VARIATION IN PATHOGENICITY OF THE FUSARIUM WILT ORGANISM OF SWEET POTATO

BY

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TABLE OF CONTENTS

Introduction 1

Literature Review

Taxonomy 3

Physiologic Specialization. 6

Pathogenicity Trials

Methods and Materials 11

Results 17

Temperature Relations. 23

Discussion 31

Summary. 35

Literature Cited 36

INTRODUCTION

The modern concept of disease takes into account the fact that neither the suscept nor the pathogen is a fixed entity. Both are plastic and vary under the influences of heredity and environment. Stakman (25) and Christensen *et al.* (7) emphasize the importance of a knowledge of variability in the pathogen and the relation of this variation to an understanding of epiphytotics, plant quarantines, and the breeding of disease resistant varieties of plants. The same authors point out that most species of plant pathogenic fungi when carefully studied have been found to be made up of a large number of biotypes. One or more of these biotypes when they react with a reasonable degree of consistency as determined by pathogenicity on a set of differential host plants are usually designated as physiologic races.

Abundant evidence of physiologic specialization in all groups of phytopathogenic fungi is available (7, 19, 20). Perhaps the most outstanding examples are to be found in the causal organisms of the cereal rusts (15) and smuts (8). In *Fusarium* species, physiologic races have been noted in the tomato wilt organism, *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder and Hansen, (1); the causal organism of headblight of small grains, *Fusarium roseum* (Lk.) Snyder and Hansen f. *cerealis* (Cke.) Snyder and Hansen, (26); the flax wilt organism, *Fusarium oxysporum* f. *lini* (Bolley) Snyder and Hansen, (6); and several others.

The objective in the present study with the causal organism of stem rot of sweet potato, *Fusarium oxysporum* f. *batatas* (Wr.) Snyder and Hansen, was to determine whether or not physiologic races of this pathogen occur. A knowledge of this point is deemed essential from a practical standpoint as a guide in further testing of sweet potato varieties developed in the

sweet potato breeding program at the Oklahoma Agricultural Experiment Station.

The problem of demonstrating possible physiologic races in this organism has been approached by testing the pathogenicity of eleven different isolates of the fungus against nine sweet potato varieties whose relative reaction to the disease was known. Temperature relations and cultural characters of the various fungus isolates have also been investigated to supplement the results of the pathogenicity trials.

LITERATURE REVIEW

Taxonomy.- The stem rot disease of sweet potatoes was first described in New Jersey by Halsted (9) in 1890. Two years later Halsted (10) stated the causal organism was the fungus *Nectria ipomoeae* Hals. In 1914 Harter and Field (11) proved that *N. ipomoeae* was not the causal organism and demonstrated that the fungus involved actually consisted of two species, *Fusarium batatatis* Wr. and *Fusarium hyperoxysporum* Wr.

In 1935 Wollenweber and Reinking (32) after many years of investigation of the genus *Fusarium* proposed a system of classification in which the genus was divided into 16 sections which in turn were subdivided into species, varieties, and forms. In this revised system *F. hyperoxysporum* became *F. oxysporum* Schl. f. 2 Wr. and *F. batatatis* became *F. bulbigenum* Cke. and Mass. v. *batatas* Wr.

In spite of the careful reworking of the genus *Fusarium* by Wollenweber and Reinking in their monograph *Die Fusarien*, there was still considerable difficulty and confusion with regard to classification of the various Fusaria encountered in pathological investigations. Snyder and Hansen (24) recognized the taxonomic difficulties involved in dealing with species of the genus *Fusarium* and felt that a major cause of the difficulties arose from the capacity for variability within species and single spore lines. As a result of their work they presented a revised concept of species in which "All members of section *Elegans* constitute one natural species group which on the bases of usage and priority may be called *Fusarium oxysporum*. We emend this species to reflect the basic morphologic features of the whole section." They consider the Fusaria causing vascular wilts as biologic forms of this one species; the separation of these forms is based on their respective pathogenicities. According to this

system the causal organism of sweet potato wilt becomes F. oxysporum f. batatas.

Sherbakoff (21) objected to the classification of Snyder and Hansen on the basis that the variability observed by them was more imagined than real. He would retain Wollenweber's original binomials using form names for the pathogenic types based on physiologic differences.

Recently McClure (16) recognized two culturally distinct groups of F. oxysporum f. batatas which he designated as group I and group II. These groups correspond to the two species F. bulbigenum var. batatas and F. oxysporum f. 2 respectively. No pathogenic differences on sweet potato were noted between these two groups.

Miller (17, 18) accepted neither Wollenweber's nor Snyder and Hansen's classification. Miller found that there occurred in the Fusarium species he studied, wild types which were characterized by abundant aerial mycelium. Kept in tubes of moist, sandy-loam soil these wild types varied little, but repeated transfers to agar produced many variations which Miller stated were due to mutations. Therefore, a taxonomic system, as presented in Die Fusarien, depending upon repeated transfers until a maximum production of macroconidia is obtained, is claimed by Miller to be invalid. Miller has found Snyder and Hansen's merging of a whole section into one genus too drastic since it has not been shown that the variation of Fusarium species with which Snyder and Hansen worked occurs in nature as well as in culture. Miller advocated a system of classification based mainly on the morphologic characters of the wild types and presented a sample key for the specimens which he studied. There appears to be little reference in the literature to Miller's findings, but certainly they deserve further consideration. In any case, a taxonomic system based on the

morphology of wild types would take many years to formulate.

Since Wollenweber and Reinking's system requires special cultural practices and techniques with which few workers are familiar, and there occurs so much variation in the Fusaria, it appears that Snyder and Hansen's simplified classification will continue to be more widely adopted. This system however, will not be as simple to develop as originally proposed. Armstrong *et al.* (3) in 1942 investigated the phenomenon of cross infection among the following hosts: coffee-weed, tomato, watermelon, cowpea, okra, Burley tobacco, and flue-cured tobacco. Fusaria from tomato, watermelon, and cowpea infected only the hosts from which they were isolated. However, the Fusaria isolated from the remaining plants in the group exhibited definite multiple-host relationships. For example, one group of isolates from tobacco infected both Burley and flue-cured tobaccos; a second group of isolates from tobacco infected cotton, Burley tobacco and coffee-weed; while a third group of isolates from tobacco infected cotton, Burley tobacco, and okra. Armstrong and his co-workers present the following problem relating to Snyder and Hansen's classification: If the organisms with which they worked, from tobacco, were compared by inoculating the susceptible Burley variety, the organisms show no differences; if these same organisms were compared by inoculating both Burley and flue-cured varieties, they could be divided into two physiologic races of the tobacco wilt organism. However, if these same organisms which had been isolated from tobacco were compared by inoculating both types of tobacco and cotton, a more complex situation resulted. One group of isolates, on a basis of priority, would become *Fusarium oxysporum* f. *vasinfectum* (Atk.) Snyder and Hansen, the cotton wilt organism also pathogenic to tobacco; the remaining isolates would be the tobacco wilt organism

pathogenic to both Burley and flue-cured tobacco.

Smith and Shaw (23) conducted similar cross inoculation tests with sweet potatoes, Burley and flue-cured tobaccos, and cotton. They found that the following three races of _F. oxysporum_ could be set up: race 1 pathogenic to Burley tobacco and sweet potato; race 2 pathogenic to flue-cured and Burley tobaccos, and sweet potato; and race 3 pathogenic to Burley tobacco and cotton. Smith and Shaw have found merit in Snyder and Hansen's system but have enumerated the following necessary conditions before such a system could be used successfully: standardized inoculation technique, maintenance of virulent cultures, and standardized genetic resistance of test plants.

Snyder and Hansen have referred to biologic forms of the species _F. oxysporum_ as determined by single host relationships; Smith and Shaw, and Armstrong _et al_. have referred to physiologic races of this same organism on a basis of multiple-host relationships. These two concepts of physiologic races, developed only within the past ten years, should be distinguished from the concept of physiologic races as determined on the basis of varietal reactions within a single host. Physiologic specialization in the following section unless otherwise noted refers to the determination of races by varietal reactions within single hosts.

Physiologic Specialization.- Physiologic specialization in phytopathogenic fungi was first suggested by Schroeter in 1879 (_cf_. Stakman 25). From the beginning of the twentieth century numerous investigators have demonstrated the occurrence of this phenomenon in ever increasing numbers of fungi. Physiologic specialization has been noted in those _Fusarium_ species pathogenic to flax (6), small grains (26), and a number of vegetable crops. Those species found on vegetables are discussed further

because of their possible bearing on the present work.

Among the earliest workers on physiologic specialization in the Fusaria was White (31) who in 1927 studied 24 isolates of the tomato wilt fungus, Fusarium oxysporum f. lycopersici, which he had obtained from various parts of the United States. He was able to divide these isolates into two groups based on the severity of wilting in inoculation tests. Thus he showed that isolates from different localities may differ in virulence. Haymaker (14), a year later, working with one isolate from each of White's two groups and two saltations from the second isolate, corroborated White's results under more carefully controlled conditions. However, Haymaker felt that it would be of no advantage to establish forms of the tomato wilt organism since the isolates of the fungus displayed such a lack of stability and the variations in isolate virulence were uniform on all the host varieties tested.

Wellman and Blaisdell (29) studying a number of isolates of F. oxysporum f. lycopersici were able to correlate cultural types with degrees of pathogenicity, the fully raised type being most virulent. However, they made no attempt to distinguish physiologic races. Wellman and Blaisdell (30) also studied the variation in cultural characteristics and pathogenicity of their isolates by means of 2,031 single spore transfers. They found that the direction of variation in the fungus on laboratory media was generally toward the more appressed growth-type which was also generally less pathogenic. They therefore considered the raised type of culture to be more truly representative and to be the basic type of the tomato wilt organism. Armstrong et al. (4) found that the wilt organism of cotton, Fusarium oxysporum f. vasinfectum (Atk.) Snyder and Hansen, also varied in culture, generally toward a more appressed and less pathogenic type.

Alexander and Tucker (1) investigating physiologic specialization in the tomato wilt fungus found an isolate of the organism that caused wilting in an otherwise resistant variety of the host. This isolate was designated as a distinct race of F. oxysporum f. lycopersici.

Blank (5) worked with 19 monoconidial or hyphal-tip isolates of the cabbage yellows fungus, Fusarium oxysporum f. conglutinans (Wr.) Snyder and Hansen, obtained from various parts of the United States. In the pathogenicity tests he used homozygous susceptible and homozygous resistant lines of cabbage in addition to F_2 progenies from crosses of resistant x susceptible; subspecies of Brassica oleracea L. were also used. No evidence of varying pathogenicity was found in the isolates of the organism with which he worked. Further, the cultures did not vary significantly in such cultural characters as growth rate, color production or sporulation.

Sleeth (22) studied pathogenic differences in the organism inciting wilt of watermelons, Fusarium oxysporum f. niveum (E.F.S.) Snyder and Hansen. While he demonstrated the occurrence of physiologic specialization in this species on the basis of differences in virulence of isolates of the causal organism, he made no attempt to classify any isolate as a permanent race because of "...sudden dissociations." Sleeth observed variants of the fungus from soil some of which were similar to variants occurring in culture. He therefore suggests that dissociation occurs in the soil "...perhaps as readily as in artificial culture." Sleeth also studied temperature relations of his isolates but found no correlation with pathogenicity.

Watanabe (28), in Japan, investigated physiologic specialization in the Fusarium spp. involved in the stem rot of sweet potato. He studied,

in culture, 40 isolates in respect to the following: effect of different media on type of growth and spore production, effect of temperature on mycelial growth, and effect of hydrogen-ion concentration on mycelial growth. In addition, he studied the pathogenicity of the various isolates and determined the toxicity of the various cultural filtrates to sweet potato slips. Through the use of each of the 5 above enumerated criteria, Watanabe was able to distinguish groups within his 40 isolates. However, the groups as determined by any one criterion, e.g., temperature, bore no relation to the groups as determined by any of the other tests.

From the pathogenicity tests, Watanabe classified his 40 isolates into 6 groups. The reaction of each isolate on each of 13 sweet potato varieties was recorded as a percentage of infected plants. These percentages were then averaged for each isolate, and the averages arbitrarily divided into 6 groups, hence the 6 pathogenic groups. While these experiments indicate the possible existence of physiologic specialization in the sweet potato wilt organism, it is felt that more consistent host-varietal reactions would strengthen this point of view. As an example, 5 of Watanabe's 40 isolates produced average percentages of infection which fell within the highest infection group, 45-50 percent; however, these isolates did not consistently produce the greatest amount of infection on any one variety of the host.

Armstrong and Armstrong (2) demonstrated the existence of physiologic races in the organism, Fusarium oxysporum f. tracheiphilum (E.F.S.) Snyder and Hansen, inciting wilt of soybeans and cowpeas. They divided 31 isolates from both hosts into two distinct races. Race 1 caused wilting of both soybeans and cowpeas, race 2 from cowpeas infected only cowpeas. In addition to this distinction there were two varieties of cowpeas which

could be employed to distinguish the races. One variety was resistant to race 1 and susceptible to race 2, the other variety was susceptible to race 1 and resistant to race 2. Two such varieties present an ideal situation in distinguishing physiologic races on a basis of pathogenicity.

The presence of physiologic specialization has been definitely established by host-varietal reactions in certain of the phytopathogenic Fusaria, and, in some cases, definite races have been established on this basis. Physiologic races have also been demonstrated to exist through cross inoculation experiments, as in the sweet potato stem rot organism. One may also consider this latter example as a single race within the species *F. oxysporum* as Snyder and Hansen have done. In either case the point remains that physiologic specialization in the *Fusarium* wilt organism of sweet potato has not been adequately demonstrated on a host-varietal reaction basis. In other wilt Fusaria physiologic specialization has been demonstrated through varietal reactions within single hosts in the organisms pathogenic to flax, tomato, watermelon, and cowpea.

PATHOGENICITY TRIALS

Methods and Materials.- Eleven different isolates of the sweet potato stem rot fungus were used in the present investigation. These were obtained from various sources as noted in table 1. All isolates except those from Oklahoma were received as pure cultures on agar slants; those from Oklahoma were isolated from diseased sweet potato stems or roots.

A mass transfer of the fungus from each source was selected as a stock culture. In order to reduce to a minimum variation and mutation, the stock cultures were maintained on potato-dextrose agar slants covered with sterile mineral oil. The cultures were stored in a 15° C. chamber. These original stock cultures were used throughout the studies reported here.

TABLE 1.- Identification, source, and isolation date of each isolate.

Isolate	Source	Date Isolated
A.	California	2/13/46
B.	California	2/13/46
C.	Idabel, Oklahoma	10/25/49
D.	Alabama	11/18/49[a]
E.	Perkins, Oklahoma	10/18/49
F.	South Carolina	12/19/49[a]
G.	Perkins, Oklahoma	10/24/49
H.	Louisiana	4/12/50[a]
I.	Okemah, Oklahoma	1/31/50
J.	Alabama	11/18/49[a]
K.	Mississippi	4/12/50[a]

[a]Date received, isolation date unknown.

The nine varieties of sweet potatoes used in the pathogenicity trials and their reactions to the stem rot fungus, as determined by greenhouse and field trials conducted over the past several years, are as follows:

Oklahoma 25, L-241, and L-37 - resistant; Oklahoma 24, Oklahoma 29, and B-2934 - tolerant; and Nancy Gold, Unit #1 Porto Rico, and L-12 - susceptible. For each of the pathogenicity trials, vine cuttings of each variety were taken from sweet potato plants growing on trellises in the greenhouse. The cuttings were trimmed uniformly and rooted for two weeks in flats of steam-sterilized sand.

The pathogenicity of each isolate was tested by inoculating about 25 plants of each of the 9 selected sweet potato varieties and observing the amount of infection that was present in each variety after the plants had grown for 28 days in the greenhouse. Each such test has been designated as a trial. The testing procedure was adapted from the method[1] employed by the U. S. Department of Agriculture at Beltsville, Maryland for testing the stem rot resistance of sweet potato varieties in the greenhouse.

Because the number of cuttings available from the various sweet potato varieties at any given time was limited, the pathogenicity trials were set up at approximately weekly intervals. Exceptions to this were trials involving isolates C and D. These two isolates were tested concurrently in trials on February 14 and again on August 26. Each fungus isolate with the exceptions of A, H, J, and K, was tested at two different times in the course of this investigation. The trials extended over a period of about 10 months.

Inoculum for each of the fungus isolates was grown in 250 ml. Erlenmeyer flasks with 150 ml. of nutrient solution in each flask. Each liter of the nutrient solution contained sucrose, 10 gm.; $MgSO_4 \cdot 7 H_2O$, 5 gm.;

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[1]Correspondence from C. E. Steinbauer.

KH_2PO_4, 1 gm.; and NH_4NO_3, 1 gm. The flasks of medium were autoclaved as prepared and then seeded by direct transfer from the stock cultures. The nutrient cultures were grown at room temperature for 7 to 9 days and agitated once a day during this period. When the inoculations were to be made, the contents of one flask were filtered through two sheets of filter paper in a Buchner funnel with suction. The fungus growth remaining on the filter paper was washed with 300-500 ml. of water and refiltered. The filter paper and fungus tissue were then minced in a Waring Blendor containing 300 ml. of water. The resulting suspension was diluted to a total volume of 3 liters and was then ready for use.

Before inoculation, the roots of the plants were washed free of sand and cut back to between 1 and 2 inches; the callus at the base of each stem was trimmed. The purpose in injuring the plants was to provide uniform opportunity for infection. Previous work had demonstrated that unless the roots were injured the results of inoculation were likely to be erratic. The plants were inoculated by dipping the roots into the fungus suspension for one minute. All the plants of each variety were inoculated at the same time and then divided into groups of 5 plants each. These groups were planted immediately according to a completely randomized design. Control plants of the susceptible Porto Rico variety were treated in the same manner except that water was used in place of the fungus suspension.

The plants were grown in sand in a greenhouse bench for 28 days following inoculation (Fig. 1). The bench was divided into four 52 X 36 inch sections with a 4 inch space between sections. The sections were filled with sand to a depth of 8 inches. Before each trial, the section of bench to be used was swabbed with Clorox, diluted 1:20, and the sand was steam

Fig. 1. Inoculated plants of a single pathogenicity trial growing in the greenhouse bench.

sterilized 6 to 8 hours. Greenhouse care following inoculation included watering the plants, removing plants as they died, and regulating sand and air temperatures.

As reported by Harter and Whitney (13) the optimum soil temperature for the development of stem rot in sweet potatoes was 30° C. For various reasons it was not possible to maintain a constant sand temperature under the conditions prevailing at the time of the present investigation. An attempt was made, however, to maintain sand temperatures as near as possible to the above reported optimum. Daily records of both sand and air temperatures were obtained from self-recording thermographs. Sand temperatures were usually in the range of 17° to 32° C. although for brief periods they ranged from 13° to 34° C. During those periods when heat was available in the greenhouse, a more uniform temperature prevailed. Air temperatures ranged from 15° to 43° C. throughout the year.

At the conclusion of each pathogenicity trial, all the surviving plants were dug and the relative severity of infection in each plant determined. This was done by splitting each stem lengthwise and observing the amount of vascular discoloration. Each plant was then assigned an arbitrary class value on the basis of the extent of discoloration. The class values used were as follows: 0 - no discoloration or discoloration extending less than 1/8 inch, 1 - discoloration involving up to 1/4 of the exposed vascular strands, 2 - discoloration of from 1/4 to 1/2 of the exposed vascular strands, 3 - discoloration involving 1/2 to 3/4 of the vascular strands, and 4 - discoloration of the vascular strands for their total length. Dead plants removed from time to time during the course of the trial were placed in class 4. A diagrammatic scale illustrating the various class values used is shown in figure 2.

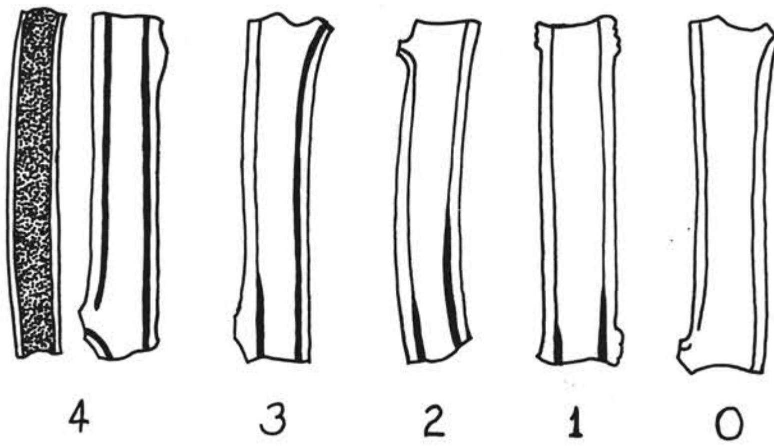

Fig. 2. Diagrammatic illustration of the class values (0-4) used in determining the severity of stem rot. (See text for explanation.)

After all the plants of a given trial had been examined, a disease index for each sweet potato variety was calculated. This was done by multiplying the number of plants in each disease class by the class value, adding the products thus obtained, and dividing this total by the number of plants originally inoculated. For example, if 20 plants of a given variety were graded in class 4 and 5 plants graded in class 3 the disease index would be

$$\frac{(20 \times 4) + (5 \times 3)}{25} = 3.80$$

Results.- The results of the pathogenicity trials are presented in table 2 as disease indexes representing the reaction of each sweet potato variety to each fungus isolate. The relative pathogenicity of each isolate is shown in table 2 as a trial mean disease index. However, as will be pointed out below, factors other than pathogenicity of the isolates have affected these values. Each trial mean disease index is an average of the 9 indexes which were obtained from testing one isolate at a given time against the 9 sweet potato varieties. Since 7 of the 11 isolates were tested in two different trials, two trial mean indexes are shown for each of these 7 isolates. Variety mean disease indexes are also shown in table 2 for each of the 9 sweet potato varieties. Each variety mean index is an average of the 18 disease indexes which were obtained from the reaction of the respective variety in each trial. The analysis of variance presented in table 3 shows that the differences between trial mean indexes are highly significant. This is true also of the variety mean indexes. Least significant differences have been included in table 2 as an aid in distinguishing differences between trial mean indexes and between variety mean indexes.

TABLE 2.- Reaction of sweet potato varieties, as indicated by disease index, to inoculation with various isolates of the stem rot fungus.

Date of Inocu- lation	Isolate	Sweet Potato Variety									
		L-12	PR[a]	OK[a] 24	L-37	NG[a]	OK[a] 29	B-2934	L-241	OK[a] 25	Trial Mean
		Disease Index	Disease Index	Disease Index	Disease Index	Disease Index	Disease Index	Disease Index	Disease Index	Disease Index	Disease Index
Jan. 20	A	2.60	3.00	1.19	0.15	0.36	0.17	0.36	0.35	0.04	0.91
Jan. 27	B	2.00	3.37	1.11	0.70	0.94	0.88	0.20	0.26	0.00	1.05
Feb. 14	C	4.00	3.38	1.37	3.19	1.72	0.44	0.44	1.84	0.27	1.85
Feb. 14	D	3.87	3.63	2.63	1.72	1.72	1.15	0.85	1.54	0.65	1.97
Mar. 4	E	3.40	3.40	0.56	0.55	0.75	0.48	0.12	0.45	0.35	1.12
July 20	F	3.56	2.04	1.40	0.84	0.40	0.72	0.72	0.76	0.28	1.19
Aug. 7	G	2.60	1.47	1.48	1.24	0.68	0.88	0.80	0.72	0.40	1.14
Aug. 16	H	3.36	3.40	2.16	1.32	1.76	1.32	1.08	0.96	0.88	1.80
Aug. 26	C	3.30	2.55	2.52	1.80	1.25	1.30	1.56	0.92	0.92	1.79
Aug. 26	D	3.40	3.00	1.84	1.88	0.90	0.85	1.52	1.00	0.64	1.67
Sept. 7	I	3.60	3.00	2.20	0.76	0.76	1.00	1.15	0.70	0.50	1.52
Sept.16	J	1.80	1.28	1.10	0.72	0.76	0.90	0.80	0.40	0.36	0.90
Sept.29	F	2.88	3.56	2.52	1.15	1.15	1.17	1.25	0.50	0.60	1.64
Sept.31	K	3.60	3.80	2.56	1.36	1.52	1.15	0.90	0.60	0.90	1.82
Oct. 6	E	1.96	1.80	1.80	0.96	1.08	0.60	0.55	0.05	1.30	1.12
Oct. 13	G	2.17	2.76	1.16	0.84	0.84	0.85	0.95	0.05	0.30	1.10
Nov. 1	I	3.55	2.70	1.56	1.30	1.35	1.14	1.00	0.25	0.56	1.49
Nov. 6	B	1.70	1.40	1.64	0.96	1.00	0.95	0.80	0.15	0.75	1.04
Mean index for varieties		2.96	2.75	1.71	1.19	1.05	0.89	0.84	0.64	0.54	

L.S.D. Values	19:1	99:1
For comparison of trial means:	0.300	0.398
For comparison of variety means:	0.424	0.561

[a]Unit #1 Porto Rico, Oklahoma, and Nancy Gold, respectively.

TABLE 3.- Analysis of variance of the pathogenicity test data of table 2.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value[a]
Total	161	164.5372		
Varieties	8	115.8910	14.4864	69.848
Trials	17	20.4444	1.2026	5.798
Variety x trials	136	28.2018	0.2074	

[a]Variety significance: F necessary at 5% point - 2.00, 1% point - 2.64.
Trial significance: F necessary at 5% point - 1.71, 1% point - 2.10.

The isolates were divided into three pathogenicity groups on the basis of their respective trial mean indexes and with the aid of the least significant difference for trial mean indexes; if an isolate was tested twice the two trial mean indexes for that isolate were averaged. The resultant groups are

Pathogenicity Group I		Pathogenicity Group II		Pathogenicity Group III	
Isolate	Trial Mean	Isolate	Trial Mean	Isolate	Trial Mean
C	1.82	I	1.51	E	1.12
D	1.82	F	1.42	G	1.12
K	1.82			B	1.05
H	1.80			A	0.91
				J	0.90

Considering that the differences between trial means are highly significant as shown in table 3, and the trial means within both Group I and Group III are relatively consistent, there is strong evidence that the differences between Groups I and III are highly significant. The differences between Groups I and II or Groups II and III are not as apparent and further testing might place isolates I and F in either group I or III. Differences between trial means presented the only basis for dividing the isolates.

The 9 sweet potato varieties used in the present tests were grouped in ascending order of resistance according to the variety mean indexes as follows: L-12 and Porto Rico, Oklahoma 24, L-37 and Nancy Gold, Oklahoma 29 and B-2934, and L-241 and Oklahoma 25.

Differences between the three pathogenicity groups of isolates and between the trial mean indexes cannot be attributed solely to differences between the isolates. The fact that all of the trials, except those involving C and D, were conducted at different dates should receive careful consideration. The factors, other than isolate differences, influencing

disease development and apparently due to running the trials at different dates have been collectively designated as the time differences. The effect of time differences between all the trials cannot be evaluated in relation to the effect of isolate differences. However, since isolates C and D were tested concurrently on February 14 and again on August 26, it was felt that an analysis of variance on the data concerning isolates C and D might give an indication of the significance of time differences in this particular case. This analysis is presented in table 4. Assuming that isolates C and D are similar (by referring to their trial mean indexes in Pathogenicity Group I) the analysis of variance shows that the time difference is not significant. The same is true of the interaction of variety and time. However, since the F value for variety x time does approach significance at the 5% level and the differences between disease index totals for varieties representing different times show considerable variation (Table 4), there is an indication that the interaction of time and variety is an important factor. The possibility that the 9 sweet potato varieties acted differently at different times is extended to include all trials in view of the following: the disease indexes for variety L-12 in the March 4th and October 6th trials involving isolate E equal 3.40 and 1.96, respectively; comparable indexes for variety Oklahoma 25 are 0.35 and 1.30 respectively (Table 2). Thus the index for L-12 was considerably less in the later trial while the index for Oklahoma 25 was considerably greater in the later trial. Examples of this type may be found throughout the pathogenicity trial data in table 2.

TABLE 4.- Reaction of sweet potato varieties, as indicated by disease index, to inoculation with two isolates of the stem rot fungus.

Date of Inoculation	Isolate	L-12	PR[a]	OK[a] 24	L-37	NG[a]	OK[a] 29	B-2934	L-241	OK[a] 25	Total
		Disease Index	Disease Index	Disease Index	Disease Index	Disease Index	Disease Index	Disease Index	Disease Index	Disease Index	
Feb. 14	C	4.00	3.38	1.37	3.19	1.72	0.44	0.44	1.84	0.27	
Do	D	3.87	3.63	2.63	1.72	1.72	1.15	0.85	1.54	0.65	
Total		7.87	7.01	4.00	4.91	3.44	1.59	1.29	3.38	0.92	34.41
Aug. 26	C	3.30	2.55	2.52	1.80	1.25	1.30	1.56	0.92	0.92	
Do	D	3.40	3.00	1.84	1.88	0.90	0.85	1.52	1.00	0.64	
Total		6.70	5.55	4.36	3.68	2.15	2.15	3.08	1.92	1.56	31.15
Grand total		14.57	12.56	8.36	8.59	5.59	3.74	4.37	5.30	2.48	

Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value[b]
Total	35	39.8104		
Subtotal	17			
Variety	8	33.6803	4.2104	
Time	1	0.2952	0.2952	1.823
Variety x time	8	2.9213	0.3652	2.256
Isolate C vs. D	18	2.9136	0.1619	

[a]Unit #1 Porto Rico, Oklahoma, and Nancy Gold, respectively.

[b]Time significance: F necessary at 5% point - 4.41
Variety x time significance: F necessary at 5% point - 2.51

TEMPERATURE RELATIONS

Temperature relations of the 11 Fusarium isolates were investigated with the objectives of determining whether the isolates could be grouped according to cardinal temperatures and whether possible temperature groups compared with groups separated on the basis of pathogenicity. Growth rates of the several isolates were determined by measuring the diameters of colonies on potato-dextrose agar plates at 48 hour intervals from the second to the sixth day inclusive. Petri dishes were seeded in triplicate for each isolate and for each temperature (15°, 20°, 25°, 30°, and 35° C.) with loops of standardized spore suspensions prepared from young transfers of the stock cultures. One loop of spore suspension was placed in the center of each dish.

The study of temperature relations indicated that there were no significant differences between isolates as to rate of growth and diameter attained after 6 days (Table 5), except for isolate J at 35° C. While all other isolates had a diameter of 0 to 6.3 mm. after 6 days at 35° C., J measured 31.3 mm. Of the temperatures tested, about 30° C. was the optimum for growth of all isolates. The temperature studies have afforded no basis for separating the isolates.

Cultural characters, such as growth types, substrate colors, and spore types were noted on the 6th day from the 30° plates. The growth types of the isolates ranged from appressed to fully raised, and the substrate colors ranged from white through various shades of lavender. These two characters bore no relation to one another nor to any other character. Figures 3 through 13 show representative colonies of each of the isolates grown at 30° C. All isolates except J produced an abundance of microconidia and very few macroconidia. The majority of spores produced by

isolate J were macroconidia.

TABLE 5.- Influence of temperature on mycelial growth of the sweet potato stem rot fungus on potato-dextrose agar plates after 6 days.

Isolate	Diameter[a] of colonies at stated temperatures (°C.):				
	15°	20°	25°	30°	35°
	mm.	mm.	mm.	mm.	mm.
A	19.0	41.5	76.3	81.0	0.0
B	6.7	40.0	70.7	81.7	0.0
C	12.7	39.7	72.0	76.3	4.3
D	19.3	42.7	70.7	71.3	5.0
E	11.7	40.0	81.7	86.3	6.3
F	10.7	48.7	83.0	90.0	0.0
G	11.7	39.0	76.0	79.3	6.0
H	11.7	44.7	77.0	75.7	0.0
I	-	-	68.0	77.0	5.0
J	7.3	32.3	62.0	71.3	31.3
K	15.3	44.7	74.7	76.5	3.3

[a]Average diameter of 3 replicates at each temperature.

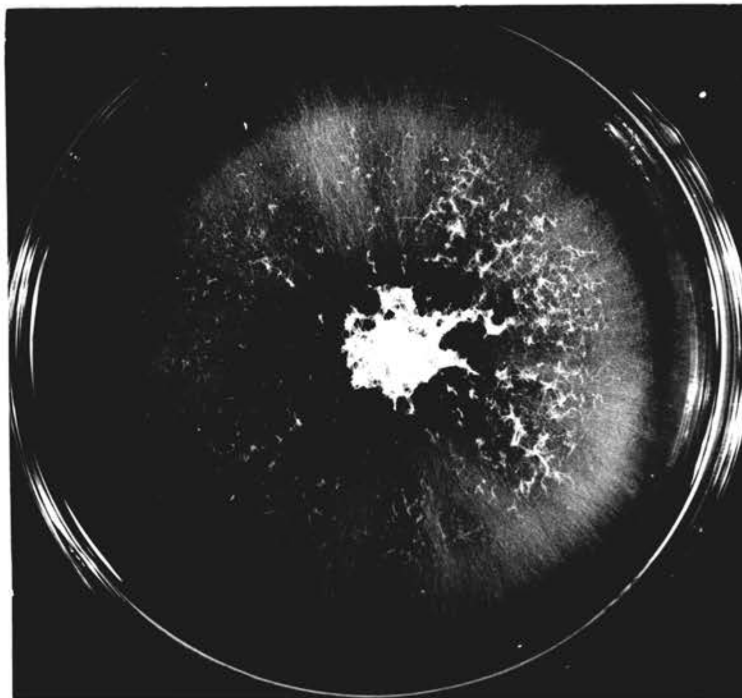

Fig. 3. Isolate A on potato-dextrose agar after 6 days at 30° C.

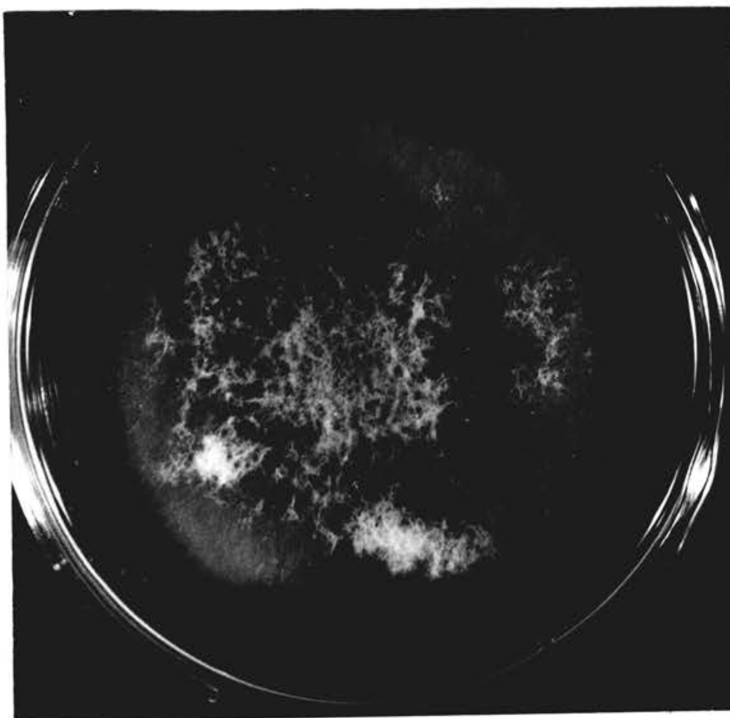

Fig. 4. Isolate B on potato-dextrose agar after 6 days at 30° C.

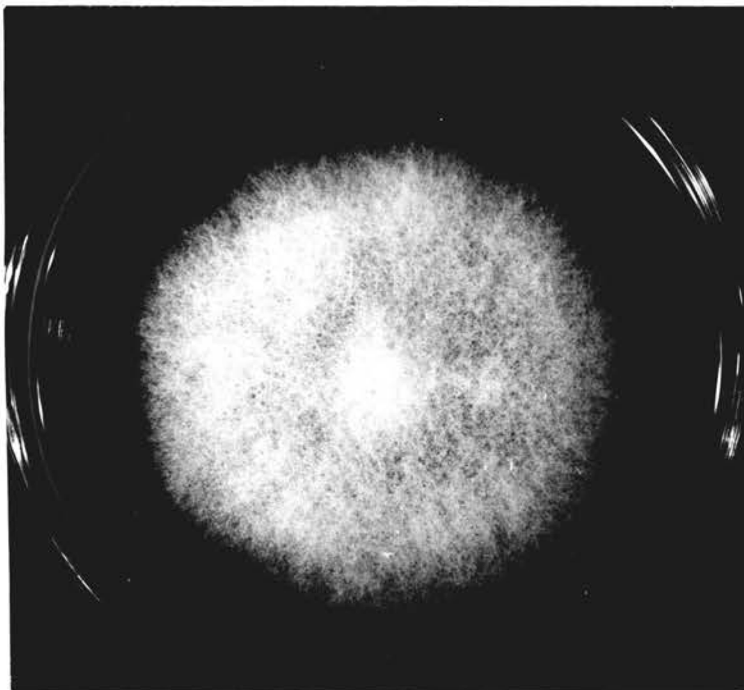

Fig. 5. Isolate C on potato-dextrose agar after 6 days at 30° C.

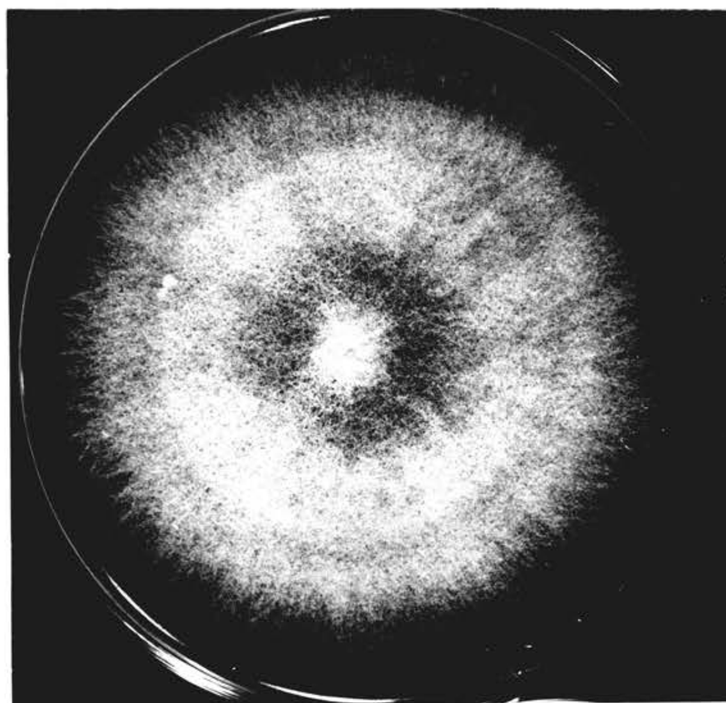

Fig. 6. Isolate D on potato-dextrose agar after 6 days at 30° C.

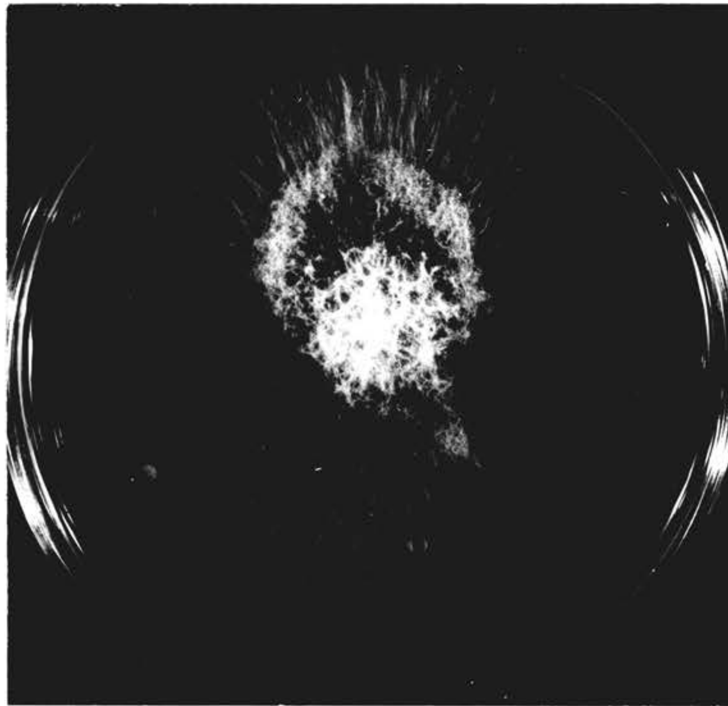

Fig. 7. Isolate E on potato-dextrose agar after 6 days at 30° C.

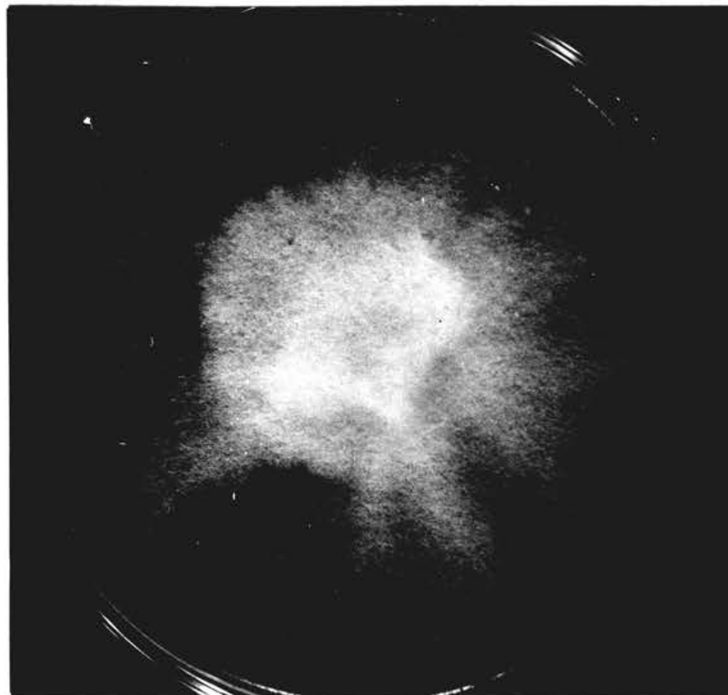

Fig. 8. Isolate F on potato-dextrose agar after 6 days at 30° C.

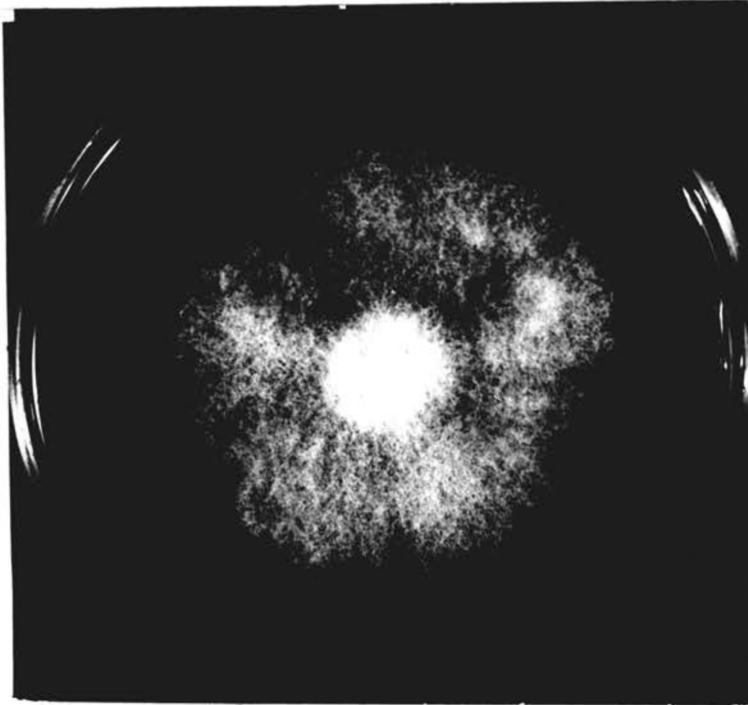

Fig. 9. Isolate G on potato-dextrose agar after 6 days at 30° C.

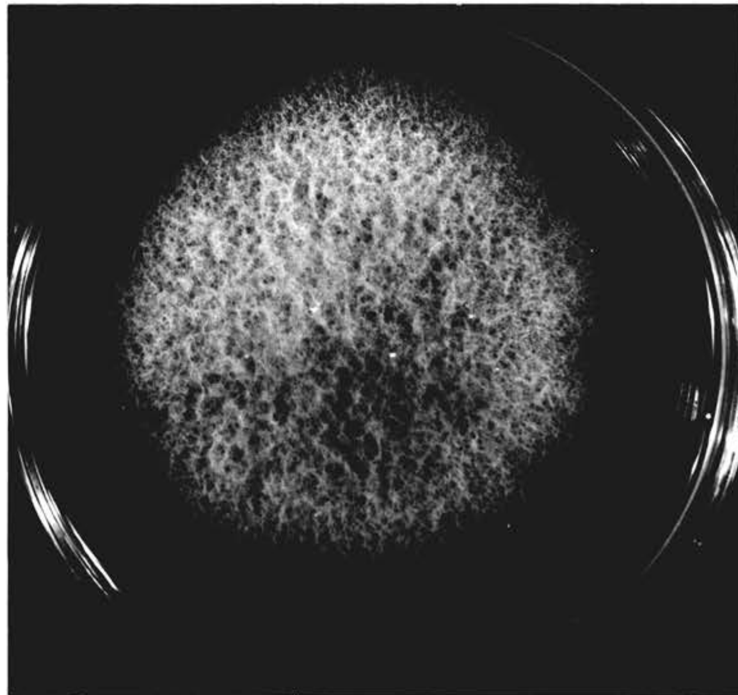

Fig. 10. Isolate H on potato-dextrose agar after 6 days at 30° C.

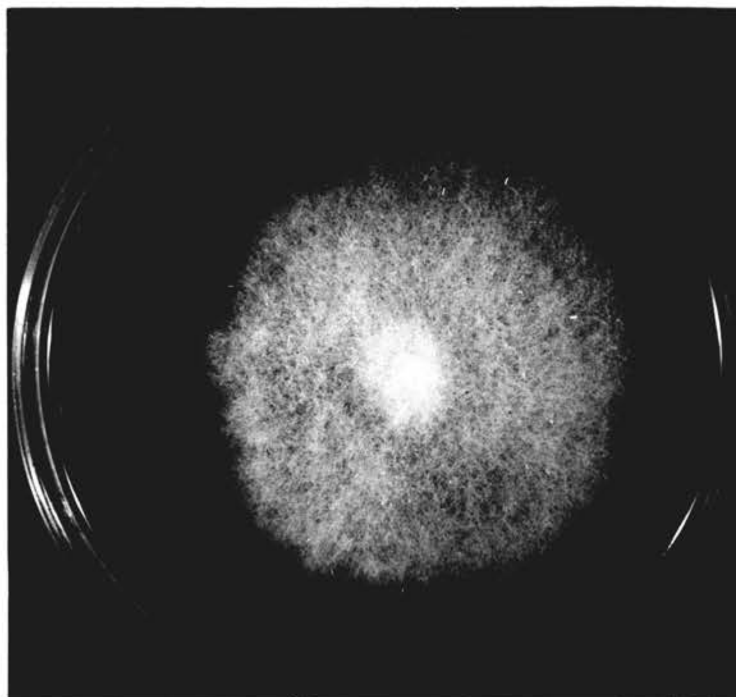

Fig. 11. Isolate I on potato-dextrose agar after 6 days at 30° C.

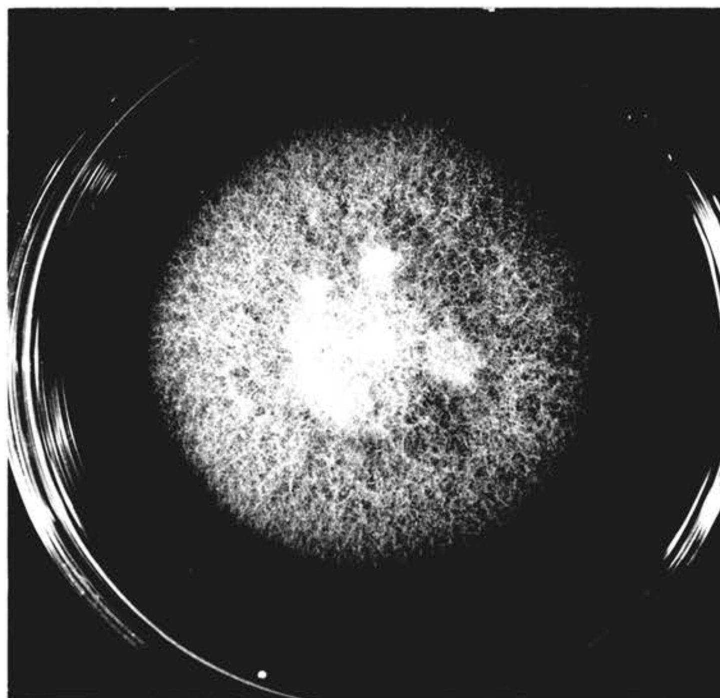

Fig. 12. Isolate J on potato-dextrose agar after 6 days at 30° C.

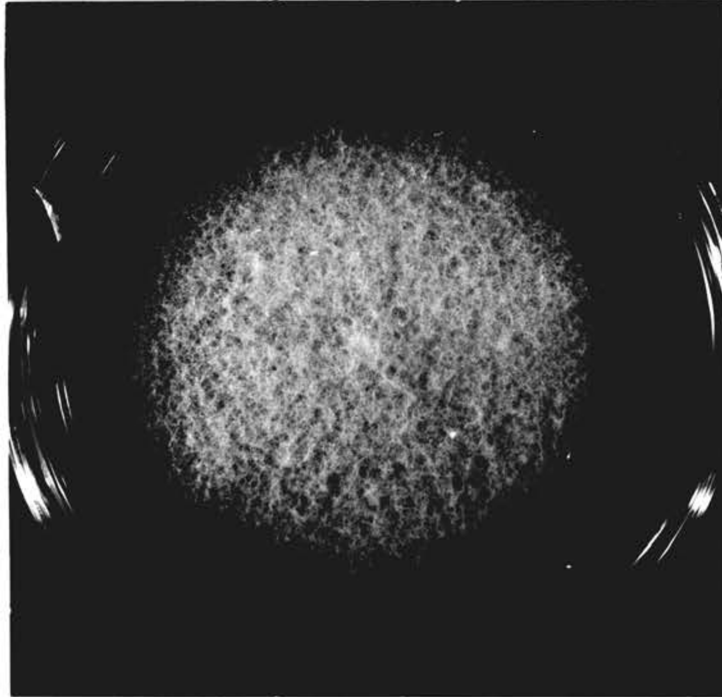

Fig. 13. Isolate K on potato-dextrose agar after 6 days at 30° C.

DISCUSSION

A knowledge of the existence or non-existence of physiologic specialization in the sweet potato stem rot organism is of value in the testing of newly developed sweet potato varieties. This point is of particular value if there is found a race of the organism which is unusually pathogenic toward one or several relatively resistant varieties or if one or more isolates are found which show unusually high pathogenicity toward all varieties. There is no evidence from the pathogenicity tests that such races exist among the eleven isolates tested in this investigation. However, neither is there conclusive evidence that races do not exist among the isolates tested since it was not possible to distinguish and evaluate the various factors contributing to infection and disease development. It seems likely though that if one or more of the isolates had been strikingly different in its pathogenic capabilities that this would have been revealed regardless of the time differences and other variables involved in the various pathogenicity trials. The observed differences in pathogenicity between isolates could be attributed to naturally occurring variation in virulence which was not sufficient, at least under the experimental conditions tried, to separate isolates or groups of isolates into physiologic races.

The temperature and cultural studies contributed nothing of significance to the pathogenicity tests. The one isolate J which exhibited an unusual amount of growth at 35° C. and produced macroconidia in abundance fell into the lowest pathogenicity group. It is not known whether this culture from Alabama is a mutation or is representative of the fungus at the time it was isolated.

The relative resistance of the 9 sweet potato varieties as deter-

mined in the present tests is similar to that determined in previous tests with two exceptions. The variety L-37, from previous tests, was considered resistant; Nancy Gold was considered susceptible. However, data from the present tests indicate that Nancy Gold and L-37 are of intermediate resistance and closely resemble each other in their reaction to the stem rot fungus. No explanation for this inconsistency is offered.

The confusion of isolate and time differences in these experiments clearly indicates that the method employed to test differences between the isolates in the greenhouse was not satisfactory. To eliminate the time difference completely would require testing the isolates simultaneously with adequate replication of both sweet potato varieties and fungus isolates. Such a method would be limited by available space. Further, it would be desirable to duplicate results of the tests. For these reasons it is deemed essential to know what the time difference is composed of and to evaluate its components in their relation to disease expression. The time difference is primarily composed of those factors predisposing the plant to disease, those producing variation within isolates, and those contributing to the environment at the time of the test.

The condition of the cuttings at the time of inoculation would be dependent upon environmental conditions affecting both the vines before the cuttings are made and the cuttings while they are rooting. No studies of this type concerning stem rot in sweet potatoes are known to the writer.

Variation in the Fusaria has been observed in culture by many workers. Mutation and segregation of genetically distinct nuclei from multinucleate cells have been suggested as the mechanism of the variation. Generally, cultural variation in the Fusaria has been toward a less patho-

genic type. Harter and Weimer (12) reported considerable loss in virulence of cultures of Fusarium hyperoxysporum after two years and complete loss of virulence after four years. In view of this evidence, the number of transfers of the isolates used in the present experiments was restricted and the stock cultures were covered with mineral oil to eliminate the necessity of further transfers. These conditions should provide a minimum opportunity for variation. It thus seems doubtful that differences observed in the pathogenicity trials could be attributed to cultural variation in the various fungus isolates. Miller (17) has suggested maintaining stock cultures in tubes of moist, sandy-loam soil. It would be of interest to compare Miller's method of storing cultures with the mineral oil technique in regard to changes in virulence.

The effect of environment on stem rot infection has been studied by Harter and Whitney (13). They have investigated the effects of soil temperature and soil moisture, but their studies were confined to one type of soil, one variety of sweet potato, and a limited number of temperatures over a wide range. Further studies on the effects of soil type, soil reaction, light, temperature, and availability of nutrients would be valuable.

It was noted earlier that the factors contributing to the time difference may cause sweet potato varieties, under varied conditions, to react differently from one another toward the stem rot fungus. Considering that resistance in sweet potatoes to stem rot is probably polygenic, the following statement by Walker (27) may explain this differing varietal reaction under dissimilar conditions: "As a rule, when resistance is polygenic in inheritance, it is relatively unstable in variable environment." In view of the above considerations there is an apparent need for evaluation of

the stability of varietal resistance under varying environmental conditions.

SUMMARY

The possibility of physiologic specialization in the sweet potato stem rot organism, Fusarium oxysporum f. batatas (Wr.) Snyder and Hansen, was investigated by testing in the greenhouse eleven isolates of the fungus, collected from various parts of the country, against nine sweet potato varieties whose relative resistance to stem rot was known. Temperature relations and cultural characters of the fungus isolates were also studied to supplement the pathogenicity tests.

None of the isolates exhibited an unusual type of pathogenicity; however, the isolates appeared to compose several groups characterized by different levels of virulence. The evidence obtained, though, is inconclusive since the effects of different isolates and the effects due to testing the isolates at different times are inseparable.

The temperature relations of all isolates were similar except for one isolate, J, which grew much more rapidly than any of the others at 35° C. This isolate was also distinguishable from the others in that it produced an abundance of macroconidia while all other isolates produced few macroconidia but many microconidia. Isolate J fell into the lowest pathogenicity group. The cultural types of the isolates were so diverse that no attempt was made to separate them on this basis.

In view of our inability to distinguish clearly the various factors contributing to disease development, it is suggested that the following studies be made and each factor evaluated as to its relative effect on infection: predisposition of the plants to infection; methods of maintaining stable fungus cultures; and environmental effects including soil type, soil reaction, light, temperature, and the stability of varietal resistance under varying environmental conditions.

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THESIS TITLE: VARIATION IN PATHOGENICITY OF THE FUSARIUM WILT ORGANISM OF SWEET POTATO

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THESIS ADVISER: F. BEN STRUBLE

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