STUDIES ON SWEETPOTATO STEM ROT OR WILT AND ITS CAUSAL AGENT

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Summary

This investigation sheds light on the cultural identity and pathogenic behavior of the sweetpotato Fusarium (F. oxysporum Schlecht. f. batatatis (Wr.) Snyder & Hansen), the causal agent of stem rot or wilt disease. A total of 36 cultural units or combinations thereof were tested 3 years in succession on three varieties -- very resistant Tinian, intermediate or moderately susceptible Triumph, and very susceptible Porto Rico. Of diverse origin, the five parent cultures were otherwise unidentified. For 3 years in succession the virulent parent and single-spore progeny strains of the fungus behaved essentially alike in degree and range of infection.

In the sporulation on sweetpotato stems macrospores predominated. Only microspores were produced by this Fusarium when it was grown in pure culture on potato-dextrose agar.

For use in the present breeding program, culture transfers at 3-month intervals, incubated at temperatures near 11° C, effectively maintained suitable pathogenic strains of the sweetpotato Fusarium.

Single-spore progeny cultures were obtained by means of the Modified Barber's Method. Of the five viable microspores isolated from the separate parent cultures the numbers that grew and gave rise to progeny cultures were: I, 5; II, 4; III, 5; IV, 5; and V, 4. Only one (I-6) of the macrospores isolated into culture was used. Thus 24 single-spore progenies were employed.

Three experiments, alike in all important particulars, were started soon after June 1 each year (1955, 1956, 1957) and ended, respectively, 21, 21, and 17 days later. Each experiment employed 108 10-inch clay pots containing a sterilized mixture of 2/3 well-composited clay loam and 1/3 clean sand. Each pot was planted with ten inoculated cuttings of one variety.

When the experiments terminated each plant was dug, examined, classified according to extent of injury, and given an arbitrary numerical "disease index" value based on five classes of injury.

The 1955 results showed a spread or range covering more than two classes, which was also true in 1956 and 1957, but to a lesser extent.

The higher temperatures in 1956 and 1957 than in 1955 apparently caused increases in severity of injury by the pathogen.

In 1955 four parents (I, II, III, IV) and their progenies behaved alike pathogenically. The means of the progenies were here consistently higher than those of the parents, though not significantly so. On the other hand the means for the progeny of Strain V were all significantly below the means of parent.

In 1956 the performance of the Fusarium parent and progeny cultures was again very similar for cultures I to IV, whereas the progeny of V made a remarkable recovery of virulence. This was

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attributed possibly to the handling of the stock cultures which had been transferred at 3-month intervals between experiments.

The results of 1957 confirmed those of 1956. An apparent decline in virulence of culture IV and its progeny was not significant.

As expected, there were significant differences in the mean wilt indices among varieties. Among years, however, the average index values were, respectively, 62, 68, and 66, not significantly different. The higher index values for Triumph in 1956 and 1957 were found to be due to contamination or incipient infections in the cutting material.

In 1957, the shortening of the incubation period from 21 to 17 days caused a marked reduction in the average rating of Porto Rico as expected. For the successive increases in the average ratings for Tinian over the 3 years, the probable explanation is the increased temperatures of the test environment.

For Tinian, the mean indices for filtered originals, unfiltered originals, and the combined means of the single-spore progenies were, respectively, 17.6, 17.0, and 19.0; for Porto Rico 92, 95, and 91; for the three varieties combined, 65, 67, and 65; when the analyses combined the data from Tinian and Porto Rico (and omitted Triumph) the means were 55, 56, and 55. None of the differences within varieties or combined varietal comparisons were significant. Filtering appears unnecessary in the procedure for evaluating stem-rot or wilt resistance in sweetpotatoes, but does reduce frothing in the preparation of inocula.

INTRODUCTION

Stem rot or wilt is still one of the most important sweetpotato diseases, and breeding for resistance to it is an important essential in crop improvement. This disease appears to be indigenous to the United States. It was originally described by Halsted (1) in 1890 as due to Nectria ipomoea. Many years later, Harter and Field (2) reported finding a Fusarium to be the true causal agent. Shortly thereafter they (3) designated as causal agents two species of Fusarium (F. batatatis Wr. and F. hyperoxysporum Wr.) described by Wollenweber (11). Complete accounts of these appear in the monograph by Harter and Weimer (4).

In reporting, in 1940, on their species concept in the genus Fusarium, Snyder and Hansen (8) suggested a single species designation for the causal agent of the sweetpotato disease; namely, Fusarium oxysporum Schlecht. f. batatas (Wr.) Snyder & Hansen. Their endeavors were noteworthy in supplying simplicity for a most complicated genus of fungi, though presumably the term batatas should here be replaced by Wollenweber's genitive batatatis.

The primary objective of this investigation, which extended over 3 years, was to become familiar with the sweetpotato Fusarium through studies on species identity, cultural purification, and the conduct of operational procedures to maintain suitable pathogenetic strains for use in the present breeding program for disease resistance.

PRELIMINARY AND BASIC OBSERVATIONS

Sporulation

A study of the incidence of the sweetpotato stem rot, or wilt, was started in the autumn of 1954 in both the greenhouse and the field at Beltsville. Fruiting specimens were collected and examined for sporulation. Macrospores predominated on the stem-rot specimens whereas only microspores were produced when the fungus isolated into pure culture. Regardless of source, the macrospores were so much alike in their promiscuously variable main dimensions as to indicate a single Fusarium species.

Stock Cultures

Five old U. S. D. A. stock cultures of the sweetpotato Fusarium were examined. These were the residue from an original collection of about 40 that had been isolated and continu-

ously held in stock culture for a decade or longer, being used year after year for determining disease resistance. These cultures had been maintained on potato-dextrose or corn-meal agar for 6 months or longer between transfers. Once each year their pathogenicity had been tested on susceptible Porto Rico plants in contaminated soil in the greenhouse.

Test Procedure

The established test procedure for determining wilt susceptibility in sweetpotatoes had regularly employed a filtered composite of the five virulent stock cultures. The cultures were labeled: F-137, 7287c, 7313a, 7339b, and B-5941. They are cited herein as I, II, III, IV, and V, respectively. To eliminate bacterial contamination in stock cultures in the past, transfers had been made to Harter's Synthetic Agar[3] which supports good mycelial growth but suppresses bacterial growth.

When first examined, early in 1955, for condition of growth, one of the five old stock cultures was slightly lower in virulence and another was contaminated with bacteria. The contaminated culture was readily purified by a simple, rapid micrurgical technique (5).

Single-Cell Isolation

The pure culture concept (5), "the origin of a culture from a single cell, spore or unit, and from one only," is generally regarded as the prime essential for studying variability in microorganisms.

To prepare the stock cultures for the isolation of single spores, they were transplanted to potato-dextrose agar three times successively at weekly intervals. In single-spore isolation microspores were employed mainly, rather than macrospores, because of their simpler genetic constitution. Only microspores were produced in culture. Previously Wellman (10) had done single-spore culture work on the tomato Fusarium and presumably had used microspores, although he did not specify the spore type.

Single-spore isolations were made by the Modified Barber's Method (5) using the micromanipulative apparatus. By single cell isolation it is possible to purify most mass cultures which are usually mixtures of strains or units. To establish the stability of the five parent cultures (isolates or strains), single-spore progeny cultures were obtained from each of them. The numbers of single microspores which grew into culture were: I, 5; II, 4; III, 5; IV, 5; and V, 4. Only one macrospore was isolated into culture; namely, I-6. By the micrurgy used it was possible to follow every stage in the development of the several spores into cultures. The mycelium produced in culture by the lone macrospore was indistinguishable from the mycelia obtained from the microspores. Two spores proved to be non-viable when isolated into microdrops and failed to develop the swelling characteristic of the viable ones. To test the germinability requirements preliminary isolations were made in distilled water. The microspores failed to germinate there, but when potato-dextrose broth was added they germinated copiously.

When isolated into microdrops of liquid media (potato-dextrose broth), the microspores germinated within 8 hours and produced enough mycelial growth and spores for the mass to be visible to the naked eye within 24 hours. As the isolated spores were mounted on the underside of a coverslip which then was placed over a deep-well slide, thereby providing a moist chamber and incubator, it was convenient to observe their germination and growth from time to time. Sterile vaseline or petroleum jelly sealed the coverslip in place. At the bottom of the well a film of sterile culture medium was installed to maintain a uniform vapor tension so as to stabilize the culture droplets at the top. When the growth became visible to the naked eye, it was transferred to test-tube culture.

Identity of the Several Isolations

The isolations from sweetpotato stem rot or wilt that were employed in this study are all held referable to one species, Fusarium oxysporum Schlecht. f. batatatis (Wr.) Snyder & Hansen. During 1955, 1956, and 1957 examination was made of the varied reproductive bodies formed on the basal portions of the stems of the potted sweetpotato plants that had been inoculated with the five parent and 24 single-spore progeny cultures. In addition to numerous

[3] Harter's Synthetic Agar: Dextrose, 200 gm.; peptone, 10 gm.; NH_4NO_3, 10 gm.; KNO_3, 5 gm.; K_2HPO_4, 2.5 gm.; $MgSO_4$, 2.5 gm.; $CaCl_2$, 0.1 gm.; shredded agar, 20 gm.; distilled water, 1000 ml. pH 5.1. Sterilized at 110°C for 15 min.

microconidia closely resembling those produced copiously in agar cultures, scrapings from a diseased stem usually showed an abundance of macroconidia. These macroconidia in some instances appeared relatively uniform in dimensions, shape and septation; but much more often they showed the rather pronounced morphological variability that has long been familiar to investigators dealing with Fusarium. Marked variations were noted even when scrapings were taken from portions of stem only a few square millimeters in area -- from sporulating tracts presumably too small to have provided wide differences in environmental conditions.

The variations observed in the several isolations obtained originally from separate sources seem rather generally of haphazard occurrence. In Figure 1 are shown five groups of five spores (I-V:0) selected in 1956 as being approximately representative of the macroconidia produced on sweetpotato stems infected with the five separate parent isolations employed (I-V). Under each of these groups are shown three groups of five spores (I-V:1-3) selected the same year as being similarly representative of the macroconidia formed on sweetpotato stems infected with three single-spore subcultures isolated from each parent culture. Among the 20 groups of spores some groups differ rather markedly from most of the other 19 groups in the whole assortment, but the deviation shown in any group is not well sustained in the three other groups placed in the same column, which represent intimately related isolations. Variations in the macroconidia taken from diseased stems in 1955 and 1957 were measured and compared and appeared mainly of like fortuitous character. As no decisive characters could be recognized as distinguishing any one series of parent and progeny isolations from the other four series of cultures, all five series of cultures used are considered to belong to a single species.

MATERIALS AND METHODS

Three varieties of sweetpotatoes were selected: very resistant Tinian (P.I. 153655), very susceptible Porto Rico, and intermediate or moderately susceptible Triumph.

Ten days to 2 weeks before an experiment was started, cuttings were placed in sand for rooting. In 1955 and 1956, three- to five-node cuttings made from greenhouse-grown plants were used. In 1957 terminal-growth cuttings were made from sprouts produced in outdoor propagating beds. When ready they were pulled from the rooting bench and rinsed in tap water to remove sand, the basal ends were freshly cut with a sharp knife to remove the protective callus, and the roots were trimmed to 2 inches to facilitate the planting operation and provide additional openings for entrance of the pathogen. This severe pruning was more drastic than in ordinary commercial practice and favored the fungus over the host.

Five days before the experiment was begun, cultures were started in 150 ml. of nutrient solution[4] in 250 ml. Erlenmeyer flasks. After 5 days the following inocula were ready for use: 5 parent cultures singly, 24 single-spore progeny cultures singly, and a composite[5] of the 5 parent cultures, making a total of 30 sources. Added also were a series of 6 filtered cultures (5 single parents and 1 composite), making a grand total of 36.

The inoculum was prepared by diluting each 150 ml. cultural unit with distilled water to a volume of 400 ml. This was more than adequate in quantity and concentration. It was applied to one bundle of prepared rooted cuttings of each variety, making a total of 108 pot units for the 36 cultural units.

The experiments were started soon after June 1 in each of 3 years (1955, 1956, 1957). In each experiment 108 10-inch clay pots were employed, each containing a sterilized mixture of 2/3 well-composted clay loam and 1/3 clean sand. Each pot was planted with ten prepared cuttings of one variety, set in holes made by a pointed redwood dibble evenly spaced over the soil surface. Immediately before planting, the cuttings were dipped into the liter beaker containing 400 ml. of inoculum.

To prevent cross-infection (7) or cross-contamination great care was taken in all details of the inoculation procedure, with the three persons simultaneously performing different phases of the operations in different rooms.

One person, the plant preparator, removed the cuttings from the rooting bench, washed

[4] A modification of Steinbauer's liquid synthetic medium (1947): NH_4NO_3, 1 gm.; KH_2PO_4, 1 gm.; $MgSO_4$, 5 gm.; sucrose, 10 gm.; distilled water, 1000 ml.

[5] "Composite" preparation involves growing the five cultures separately and then mixing all the cultures, filtering off and discarding the liquid medium, macerating the filter paper and fungus growth in distilled water in a Waring Blendor and making the blend up to volume with distilled water for inoculation purposes.

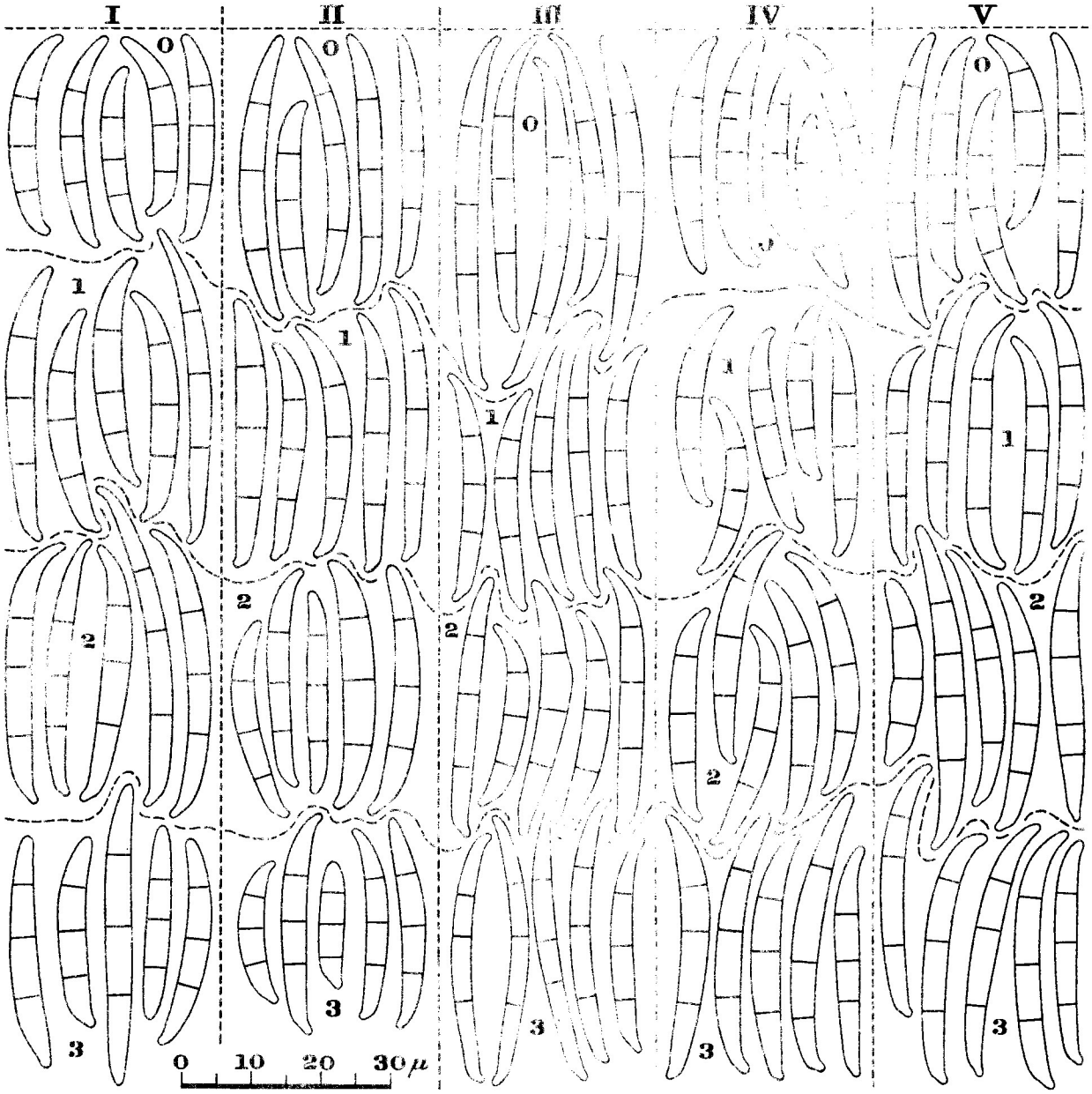

FIGURE 1. Groups of five macroconidia of Fusarium oxysporum f. batatatis produced on sweetpotato stems by the several parent isolations, I-V, and by three single-spore cultures obtained from each of the parent isolations, I-V; 1-3.

off adhering sand, trimmed roots and the basal stem callus; and delivered these freshly prepared healthy plants to the inoculation greenhouse. (In the 1956 and 1957 experiments, a fourth person carried the tray to the inoculation room.)

A second person, the culture preparator, assembled the several units of inoculum in the order of use according to the following procedure. The contents of each culture flask in most instances were emptied directly into a blending jar. In some fewer instances they were placed onto the filter in a Büchner funnel and after removal of the liquid by suction the filter paper together with the mycelial mat were placed in the blending jar. Then sterile distilled water was added to bring the volume to 400 ml. and the blender was operated for 30 seconds. The mycelial suspension was poured into a sterilized beaker and delivered to the greenhouse for use in inoculation. After each use the contaminated utensils were decontaminated, washed, rinsed successively in distilled water, 95% ethyl alcohol, and petroleum ether, and then drained dry before reuse.

A third person, the plant inoculator, used a sterilized dibble to make ten holes in the soil in each pot. The ten prepared cutting plants were dipped into the inoculum and then planted in the designated pots. The operator removed the contaminated beaker and dibble, thoroughly washed his hands, and dealt with the next lot of inoculum. This phase of the experiment required about 4 hours for completion.

After planting, the soil was watered and kept moderately moist during the test periods (21 days in 1955 and 1956, 17 days in 1957). Care was taken when watering to prevent cross-contamination by contact or splashing.

In the 1955 experiment, the test pots were arranged in numerical order with respect to the Roman and the Arabic numerals designating the isolations and subcultures used for inoculation, so that the three sweetpotato varieties were in juxtaposition on the benches. In the 1956 experiment, which duplicated the first one in most particulars, the sweetpotato varieties were placed on separate benches and the test pots or units within varieties were completely randomized. In 1957 the planting and pot arrangement were the same as in 1955.

At the termination of the experiment each plant was dug, examined with the aid of a sharp knife, classified according to the extent of injury from stem rot or wilt and given an arbitrary numerical value[6] based on severity of injury. The sum of the values for all plants in one pot gave an injury index number for that lot.

For confirming the identity of the causal agent in each of the 36 lots of diseased plants at digging time, several sporulating stem specimens were collected from the susceptible host series, placed on moistened filter paper in sterile Petri dishes and stored in the refrigerator (6° C).

Under these conditions the macrospores, which were produced in great abundance by all 36 cultural units during the third week of growth, seemed to survive very well. It was estimated that over 90 percent were alive at the end of 5 months' storage and that a considerable number, or over 30 percent, were still viable after 10 months' storage.

The severity of the disease on cuttings of the Triumph variety appeared to be greater in 1956 than in 1955. However, in 1957 some plants of this variety were found coming down with infection before any of the others. Thus the stock of this variety was found to be contaminated with incipient infections, giving higher values than expected in 1956 and 1957.

EXPERIMENTAL RESULTS

The experimental results are summarized in Table 1. This was essentially a comparative study that tested the established wilt-testing procedure and several alternatives. The several modifications took into account the identity and pathogenicity of the five parent cultures singly and in composite and 24 of their single-spore progeny, individually. The established procedure employed a filtered composite of five parent cultures, labeled VI-OF, which was compared with the effect of filtration on the five parent cultures, individually. The index number[6] represents the severity of disease induced by each of the 36 Fusarium culture units on the three sweetpotato varieties in 1955, 1956, and 1957.

The 1955 results, when examined for frequency distribution of inoculated plants among

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[6] The five classes of injury and their assigned numerical values for the 10-plant lots were: 1 -- dead, 10.0; 2 -- severe, 7.5; 3 -- moderate, 5.0; 4 -- slight, 2.5; and 5 -- none, 0.0. This is an adaptation of the commonly used indexing system which employs 25-plant samples in which the index values are, respectively, 4, 3, 2, 1, and 0. With either scale of values there is a maximum disease index of 100, if all plants are killed, and of 0 if no injury is evident.

Table 1. Summary compilation of the wilt index values for the experiments conducted during three successive seasons, representing the degree of severity induced by each of the 36 cultural units of Fusarium oxysporum f. batatatis on resistant Tinian, intermediate Triumph, and susceptible Porto Rico varieties of sweetpotato.

Varieties	Filtered Original				Unfiltered Original				Unfiltered Single-spore Progeny Cultures																							
									1				2				3				4				5				6				Progeny Mean			
Resistant Tinian	'55	'56	'57	Mean	'55	'56	'57	Mean	'55	'56	'57	Mean	'55	'56	'57	Mean	'55	'56	'57	Mean	'55	'56	'57	Mean	'55	'56	'57	Mean	'55	'56	'57	Mean	'55	'56	'57	Mean
I	20	28	23	24	13	20	28	20	18	15	30	21	20	18	30	23	28	30	20	26	15	35	18	23	18	15	18	17	25	15	13	18	21	21	21	21
II	18	15	23	19	13	18	35	22	25	20	15	20	18	25	10	18	18	23	33	24	28	38	30	32									22	26	22	23
III	10	23	13	15	5	20	20	14	18	30	23	24	13	15	33	20	13	15	23	17	13	10	23	15	15	18	33	22					14	18	27	20
IV	20	18	10	16	5	8	17	10	20	8	25	18	15	8	25	16	8	15	13	12	13	13	10	12	8	18	5	9					13	12	16	13
V	13	20	10	14	10	20	23	18	5	33	23	20	10	18	20	16	10	23	8	14	15	15	30	20									10	22	20	18
VI (I-V)	10	25	30	22	5	20	38	21																												
[illegible]																																				
I	88	96	95	93	90	95	93	93	95	89	90	91	95	83	88	89	90	91	80	87	98	93	90	93	93	93	93	91	93	85	[illegible]	88	94	89	87	90
II	85	90	90	88	88	91	88	89	90	88	75	84	85	88	73	82	90	83	100	91	100	96	100	99									91	89	87	89
III	68	85	88	80	80	86	95	87	75	95	95	88	70	85	95	83	73	83	95	84	88	83	88	86	88	85	76	83					79	86	90	85
IV	53	89	85	76	78	83	75	79	88	80	83	84	75	79	85	80	73	83	88	81	55	71	70	65	80	90	78	83					74	81	81	79
V	48	95	78	74	85	92	100	92	63	86	85	78	23	91	95	70	40	83	90	71	20	80	83	61									37	85	88	70
VI (I-V)	88	100	93	94	80	100	98	93																												
[illegible]																																				
I	100	100	95	98	100	100	88	95	100	90	88	93	100	95	90	95	100	100	80	93	100	100	93	98	100	100	95	98	100	100	85	95	100	97	89	95
II	98	100	90	96	100	100	93	98	98	100	85	94	100	98	85	94	100	100	98	99	100	100	100	100									100	100	92	97
III	98	100	85	94	100	98	95	98	100	100	88	96	95	93	98	95	93	98	90	94	100	96	93	97	100	95	90	95					98	97	92	95
IV	85	98	80	88	100	100	72	91	100	100	88	96	100	93	68	87	98	98	70	89	93	90	78	87	98	85	90	91					98	93	79	90
V	93	93	73	86	90	100	88	93	85	98	80	88	65	98	88	84	65	95	85	82	50	68	80	66									66	90	83	80
VI (I-V)	98	100	88	95	95	100	98	98																												
Variety Totals R-I-S																																				
I	208	224	213	215	203	215	209	209	213	194	208	205	215	196	208	206	218	221	180	206	213	226	201	213	211	212	196	206	218	200	188	202	215	208	197	207
II	201	205	203	203	201	209	216	209	213	208	175	199	203	211	168	194	205	206	231	214	228	234	230	231									213	215	201	206
III	176	208	186	190	185	204	210	203	193	225	206	208	178	193	226	199	179	196	208	194	201	191	204	199	203	196	201	200					191	201	209	200
IV	158	205	175	179	183	191	164	179	208	188	196	197	190	180	178	183	179	196	171	182	161	174	158	178	186	190	173	183					185	186	176	182
V	154	208	161	174	185	212	211	203	153	217	188	186	98	207	203	169	115	201	183	166	85	163	193	147									113	197	191	167
VI (I-V)	196	225	211	211	180	220	234	211																												

the five injury classes, showed a spread or range often covering more than two classes as was also true in 1956 and 1957, but to a less extent. Such variability was believed due to insufficient uniformity among the cuttings. By more precise selection for age and maturity (Steinbauer, 9), this variability was demonstrably reduced in the cuttings. By repeating the experiment 3 years in succession, it was possible to observe the effects of time and frequency of transfer on the pathogenic stability of identical stock cultures. The higher temperatures in 1956 and 1957 appeared to favor the pathogen over the host, either through increase in virulence of the pathogen or decrease in resistance of the sweetpotato host, since there was an increase in severity of injury.

In Table 1 the index values, which represent the degree of severity, are arranged in juxtaposition for ready comparison of the three experiments. As a further aid in making comparisons, a cumulative index or sum of the variety totals is shown at the bottom of the table.

Four of the parents (I, II, III, IV) and their progeny cultures, taken individually in 1955, behaved pathogenically in similar fashion. From the cumulative index values the parents and their respective progeny means were: I, 203 and 215; II, 201 and 213; III, 185 and 191; and IV, 183 and 185. While the progeny means were consistently higher than those of these parents, the differences are small and not significant. The V values were 185 and 113. The strain V progeny means were significantly below those of the parent.

In 1956 the performance of cultures I to IV and their progeny was essentially the same as in 1955; whereas culture V and its progeny had apparently made a remarkable increase in pathogenicity: I, 215 and 208; II, 209 and 215; III, 204 and 201; IV, 191 and 186; and V, 212 and 197. The increase in pathogenicity of the single-spore progeny of V can possibly be attributed to the handling of the stock cultures, which had been simply transferred four times or at 3-month intervals between the two experiments.

The results in 1957 were: I, 209 and 197; II, 216 and 201; III, 210 and 209; IV, 164 and 176; and V, 211 and 191. In this experiment there was an apparent decline in virulence of culture IV and its progeny. However, when the values for all 3 years were taken into account, (Table 1) the differences between the years were not significant.

As expected, there were significant differences between varieties in regard to the mean wilt indices (Table 2). The average index values for the 3 years were 62, 68, and 66, respectively. Higher temperatures during the 1956 and 1957 seasons may account for at least

Table 2. Averages of index numbers for stem rot or wilt of the three sweetpotato varieties and for the three years of this investigation.

Variety	1955	1956	1957	3 Years
Tinian	15	20	22	19
Triumph	77	88	88	84
Porto Rico	94	97	87	93
Average	62	68	66	65

a part of this increase. The high index values for Triumph in 1956 and 1957 were found to be due to presence of incipient infections in the cutting materials. For Tinian to have shown progressive increases in the average ratings over the 3-year period, we have no explanation other than increased temperature. However, shortening the incubation period during 1957 from 21 to 17 days caused a marked reduction in the average rating of only the highly susceptible Porto Rico, which was expected. No similar reduction was noted in the other two varieties.

In the analysis of Tinian data alone, the differences between mean indices for filtered originals and unfiltered originals and the combined means of the single-spore progenies were not statistically significant, the means being, respectively, 17.6, 17.0, and 19.0. Also there were no significant differences between culture means within the filtered or unfiltered originals, which suggests that any one of these five parent cultures alone or two or more in composite can be used satisfactorily as sources of test inoculum. However, among the prog-

enies of different origin, there were some significant differences among the means, with the progeny of IV averaging significantly lower than the progenies of I, II, and III. Within parent cultures there were no significant differences among their single-spore progenies. For Tinian there were very highly significant differences among the means for the 3 years.

In the analyses of the Porto Rico variety by itself, no significant differences occurred between the filtered and unfiltered originals, and the single-spore progenies, with the means being, respectively, 92, 95, and 91.

In the analyses of all three varieties combined, the differences among the filtered originals, the unfiltered originals, and the single-spore progenies were not significant. The three means were, respectively, 65, 67, and 65. When the analyses combined the data from resistant Tinian and very susceptible Porto Rico (and omitted Triumph) the means were, respectively, 55, 56, and 55. Thus again the differences were not significant between the filtered and the unfiltered cultures.

Finally, filtration did not materially alter the extent of wilt injury, judging from the performance of the several Fusarium cultures on the three sweetpotato varieties in three experiments conducted during 1955, 1956, and 1957. Therefore, this step appears unnecessary and it can be discontinued in the procedure for evaluating stem rot or wilt resistance in sweetpotatoes. However, filtration is a convenience in preparing inocula since it eliminates frothing during maceration in the Waring Blendor.

DISCUSSION AND CONCLUSIONS

How many Fusarium cultures are necessary to guarantee successful evaluation of test materials for disease susceptibility or resistance? For the tomato, Porte and Wellman (6) found one stable culture of Fusarium oxysporum f. lycopersici (Sacc.) Snyder & Hansen satisfactory for determining wilt resistance in greenhouse tests. In this study it appears that any one of the five parent cultures used, or its progeny in a high state of virulence, gave dependable information on the relative resistance or susceptibility of the sweetpotato varieties to stem rot or wilt. Also, filtering was shown to have no significant effect on results. Thus the standard procedure can be simplified by omitting the filtration step. The stock cultures should be transferred quarterly or at 3-month intervals for maintaining the cultures in a high state of virulence. The possibility of occurrence of significant variation within a single culture must be kept in mind although the probability may be low.

Literature Cited

1. HALSTED, B. D. 1890. Some fungus diseases of the sweetpotato. New Jersey Agr. Exp. Sta. Bul. 76: 1-32.
2. HARTER, L. L., and E. C. FIELD. 1913. Fusarium batatatis Woll. Ms. not Nectria ipomoeae Hals. the cause of sweetpotato stem rot. Phytopathology 3: 68.
3. HARTER, L. L., and E. C. FIELD. 1914. The stem-rot of the sweetpotato (Ipomoea batatas). Phytopathology 4: 279-304.
4. HARTER, L. L., and J. L. WEIMER. 1929. A monographic study of sweet-potato diseases and their control. U. S. Dept. Agr. Techn. Bul. 99.
5. HILDEBRAND, E. M. 1938. Techniques for the isolation of single microorganisms. Bot. Rev. 4: 628-664.
6. PORTE, W. S., and F. L. WELLMAN. 1941. Development of interspecific tomato hybrids of horticultural value and highly resistant to Fusarium wilt. U. S. Dept. Agr. Circ. 584.
7. REYNIERS, J. A. 1943. Chap. IX: pages 205-232. The control of cross infection among limited populations. The use of mechanical barriers in preventing cross infection among hospitalized infant populations. In: REYNIERS, J. A. Micrurgical and Germ-Free Methods. Charles C. Thomas, Springfield, Ill. 274 pp.
8. SNYDER, W. C., and N. E. HANSEN. 1940. The species concept in Fusarium. Amer. Jour. Bot. 27: 64-67.
9. STEINBAUER, C. E. 1956. Types of sweetpotato cuttings for most precise evaluation of Fusarium wilt susceptibility. Proc. Amer. Soc. Hort. Sci. 68: 394-399.

10. WELLMAN, F. L. 1940. Differences in growth characters and pathogenicity of Fusarium wilt isolations tested on three tomato varieties. U. S. Dept. Agr. Techn. Bul. 705: 1-28.
11. WOLLENWEBER, H. W. 1914. Identification of species of Fusarium occurring on the sweetpotato, Ipomoea batatas. Jour. Agr. Res. 2: 251-285.

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