis of the leaves were also evident.

An expression of resistance to the disease was the survival and normal growth of the inoculated plant, but with the presence of internal vascular discoloration. Viable spores and mycelia of Fusarium oxysporum f. batatis which were pathogenic to the more susceptible varieties could be isolated from areas of these stems near the soil line.

And finally, highly resistant varieties demonstrated no symptoms or effects of the disease even though spores and mycelia that were pathogenic to more susceptible varieties could be isolated from their stems a month after inoculation. In these varieties the pathogen only colonized the host in a limited area of the vascular system and was not capable of causing pathogenic effects such as vascular discoloration, chlorosis, and stunting.

Considering the magnitude of the differences between the highly resistant response and the highly susceptible response, it was reasoned that

investigations of a relatively general nature might furnish some information concerning whatever factor in the physiology or histology of the host-parasite relationship which was responsible for this resistant reaction.

The highly resistant foreign introduction "Tinian" and the highly susceptible variety Porto Rico were selected as representative hosts for this investigation.

Preliminary investigations on the nature of infection demonstrated that spores and mycelia of Fusarium oxysporum f. batatis became established in the vascular system of "Tinian". The presence of the pathogen, however, was limited to the area of the vascular system immediately around the site of invasion, which was, at the base of the vine cutting. None could be isolated from areas more than a few centimeters removed from the invasion site (Figure 11). This suggested that a defense mechanism existed in the resistant plant which restricted the pathogen to the invasion area. Furthermore, this mechanism could not be highly fungitoxic since Fusarium oxysporum f. batatis isolated from infected "Tinian" was viable and pathogenic to susceptible varieties. And finally, in the susceptible plant this mechanism must have been absent because of the rapid and progressive spread of the pathogen throughout the vascular system of such plants.

The probability was first investigated of this factor being either a) a nutritional one for the pathogen, or b) a fungitoxic substance present in the resistant plant which would inhibit fungal growth.

These had been suggested by reports of the detection of such factors in various wilt diseases. Reynolds (30) reported that extracts from flax plants which were resistant to Fusarium wilt of flax, caused by Fusarium oxysporum f. lini (Bolley) Snyder & Hansen, supported less mycelial growth than did extracts from susceptible plants. Fisher (20) in 1935 investigated

Fusarium wilt of tomato, caused by Fusarium oxysporum f. lycopersici (Sacc.) Snyder & Hansen, and reported that expressed sap from certain resistant tomato varieties retarded the growth of the fungus. The importance of high carbohydrate levels in tomato varieties resistant to Verticillium wilt (Verticillium albo-atrum Reinke & Berth.) another vascular disease, had been observed by Salman and Buckley (31). Kalyanansundaran (26) drew the same conclusions with Fusarium wilt of cotton, caused by Fusarium oxysporum f. vasinfectum (Atk.) Snyder & Hansen.

A number of tests were performed in this research which indicated that tissue extracts of Porto Rico and of "Tinian" both supported nearly the same degree of mycelial growth and spore germination (Tables 3-6). No significant or consistent differences were found, within the limits of this investigation, which would suggest that the chief basis of resistance was nutritional or due to a fungitoxin.

In the wilt diseases of other crops, the suppression of a segment of the metabolism of the pathogen with the effect of inhibiting the production of a vital enzyme or toxicant has been considered as the basis for resistance by some investigators (7,21,22). Proof, however, of such a defense mechanism has not been conclusively demonstrated, and within the limits of this present investigation, no such mechanism could be detected in the resistance of "Tinian" to Fusarium wilt.

Test plants of "Tinian" and of Porto Rico responded no differently to metabolic products of the pathogen, whether the pathogen was cultured in artificial media or in media specially prepared from extracts of the same plants (Table 7). Toxic effects from all culture filtrates were produced on both "Tinian" and Porto Rico vine cuttings. In no case was a filtrate capable in inducing in either variety the syndrome typical of Fusarium wilt (Figures 12,13 & 14).

Table 3.--Extent of mycelial growth of *Fusarium oxysporum* f. *batatis* on sweet potato stem tissues exposed by splitting open the stems and inoculated with seeded agar blocks.

Sweet Potato Line	Mean growth in mm from the area of inoculation	
	7 days	13 days
Porto Rico.	13.3[1]	32.5
"Tinian".	14.2	30.5

1. Mean of 10 replications.

Table 4.--Mean colony diameters of Fusarium oxysporum f. batatis cultured on agar-extracts of resistant "Tinian" and P.I. 250120, and of susceptible Porto Rico sweet potato stems.

Percent extract in agar	Mean colony diameter in mm					
	"Tinian" (resistant)		P.I. 250120 (resistant)		Porto Rico (susceptible)	
	2 days	3 days	2 days	3 days	2 days	3 days
0	6.0[1]	13.5	6.0	12.8	6.0	17.0
1	8.0	17.5	8.3	15.5	8.0	19.0
10	10.0	17.8	10.0	15.5	10.0	19.0
20	9.5	17.5	10.0	15.5	10.3	18.8
30	9.0	17.5	9.0	15.5	10.5	19.0
40	8.0	17.5	8.5	15.3	10.0	19.0

1. Means of 3 replications.

Table 5.--Germination of Fusarium oxysporum f. batatis spores after 16 hours of incubation in a 1% glucose-sap extract medium prepared from sweet potato stems of "Tinian" and Porto Rico.

	Mean percent germination
Sap from healthy "Tinian stems.	38[1]
Sap from infected "Tinian" stems.	9
Sap from healthy Porto Rico stems	33
Sap from infected Porto Rico stems.	5
Glucose control	61

1. Mean of 3 replications; 500 spores counted per replicate.

Table 6.--Germination of Fusarium oxysporum f. batatis spores after 16 hours of incubation in an aqueous extract from healthy and diseased sweet potato stems of "Tinian" and of Porto Rico.

	Mean percent germination
Extract from healthy "Tinian" stems.	76.5[1]
Extract from infected "Tinian" stems	80.5
Extract from healthy Porto Rico stems.	72.0
Extract from infected Porto Rico stems	83.0
Glucose control.	68.0
Distilled water control.	24.0

1. Mean of 2 replications; 500 spores counted per replicate.

Table 7.--Effects of culture filtrates of Fusarium spp. after 3 week

sweet potato vine cuttings of "Tinian" and Porto Rico.

Treatment	Mean scores "Tinian"	Por
1. F. oxysporum f. batatis cultured in artificial media:		
a. Culture filtrate	1[2]	
b. Spore suspension	0	
2. F. oxysporum f. batatis cultured on "Tinian" stem media:		
a. Culture filtrate	4	
b. Spore suspension	1	
3. F. oxysporum f. batatis cultured on Porto Rico stem media:		
a. Culture filtrate	1	
b. Spore suspension	0	
4. F. oxysporum f. lycopersici cultured in artificial media:		
a. Culture filtrate	1	
b. Spore suspension	0	
5. Controls:		
a. Uninoculated artificial media.	1	
b. Distilled water.	0	

1. Mean of 5 plants tested.
2. Interpretation of scoring system:
   0.- no symtoms
   1.- chlorosis only
   2.- severe chlorosis only
   3.- vascular discoloration
   4.- death of plant

Table 7.--Effects of culture filtrates of *Fusarium* spp. after 3 weeks on sweet potato vine cuttings of "Tinian" and Porto Rico.

Treatment	Mean scores[1] "Tinian"	Porto Rico
1. *F. oxysporum* f. *batatis* cultured in artificial media:		
a. Culture filtrate	1[2]	1.5
b. Spore suspension	0	4
2. *F. oxysporum* f. *batatis* cultured on "Tinian" stem media:		
a. Culture filtrate	4	4
b. Spore suspension	1	4
3. *F. oxysporum* f. *batatis* cultured on Porto Rico stem media:		
a. Culture filtrate	1	1
b. Spore suspension	0	4
4. *F. oxysporum* f. *lycopersici* cultured in artificial media:		
a. Culture filtrate	1	2
b. Spore suspension	0	0
5. Controls:		
a. Uninoculated artificial media	1	1
b. Distilled water	0	0

1. Mean of 5 plants tested.
2. Interpretation of scoring system:
   - 0.- no symtoms
   - 1.- chlorosis only
   - 2.- severe chlorosis only
   - 3.- vascular discoloration
   - 4.- death of plant

Histological studies.--The possibility of anatomical structures or characteristics of the host participating in or constituting a defense mechanism against Fusarium wilt has also been considered.

Cox (5) reported that tyloses and gel occlusions occurred in cantaloup and cucumber plants infected with Fusarium wilt, caused by Fusarium oxysporum f. melonis (Leach & Currence) Snyder & Hansen, and they occurred in both resistant and susceptible varieties of these species. The healthy uninoculated plants were reported not to have any vascular occlusions.

Beckman (1,2,3) reported that banana varieties resistant to Fusarium wilt of banana, caused by Fusarium oxysporum f. cubense (E. F. Sm.) Snyder & Hansen, produced vascular occlusions when invaded by the pathogen.

In the present investigation, a similar phenomena has been found to occur in Fusarium wilt of sweet potato.

The anatomy of healthy "Tinian" and Porto Rico stems is fundamentally alike (Table 8). The steles are of the type known as ectophloic siphonosteles (9) and they have extensive cortex and pith parenchyma tissues. Perhaps a significant observation is the slight difference in vessel diameters in "Tinian" and Porto Rico (Figures 15-18). Vessels in the variety Porto Rico average 40x53 microns in cross-section while they average somewhat less, 16x26 microns, in "Tinian". Occasional giant vessels occur in "Tinian", however, 120x150 microns in diameter.

Invasion by Fusarium oxysporum f. batatis in the vascular tissues of a host induces certain changes in the anatomy of the sweet potato plants.

Watanabe (37) and McClure (28) observed that the xylem of sweet potato stems infected with Fusarium wilt had abnormally high numbers of tyloses. Both investigators also noted that these tyloses occurred not only in the region of infection but also in advance of the pathogen.

Table 8.--Comparative anatomy of the steles of Porto Rico and of "Tinian" sweet potato.

Structure	"Tinian"	Porto Rico
1. Epidermis	Structurally intergrades with cortex.	Structurally distinct from cortex.
2. Cortex	10 to 15 cells in width.	15 to 30 cells in width.
3. Phloem	Interxylary phloem present (33).	No interxylary phloem.
4. Phloem fibers	Present	Present
5. Xylem	Irregular in width.	Regular in width.
6. Vessel elements	Average 16x26 microns in cross-section.	Average 40x53 microns in cross-section.
	Occasional larger ones 120x150 microns.	No giant cells
7. Tracheids	Numerous with bordered pits.	Numerous with bordered pits.
8. Pith	Large, unlignified, very spongy.	Large, unlignified, less spongy.

In this present investigation a study was made of the comparative histology of Fusarium wilt in the resistant host and in the susceptible host. Results indicated that fundamental differences exist between the 2 hosts with respect to a) the pattern of mycelial penetration, and b) the pattern of tylose development.

In general, the vascular system of the uninoculated sweet potato plants were essentially free of tyloses (Figures 19-20). Less than 3% of the xylem vessels and tracheids of "Tinian" and of Porto Rico contained naturally-occurring tyloses (Figure 21).

In the infected plants tyloses were produced in abundance and constituted a distinct anatomical symptom of the disease (Figure 22).

Tyloses first appear in the xylem conducting cells as numerous small protrusions of protoplasm from the adjoining xylem parenchyma cells (Figure 23). They pass into the vessels and tracheids through the bordered pits of the cell walls (Figure 24), and it is not uncommon for the entire protoplasmic content of a parenchyma cell to evacuate in this manner into a vessel or tracheid.

In "Tinian" tyloses were produced in greater numbers and more extensively throughout the xylem than in the variety Porto Rico.

Six days after inoculation, tyloses in "Tinian" occurred in 25 to 50% of the xylem cells located 2 to 3 cm from the invasion site at the base of the plant. In the variety Porto Rico, tyloses occurred in less than 3% of the xylem vessels located in the same area. This percentage was no greater than that in the uninoculated controls (Figure 1).

Nine days after inoculation the differences between tylose-development in "Tinian" and in Porto Rico were more pronounced (Figure 2).

After 12 days the differences had reached their maximum extent (Figure 3). In "Tinian", 88 to 94% of the vessels and tracheids 2 to 3 cm

Figure 1. Tylose development and mycelial penetration 6 days after inoculation of "Tinian" and Porto Rico sweet potato cuttings with *Fusarium oxysporum* f. *batatis*. (Refer to Materials and Methods for interpretation of scoring system.)

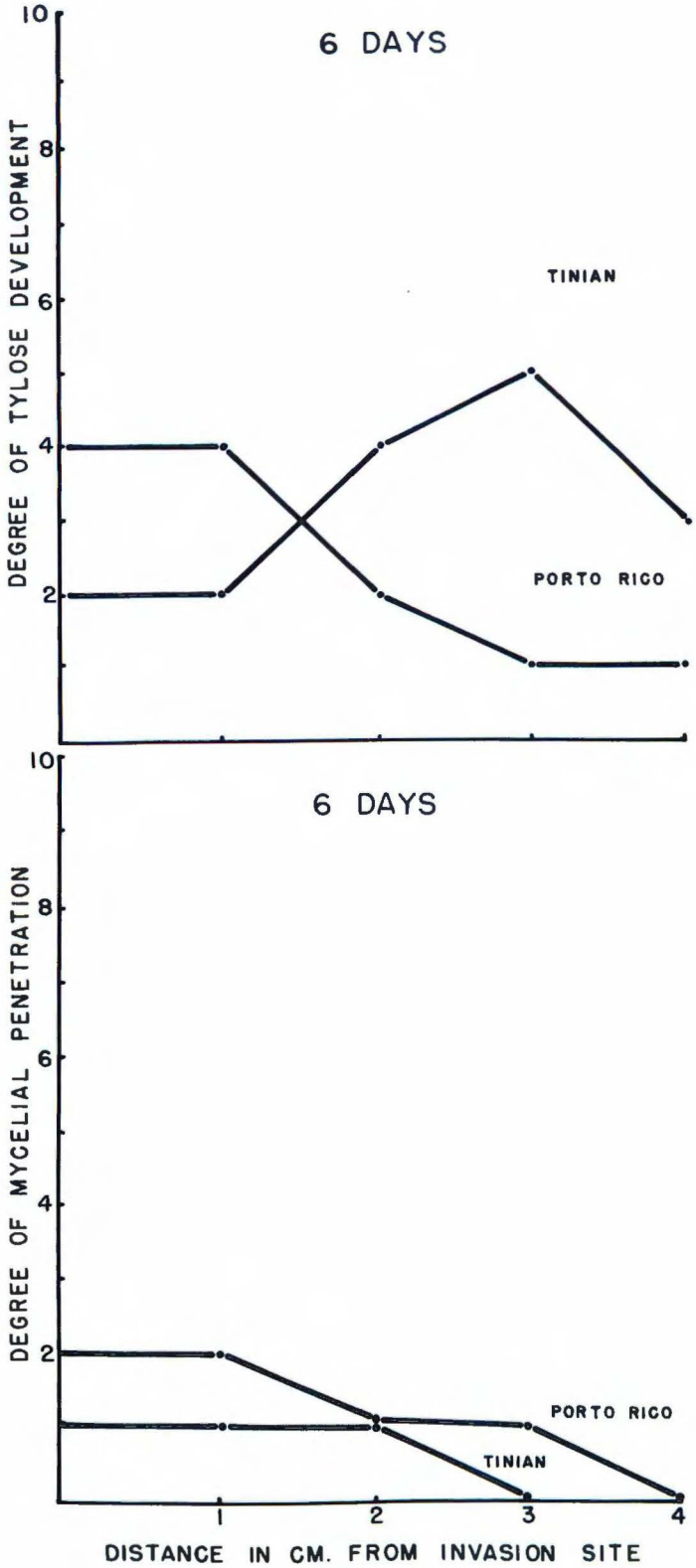

6 DAYS
DEGREE OF TYLOSE DEVELOPMENT
10
8
6
4
2
TINIAN
PORTO RICO
6 DAYS
DEGREE OF MYCELIAL PENETRATION
10
8
6
4
2
PORTO RICO
TINIAN
1
2
3
4
DISTANCE IN CM. FROM INVASION SITE

Figure 2. Tylose development and mycelial penetration 9 days after inoculation of "Tinian" and Porto Rico sweet potato cuttings with Fusarium oxysporum f. batatis. (Refer to Materials and Methods for interpretation of scoring system.)

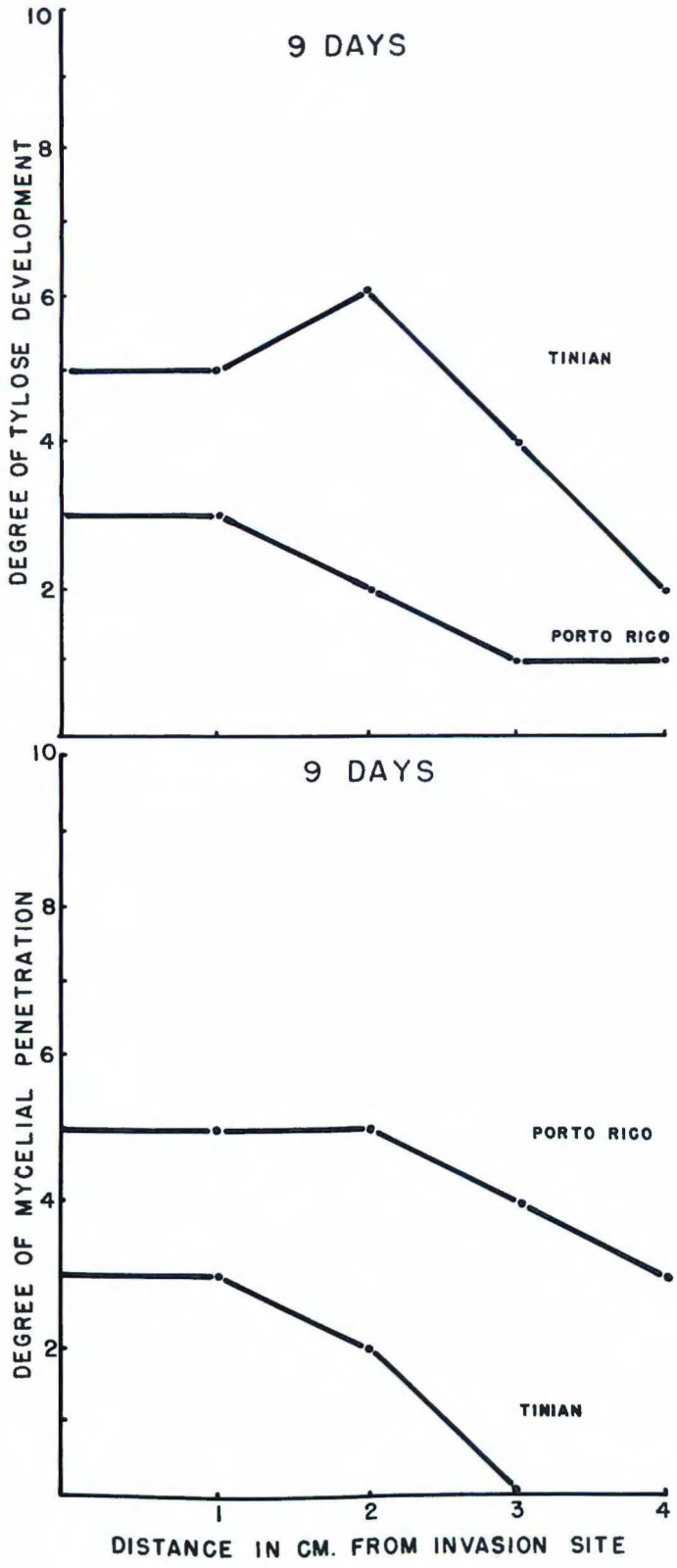
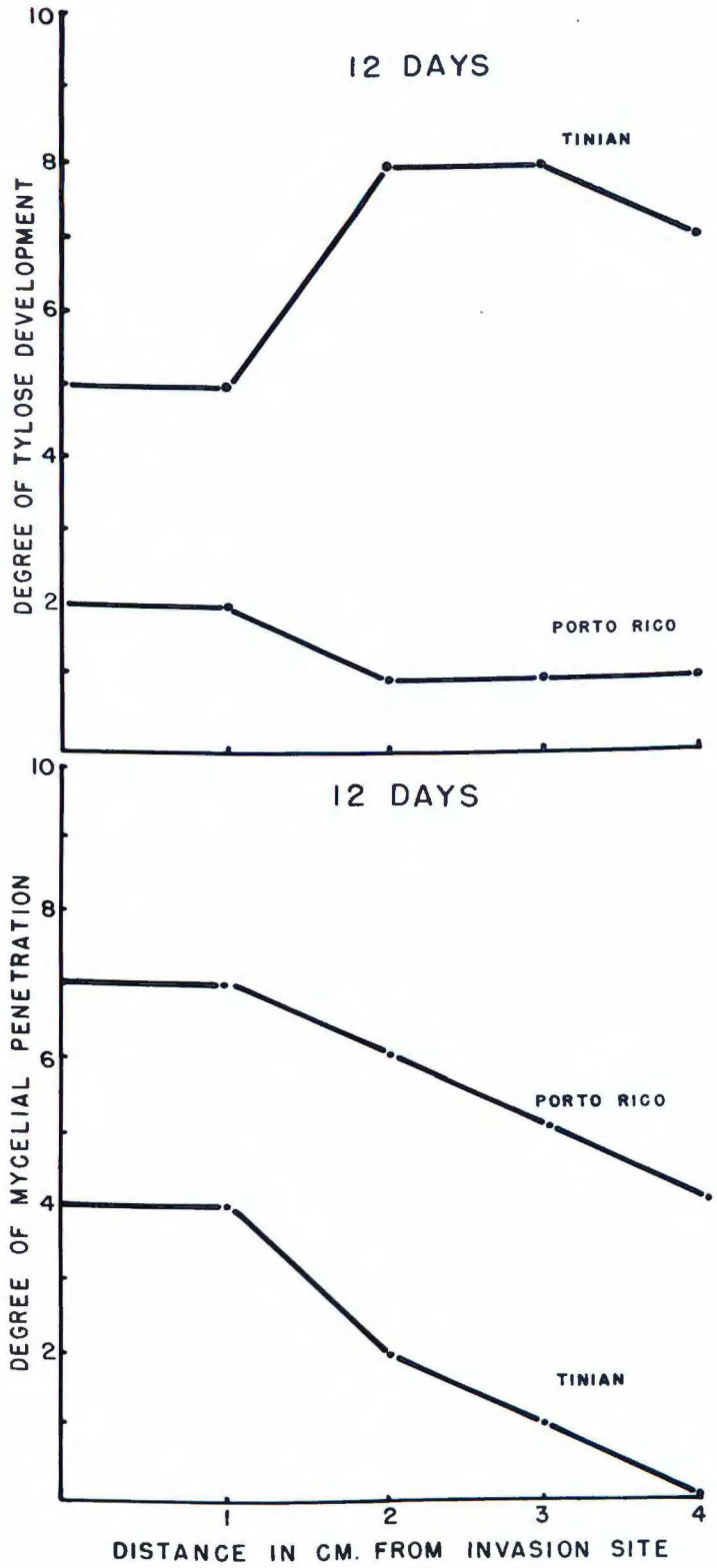

9 DAYS
DEGREE OF TYLOSE DEVELOPMENT
10
8
6
4
2
TINIAN
PORTO RICO
9 DAYS
DEGREE OF MYCELIAL PENETRATION
10
8
6
4
2
PORTO RICO
TINIAN
1
2
3
4
DISTANCE IN CM. FROM INVASION SITE

Figure 3. Tylose development and mycelial penetration 12 days after inoculation of "Tinian" and Porto Rico sweet potato cuttings with Fusarium oxysporum f. batatis. (Refer to Materials and Methods for interpretation of scoring system.)


12 DAYS
TINIAN
PORTO RICO
DEGREE OF TYLOSE DEVELOPMENT
10
8
6
4
2
12 DAYS
PORTO RICO
TINIAN
DEGREE OF MYCELIAL PENETRATION
1
2
3
4
DISTANCE IN CM. FROM INVASION SITE

from the invasion site were congested with tyloses (Figure 25). They plugged the major vessels which were the chief routes of mycelial penetration and spore dissemination, and they frequently sealed off the vessel passages entirely (Figures 26-28).

In the variety Porto Rico the tyloses were small and scattered. They seldom blocked a vessel passage and, in any event, were by-passed by the fungus which continued on to infect the plant systemically (Figure 29).

The possible significance of the tylose-producing response becomes clearer after a similar analysis of the extent of mycelial penetration in these same tissues.

In general, mycelia of Fusarium oxysporum f. batatis was found to penetrate the vascular system of Porto Rico quickly and systemically. In "Tinian" the mycelia are confined to areas close to the invasion site.

Six days after inoculation of Porto Rico cuttings, mycelial strands of Fusarium oxysporum f. batatis were observed up to 4 cm away from the invasion site (Figure 30). In "Tinian" 12 days elapsed before the mycelia could be detected the equivalent distance away from the invasion site.

The most significant observation, however, is that in "Tinian" the area of highest tylose development consistently coincided with, or occurred in advance of, the area where mycelial penetration was at its maximum. Furthermore, mycelia in "Tinian" did not extend more than 2 cm from this critical area of maximum tylose formation at any time.

In the variety Porto Rico this relationship between mycelial-penetration and tylose-formation did not exist. Mycelia were observed around and well beyond the area where tylose development occurred. For example, after 9 days, mycelia could be detected up to 7 cm away from

the invasion site, 4 cm beyond the area where the last pathologically-induced tyloses occurred (Figure 31). And after 12 days, mycelial penetration was systemic (Figure 29).

This evidence suggests, then, that the basis for the resistance of "Tinian" to Fusarium wilt is related to its capacity to develop tyloses well in advance of the fungus.

Beckman regarded the vascular occlusions in banana plants which were infected with Fusarium oxysporum f. cubense as functioning so as to retard the distribution of the spores of the pathogen throughout the vascular system of the plant. It was his conclusion that this response on the part of the host was a protective mechanism which delayed the progress of the pathogen. This delay was interpreted by him to be important in providing time for the other defensive mechanisms of the host to become effective.

The same situation may apply with regard to the resistance of "Tinian" to Fusarium wilt. The abundant tyloses may act as physical barriers against the dissemination of the pathogen as spores carried in the transpiration stream, and possibly directly against the further growth of mycelial strands into the upper regions of the vascular system. Or they may merely check the progress of the pathogen temporarily so as to allow for other host defensive mechanisms to become effective.

It is the conclusion of this study that tylose formation by the resistant plant may represent one of the defensive mechanisms against systemic infection by the pathogen.

As to the presence of this mechanism in all varieties of sweet potato resistant to Fusarium wilt, and as to whether resistance can be correlated to the tylose-forming response, further investigations of a broader selection of sweet potato hosts must be conducted. If such correlation can be established experimentally, the detection of this factor in the

laboratory could constitute an efficient technique for the screening of sweet potato plant material for resistance to the wilt pathogen.

Figure 4. General view of the sweet potato planting for the field resistance tests conducted during the summers of 1961 and 1962 at the University of Maryland Vegetable Research Farm, Salisbury, Maryland.

Figure 5. Complete survival of 10 "Tinian" sweet potato plants inoculated prior to planting with Fusarium oxysporum f. batatis in the field resistance test of 1962.

Figure 6. Complete loss of sweet potato plants of the susceptible breeding line B-651-1 inoculated with Fusarium oxysporum f. batatis in the field resistance test of 1962.

Figure 7. Severe reduction of plants of the susceptible sweet potato variety Porto Rico inoculated with the wilt pathogen Fusarium oxysporum f. batatis in the field test of 1962.

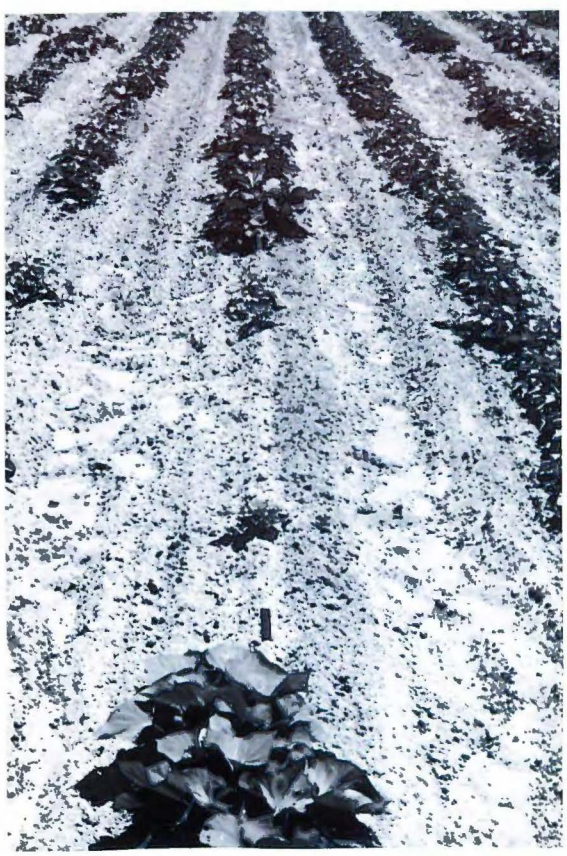
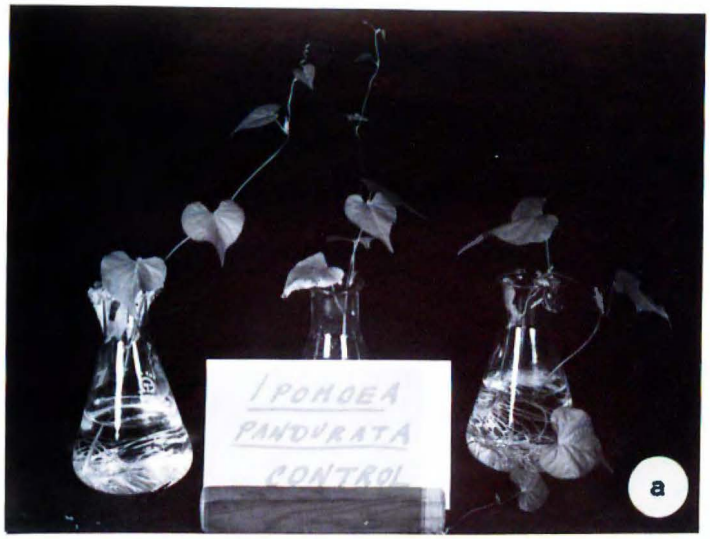


Figure 8. Susceptibility of *Ipomoea pandurata*, a common Morning Glory, to *Fusarium oxysporum* f. *batatis*:

a) uninoculated control plants,

b) inoculated test plants.


IPOMOEA
PANDURATA
CONTROL
a

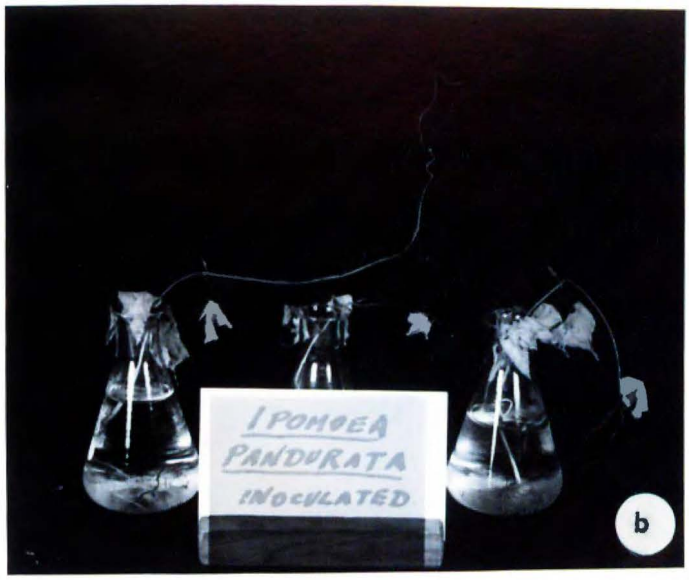
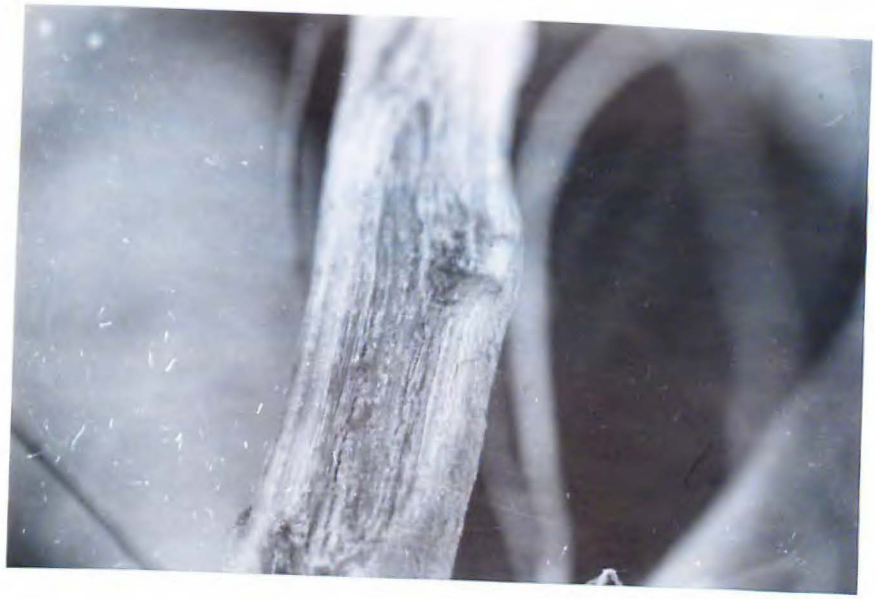

IPOMOEA
PANDURATA
INOCULATED
b

Figure 9. Discoloration of the vascular system of a stem of a susceptible sweet potato plant inoculated with Fusarium oxysporum f. batatis.

Figure 10. Disease symptoms of Fusarium wilt of sweet potato on an infected plant of the variety Nancy Hall.



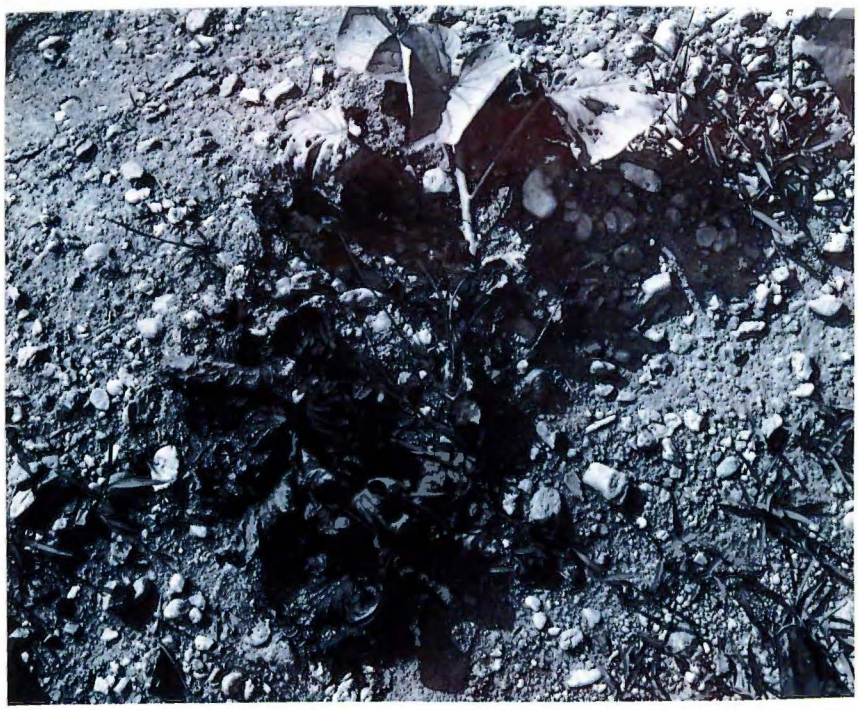
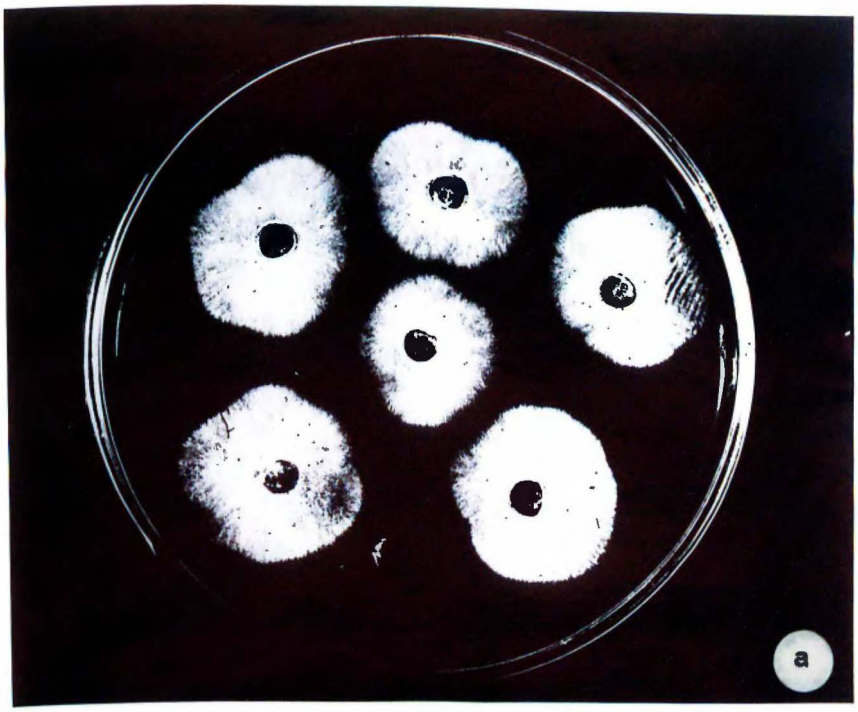


Figure 11. Isolation of *Fusarium oxysporum* f. *batatis* from the vascular system of the sweet potato foreign introduction "Tinian":

a) isolations from infected stems,

b) uninoculated controls.


a

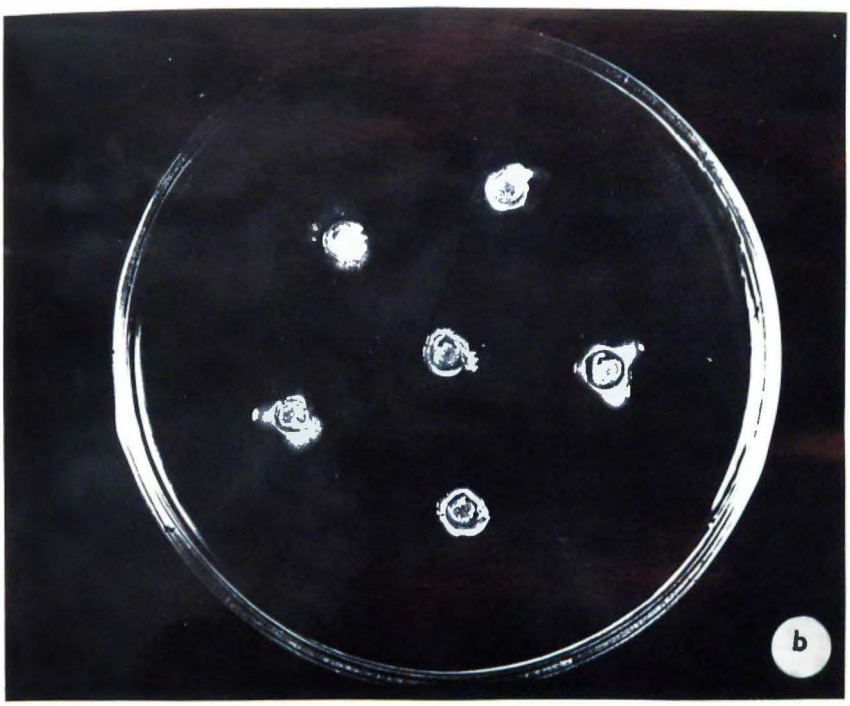
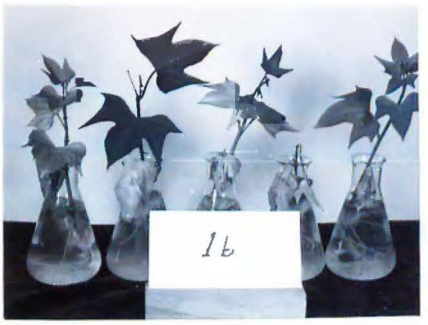

b

Figure 12. Chlorosis and wilting induced in both Porto Rico (a) and "Tinian" (b) sweet potato vine cuttings by culture filtrates of *Fusarium* spp.:

1. Uninoculated vines in artificial media,
2. Vines in a culture filtrate of *Fusarium oxysporum* f. *batatis*,
3. Vines in a culture filtrate of *Fusarium oxysporum* f. *lycopersici*.


1a


1b


2a


2b


3a


3b

Figure 13. Effects of spores of Fusarium oxysporum f. batatis on sweet potato vine cuttings of Porto Rico (a) and "Tinian" (b).

Figure 14. Effects of spores of Fusarium oxysporum f. lycopersici on sweet potato vine cuttings of Porto Rico (a) and "Tinian" (b).


4a

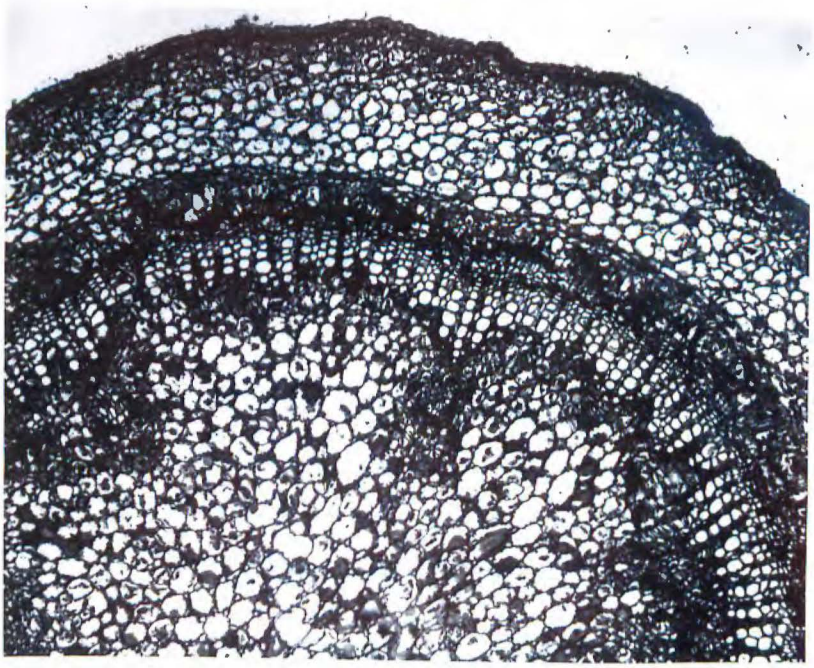

4b


5a


5b

Figure 15. Cross-section of a healthy Porto Rico sweet potato stem showing well-developed xylem (40x).

Figure 16. Typical xylem vessels of a healthy Porto Rico sweet potato plant (115x).



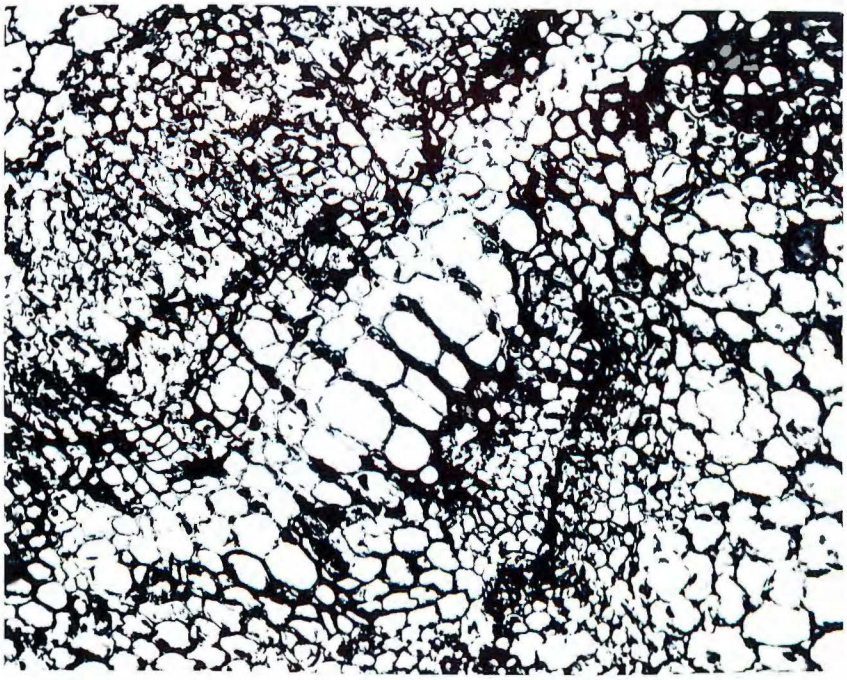
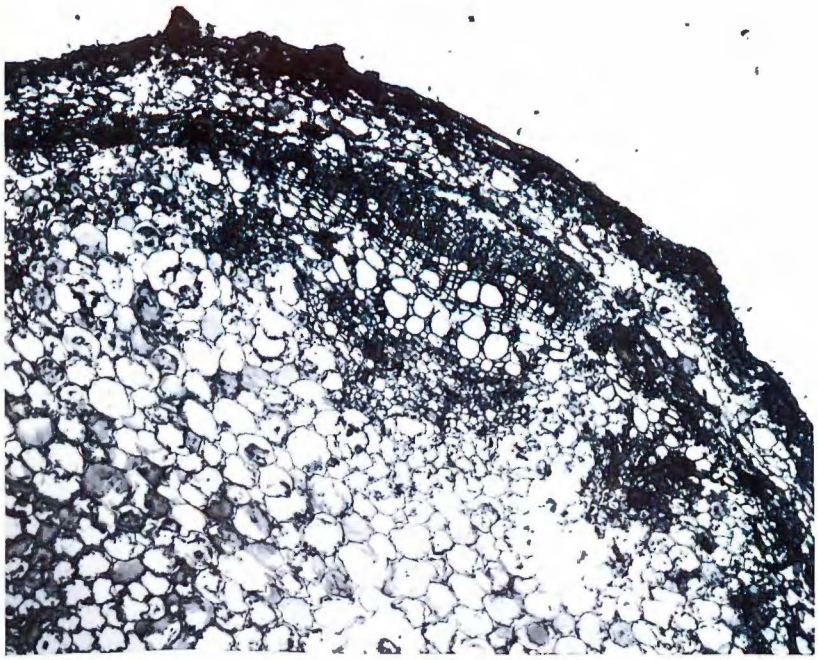


Figure 17. Cross-section of a healthy "Tinian" sweet potato stem showing a xylem of irregular structure and width (40x).

Figure 18. Xylem of a healthy "Tinian" sweet potato stem showing in detail an area where unusually large vessels occur (115x).



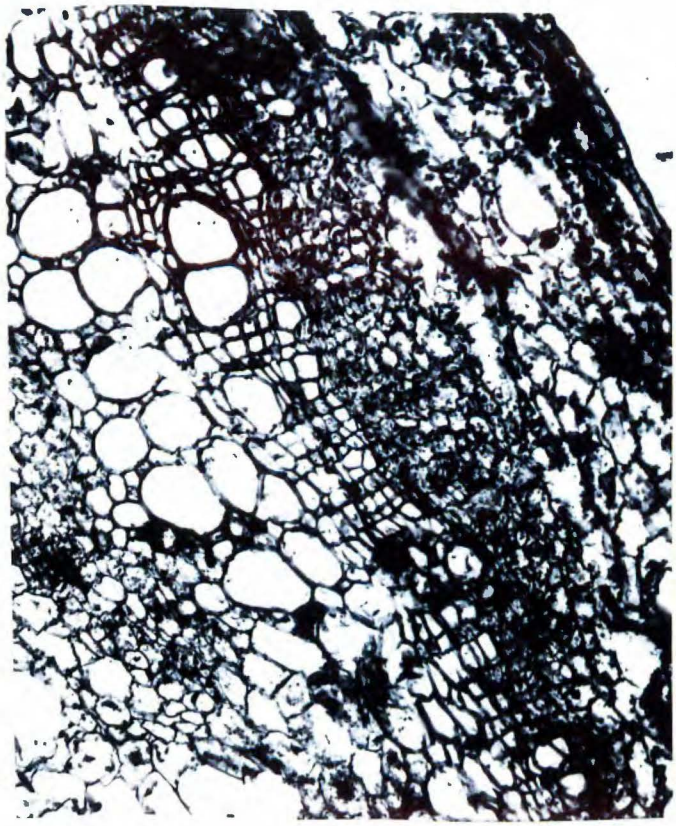
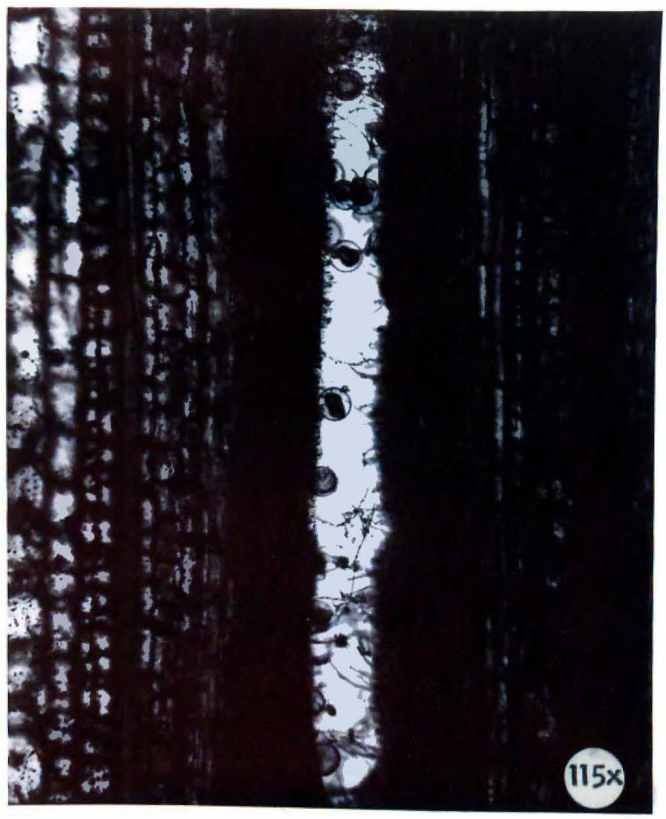


Figure 19. Longitudinal section of the xylem of an uninoculated "Tinian" sweet potato stem. Note the absence of tyloses (115x).

Figure 20. Longitudinal section of the xylem of an uninoculated Porto Rico sweet potato stem. Note the absence of tyloses (115x).

Figure 21. Longitudinal section of the xylem of an uninoculated "Tinian" sweet potato stem. Note the naturally-occurring tyloses (115x).

Figure 22. Longitudinal section of the xylem of a Porto Rico sweet potato stem inoculated with Fusarium oxysporum f. batatis. Note that tyloses are produced in abundance and constitutue an important anatomical symptom of the disease. Also, note the mycelial strands of the pathogen.


115x

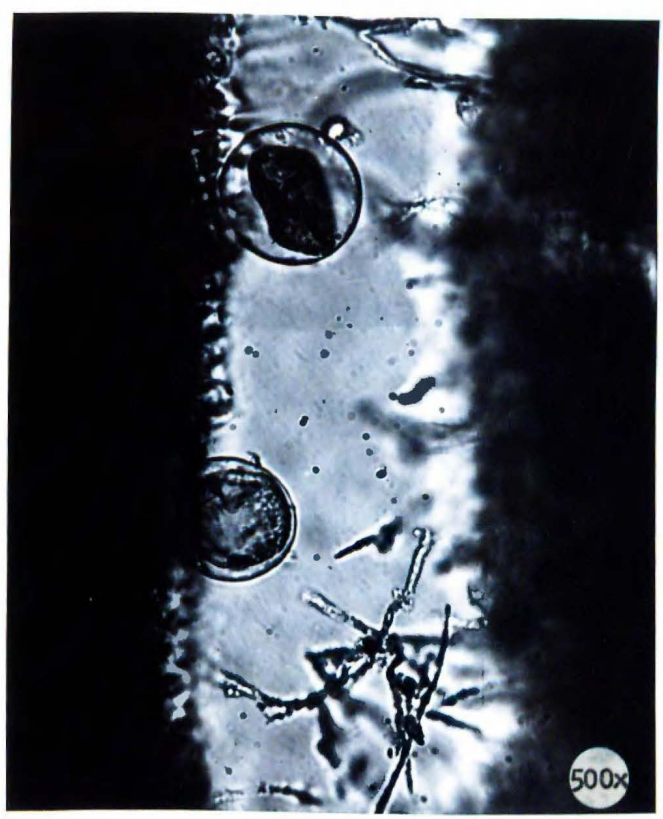

500x

Figure 23. Longitudinal section of the xylem of a "Tinian" sweet potato stem 6 days after inoculation with Fusarium oxysporum f. batatis. The area is 2 cm from the site of invasion. Note the initiation of tyloses (115x).

Figure 24. Longitudinal section of a xylem vessel element of a Porto Rico sweet potato stem 6 days after inoculation with Fusarium oxysporum f. batatis. Note the tyloses which originate as protoplasmic protrusions through the bordered pits of an adjoining parenchyma cell (500x).

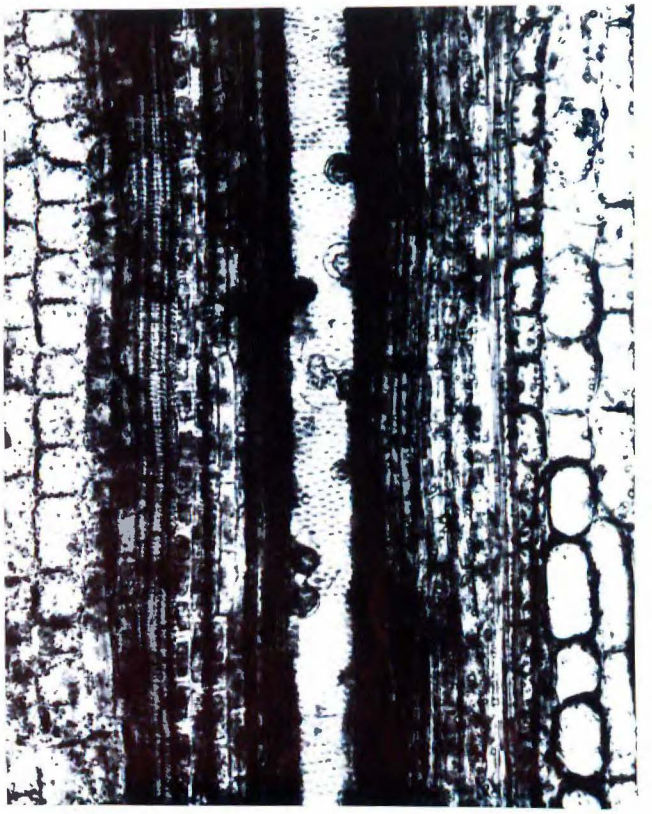
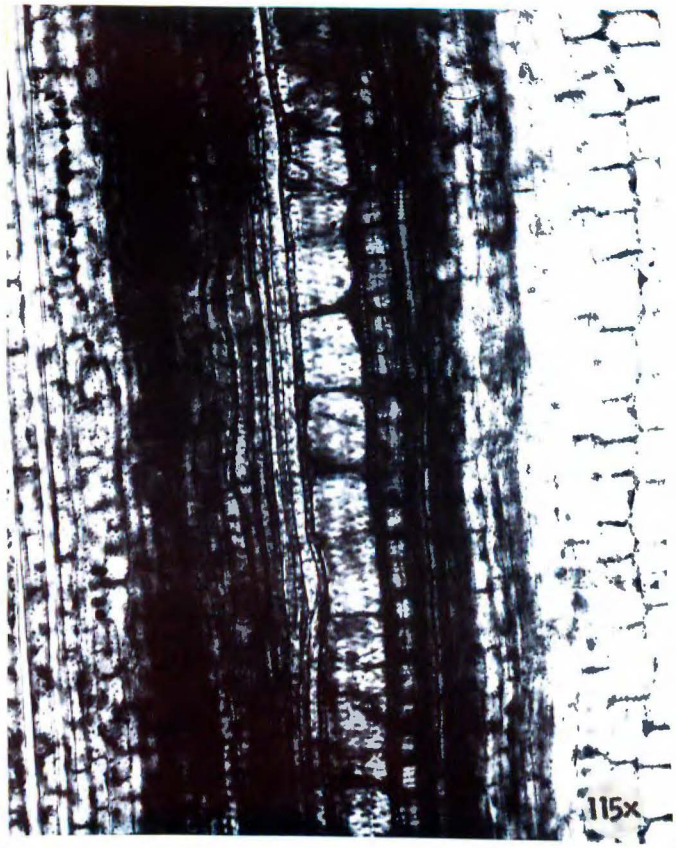


Figure 25. Longitudinal section of the xylem of a "Tinian" sweet potato stem 12 days after inoculation with Fusarium oxysporum f. batatis. In this area 2 - 3 cm from the invasion site, 88 - 94% of the vessels and tracheids are congested with tyloses (115x).

Figure 26. Longitudinal section of the xylem of a "Tinian" sweet potato stem 6 days after inoculation with Fusarium oxysporum f. batatis. Note in this section, 3 cm from the invasion site, the near-total obstruction of vessels by tyloses (115x).

Figure 27. Longitudinal section of the xylem of a "Tinian" sweet potato stem 9 days after inoculation with Fusarium oxysporum f. batatis. Note in this section, 4 cm from the invasion site, the sealing-off of the vessels by tylose formation.


115x

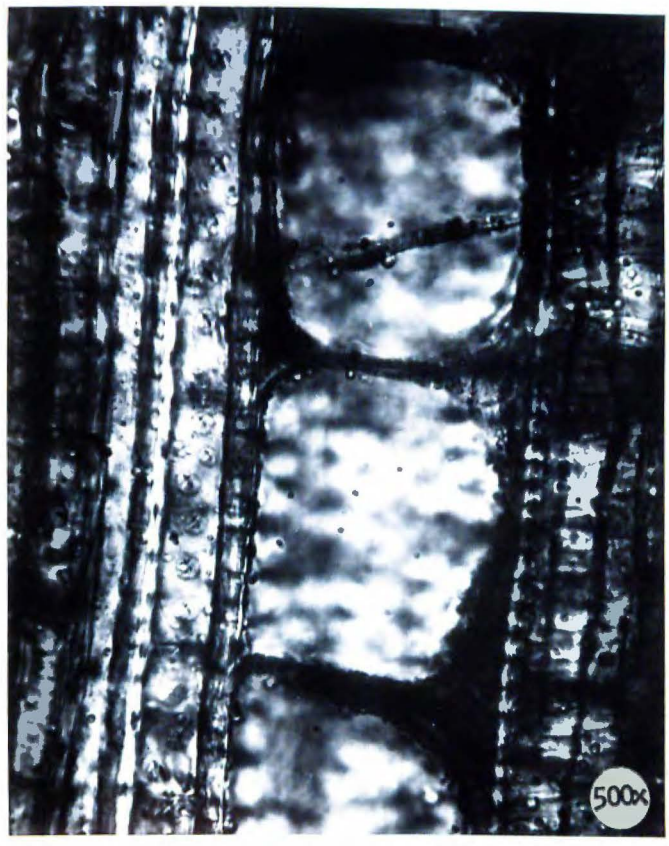

500x

Figure 28. Longitudinal section of the xylem of a "Tinian" sweet potato stem 9 days after inoculation with *Fusarium oxysporum* f. *batatis*. Note in this section, 7 cm from the invasion site, that the vessel passage is plugged by tyloses (500x).

Figure 29. Longitudinal section of the xylem of a Porto Rico sweet potato stem 12 days after inoculation with *Fusarium oxysporum* f. *batatis*. Note the systemic penetration by mycelia of the pathogen in this area 8 cm from the invasion site.


115x


500x

Figure 30. Longitudinal section of the xylem of a Porto Rico sweet potato stem 6 days after inoculation with Fusarium oxysporum f. batatis. Note the mycelial strands of the pathogen with the absence of any tylose-forming response in the vessel by the plant (500x).

Figure 31. Longitudinal section of the xylem of a Porto Rico sweet potato stem 9 days after inoculation with Fusarium oxysporum f. batatis. Note that in this area, 7 cm from the invasion site, mycelia of the pathogen can be detected and that there is no effective tylose-forming response by the plant (115x).

SUMMARY

Field resistance tests indicated that from among 94 different lines of sweet potato tested, 10 were highly resistant to Fusarium wilt of sweet potato caused by Fusarium oxysporum f. batatis. Among these were the foreign plant introductions P.I. 153655 ("Tinian"), P.I. 153906, and P.I. 153907; the variety Pelican Processor; and the breeding selection B-6842 from the United States Department of Agriculture Plant Industry Station at Beltsville, Maryland.

Host range studies indicated that the pathogen did not produce symptoms in many different crop plants. Two species of Morning Glory, however, were tested and shown to be susceptible.

Germination tests indicated that extracts from the highly resistant "Tinian" did not contain a fungitoxin. Nor could it be shown that culture filtrates of the pathogen contained toxins to which the resistant "Tinian" was immune.

Histological examination of stem sections from susceptible Porto Rico plants and from resistant "Tinian" plants indicated that "Tinian" responds to infection by the production of tyloses in the vascular system in advance of the fungus. This response does not occur in the variety Porto Rico. Furthermore, the pathogen in "Tinian" is restricted to the area of the stem near the invasion on site. In Porto Rico, the pathogen spreads systemically throughout the vascular system.

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