DIFFERENCES IN GROWTH CHARACTERS AND PATHOGENICITY OF FUSARIUM WILT

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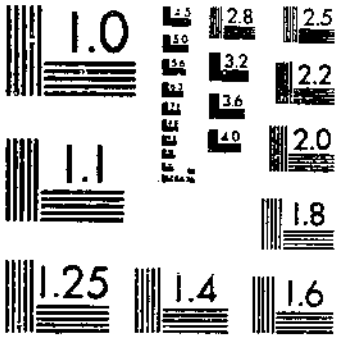

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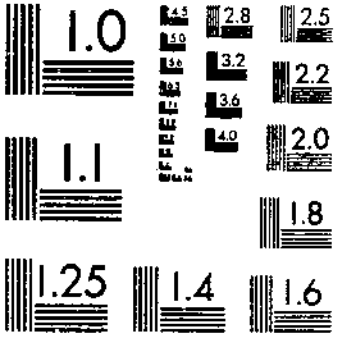

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Technical Bulletin No. 705 February 1940

UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

DIFFERENCES IN GROWTH CHARACTERS AND PATHOGENICITY OF FUSARIUM WILT ISOLATIONS TESTED ON THREE TOMATO VARIETIES[1]

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CONTENTS

INTRODUCTION

The fusarium wilt of tomatoes caused by *Fusarium bulbigenum* var. *lycopersici* (Brushi) Wr. and R., is probably the most important disease of this crop in the United States. It occurs in all the principal tomato-growing sections and is often found in greenhouses. Even with the widespread use of several wilt-tolerant tomato varieties, the losses from fusarium wilt probably reach one to two million dollars each year.

Fusarium wilt has been known for a long time, has been reported from countries in many parts of the world, and needs little description since it has been discussed and illustrated in numerous publications. Plants affected by the disease suffer from a yellowing and wilting of the foliage, which begins with the older leaves and eventually results in the death of the plant. When the stems of diseased plants are cut near the base, the water-conducting vessels show a brownish discoloration, which is very characteristic of the disease. Although it may be occasionally carried on the seed, the organism is chiefly a soil inhabitant. When plants are grown on infested soils, the fungus enters the plant through the roots and grows up through the vascular elements. The wilting that results from the fungus invasion is apparently due in part to some toxic principle secreted by the fungus, and perhaps in part to obstruction of the conducting tissues.

Tomato growers in some sections of the United States had, in the past, almost abandoned the crop until work by Federal and State agencies resulted in the development of varieties tolerant to the disease. The use of these varieties has been of the utmost value to the

[1] Submitted for publication May 22, 1939.

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tomato industry, and in general they have shown a fairly high degree of resistance. However, when grown in certain localities outside the region in which they were produced, some wilt-resistant or tolerant varieties occasionally have appeared to be relatively susceptible, although the same stocks have shown satisfactory resistance when tested in other regions under what appeared to be similar environmental conditions. Every care has been exercised to maintain the hereditary resistance of these varieties, but the suggestion is occasionally made that certain varieties are losing this valuable character. The purpose of this bulletin is to report on studies of differences found in *Fusarium bulbigenum* var. *lycopersici* that might explain the divergent results obtained with strains of wilt-tolerant tomatoes grown in some localities. An abstract of some of the results of this work has recently appeared (*25*).[2]

It is known that some of the species of *Fusarium* are particularly characterized by variability in culture, and the works of White (*26*), Leonian (*10*) and Haymaker (*7*, *8*) have already demonstrated this to be true of the tomato-wilt organism. Preliminary studies by Wellman (*24*) have confirmed these findings and it has seemed possible that the variability of the organism in culture might be correlated with differences in pathogenicity, a situation already suggested by the somewhat limited experimentation along this line reported by White and by Haymaker.

MATERIALS AND METHODS

For a number of years, the senior author has collected cultures of *Fusarium* isolated from wilt-diseased tomato plants from various regions of the United States, and in addition several pathologists have furnished cultures on request. From these, 127 cultures originating in about 35 localities have been retained wholly without regard to cultural appearance. For the present studies, 30 of the 127 isolates were picked at random, except for 2 that were retained for historical reasons (table 1).

A number of single-spore isolations were made from each of the 30 cultures. After a week's incubation on potato-dextrose agar, the prevailing type of fungus colony was noted and one typical of the group was arbitrarily picked out, given a selection number, and the related cultures were discarded. Thus each selection number designates a progeny of a distinct single-spore isolation from a separate isolate of a region. These numbers have been listed in table 1, with appropriate data regarding description and origin of the *Fusarium* selections they represent. The identity of the cultures dealt with in this bulletin was first based on the fact that all came from stems of tomato plants diseased with wilt, and that all caused wilt on reinoculation to tomatoes under controlled greenhouse conditions. Cultural and microscopic studies on 29 of the selections also proved they were *Fusarium bulbigenum* var. *lycopersici*. This identification was further supported for 19 of these selections that came from cultures, 1 or more of which had been previously designated as the tomato Fusarium wilt organism by one or another of the following workers: L. J. Alexander, Alice A. Bailey, W. Bohn, S. H. Essary, L. L. Harter, G. K. K. Link, W. S. Porte, F. J. Pritchard, O. A. Reinking, C. D.

[2] Italic numbers in parentheses refer to Literature Cited, p. 27.

Sherbakoff, Miriam C. Strong, F. Van Haltern, H. W. Wollenweber, and M. W. Woods. Isolations of widely variant forms were sent to Reinking who cultured and studied them and determined that they belonged under the *lycopersici* rank. As the work progressed, microscopic studies were made of all the selections and compared with the description of *F. bulbigenum* var. *lycopersici*, as given by Wollenweber and Reinking (*27*), and in addition, Reinking, on visiting this laboratory, examined all cultural material and notes and concurred with the opinion that 29 of the 30 selections were definitely the common tomato fusarium wilt organism. One wilt organism was found to be another species of *Fusarium*.

TABLE 1.—*Culture selections of tomato fusarium wilt isolations used to study variation in growth characters and pathogenicity* [1]

Selection or culture No. [2, 3]	State of origin	From whom obtained [2]	Isolator	Date of isolation	Remarks
1	Florida	F. L. Wellman	F. L. Wellman	1933	
2	California	S. P. Doolittle	S. P. Doolittle	1936	
3	Virginia	W. S. Porte	W. S. Porte	1920	
4	Oregon	S. P. Doolittle	S. P. Doolittle	1936	
5	Missouri	W. Bohn	W. Bohn	1934	From Bohn's No. 7.
6	Florida	F. L. Wellman	F. L. Wellman	1938	Reisolation of No. 1 from artificially infected Bonny Best.
7	Virginia	S. P. Doolittle	F. S. Beecher	1934	
8	Missouri	W. Bohn	W. Bohn	1936	From Bohn's No. 40.
9	Texas	P. A. Young	P. A. Young	1937	
10 [4]		G. K. K. Link	H. W. Wollenweber.	Prior to 1924.	Wollenweber's "#5001, *F. lycopersici* (Sacc.) Wr. tomato wilt."
11	Florida	F. L. Wellman	F. L. Wellman	1933	
12	Wisconsin	S. P. Doolittle	S. P. Doolittle	1936	
13	Missouri	W. Bohn	C. M. Tucker	1926	From Bohn's No. 11.
14	Georgia	F. Van Haltern	F. Van Haltern	1934	
15	Florida	W. S. Porte	F. L. Wellman	1937	
16	Ohio	L. J. Alexander	L. J. Alexander	1938	
17	Michigan	M. C. Strong	M. C. Strong		
18	do	do	do		
19	Utah	C. D. Sherbakoff	H. L. Blood	1935	
20	Tennessee	do	C. D. Sherbakoff	1936	
21	do	do	do	1936	
22	do	do	do	1936	
23	Indiana	V. Wright	V. Wright	1937	
24	Maryland	M. W. Woods	M. W. Woods	1936	
25 [5]	Indiana	V. Wright	V. Wright	1936	From darkened vascular's in fruit from wilted plant.
26	Michigan	F. Weiss	G. H. Coons	Prior to 1924.	Culture used at Madison, Wis., in fusarium conference. Bailey No. 168 F.
27 [1]	Tennessee	C. D. Sherbakoff	S. H. Essary	Prior to 1919.	
28	Oregon	do	H. P. Barss	1931	
29	Georgia	W. D. Moore	F. L. Wellman	1938	
30	Florida	G. D. Kolbert	do	1938	

[1] All culture selections originated from isolations from diseased tomato plants.
[2] Each cultural selection represents a single spore isolation progeny from the original culture in the senior author's collection (p. 2).
[3] These 30 cultures were from tubes selected at random from the collection of tomato *Fusarium* isolates.
[4] Included specifically because of historical interest. Wollenweber on a visit to this laboratory told the senior author that culture 10, his 5001, was an original isolate from Erwin F. Smith who cultured it many years before 1924.
[5] All cultures, with this exception, were found to belong to the wilt *Fusarium*, *F. bulbigenum* var. *lycopersici*.

The 30 selections were studied in culture over a period of 11 months. They were grown under laboratory conditions in parallel series, comparing characters and variations on several different media, including liquids, agars, and cooked plant tissues. The most obvious differ-

ences studied consisted of mat character, rapidity of growth, color developed, sclerotial formation, and spore production.

Three varieties of tomatoes were used for testing pathogenicity: two of *Lycopersicon esculentum* Mill., namely, Bonny Best, wilt-susceptible, and Marglobe, wilt-tolerant under most field conditions; and a strain of Red Currant [3] (*L. pimpinellifolium* Mill.) selected by W. S. Porte and known to be practically immune from wilt injury under very severe field conditions. All plants were grown in steam-sterilized soil and were approximately a month old before inoculation. The method of inoculation, with the definition of disease evaluations, is described and discussed in detail in another paper (*24*). In brief the inoculum consisted of a standardized water suspension of mycelial bits and spores from fusarium mats grown in a liquid medium for 5 days. Roots washed free from soil were dipped in the suspension and planted immediately in freshly sterilized soil in benches and soil pans, kept at about 26° C. by thermostatic control. Air temperatures were kept as near 28° as possible. After a period of about a week, which varied from 5 to 9 days depending upon intensity of sunlight and relative humidity, plants were removed from the soil, observed, and dissected, and each evaluated for intensity of disease symptoms.

Increasing amounts of disease invasion and injury were designated as follows: 0, no apparent infection; 1 and 2, first infection, browning of vessels in taproot; 3, 4, and 5, mild disease, infection confined to base of plants; 6, 7, and 8, serious wilting, darkened vessels extending into main body of stem from base nearly to tip; 9 and 10, very severe wilting; 15, dead at an early date, collapse of plant with darkened vascular elements extending the full length of the stem.

VARIATIONS FOUND IN CULTURE TYPES

Cultural differences have been studied in the tomato wilt *Fusarium* by other workers (*7*, *10*, and *26*). It was the aim of the present work (1), to study differences in virulence among *Fusarium* strains; and (2), to make, if possible, groupings according to cultural types and virulence. It was found that the cultures studied could be divided into five general classes, based on macroscopic characters of so obvious a nature that they could be grouped after the first series of cultural studies had been made. This easy grouping was developed incidental to the preparation of cultures for the comparative pathogenicity studies. Because this grouping proved such a convenience in the conduct of the entire study and is so closely correlated with the results on pathogenicity tests, its discussion here should furnish the reader a convenient point of orientation. In order to determine the range of variability as well as the constancy of the cultural characters of the 30 *Fusarium* selections used, a single-spore representative of each one was grown on many types of agar media.

Five different kinds of agar [4] were employed. There was considerable variation in the success with which these agars could be used.

[3] Seeds of this strain were supplied by W. S. Porte.

[4] Potato-dextrose agar: Potatoes cubed, 500 gm.; water, 1,000 cc., steamed 30 minutes, filtered through cotton, enough water added to filtrate to make up to 1,000 cc. Dextrose, 20 gm.; agar, 20 gm.; added, steamed 1 hour, put in tubes or flasks and sterilized 30 minutes at 15 pounds pressure. Acidified potato-dextrose agar: Potato-dextrose agar was melted and 150 cc. of 20-percent lactic acid solution was added just before the agar was poured into the Petri dishes. Prune agar: 200 gm. dried prunes, water 1,000 cc., steamed 30 minutes, filtered through cotton, enough water added to filtrate to make up to 1,000 cc.; 30 gm. agar added, steamed 1 hour, put in flasks and sterilized 20 minutes at 15 pounds pressure. Starch agar: Rice starch, 10 gm.; agar, 20 gm.; water, 1,000 cc.; sterilized 20 minutes at 15 pounds pressure. Malt agar: Malt extract, 25 gm.; agar 20 gm.; water, 1,000 cc.; sterilized 20 minutes at 15 pounds pressure.

On prune agar and malt agar, all cultures tended to be markedly appressed in growth character. On prune agar, colors [5] were either faint or almost entirely lacking, while on malt agar the color of all selections was uniformly dark, ranging around dark mineral red and deep purplish vinaceous. On starch agar, the mat characters were practically all the same, with occasional minor variations that were not consistent. On acidified potato-dextrose agar, growth was somewhat inhibited in comparison with the same medium nonacidified. In some cases colors were slightly more brilliant on the acidified agar than on the nonacid medium. However, no greatly significant changes in actual colors developed, and in some cultures the time for color appearance was somewhat delayed over that seen on nonacid agar.

The greatest differences in growth characters of *Fusarium* selections were observed on 14-day-old growth on potato-dextrose agar (table 2). Macroscopic examinations were made on 12 or more generations of each of these isolates grown in comparable series on potato-dextrose agar in Petri dishes, under identical conditions, during the same periods of time, in diffused light on the laboratory table, and at room temperature that fluctuated between 24° and 28° C. About 10,000 cultures were examined in connection with these studies.

TABLE 2.—*Macroscopic growth [1] characters of 30 culture selections of tomato wilt Fusarium [2] grouped according to culture classes*

[For descriptions of culture groups see pp. 6–8]

Culture group	Selection or culture No.	Type of mat growth	Aerial mycelium	Color [3]	Production of sclerotia	Sporodochia developed	Occurrence of striking saltation	Growth at room temperature
								Centimeters [4]
R	5	Raised	Compact cottony to felty.	White with ring of pale lavender violet.				7.9
	6	do	do	do			(5)	7.0
	17	do	Downy to felty.	White with trace of vinaceous purple at center.			+	6.4
	21	do	Compact cottony to felty.	White with ring of pale lavender violet.			(5)	7.5
RS	2	do	Woolly to cottony.	White with trace of vinaceous lavender.	+	+	+	7.5
	4	do	do	do	+	+	+	7.9
	7	do	Woolly	do	+	+	+	7.8
	9	do	do	White or pale salmon	+	+	+	7.9
	12	do	do	White with trace of vinaceous lavender.	+	+	+	7.7
	16	do [6]	do	do	+	+	+	8.2
	20	do	do	do	+	+	+	7.4

[1] Grown on potato-dextrose agar (30 cc. in Petri dishes 8.5 by 1.5 cm. inside measurements) 14 days at room temperatures 24° to 28° C., and in diffuse light.
[2] Single spore cultures obtained from isolations from diseased tomatoes, see table 1. Pathogenicity proved in all cases. All selections *Fusarium bulbigenum* var. *lycopersici* except No. 25.
[3] Color nomenclature according to Ridgway (*16*).
[4] Average diameter of 4 colonies, 7 days old.
[5] Saltation very infrequent.
[6] Raised condition somewhat reduced.

[3] Colors given according to Ridgway (*16*).

TABLE 2.—*Macroscopic growth characters of 30 culture selections of tomato wilt Fusarium grouped according to culture classes*—Continued

Culture group	Selection or culture No.	Type of mat growth	Aerial mycelium	Color[1]	Production of sclerotia	Sporodochia developed	Occurrence of striking saltation	Growth at room temperature
								Centimeters[2]
IR	11	Intermediate raised.[3]	Coarse	White and vinaceous purple.				7.1
	13	do	Coarse matted, cottony.	Vinaceous purple with white or vinaceous gray.				7.4
	14	do	do	do				7.1
	20	do	do	do				7.7
	23	do.[4]	Coarse cottony.	Vinaceous purple and white.				7.5
	26	do.[4]	do	do				7.2
	3	Intermediate appressed.	do	do				7.4
IA	10	do.[5]	Felty to cottony.	Vinaceous purple to white and vinaceous lavender.				7.6
	19	do.[5]	Compact felty.	Vinaceous purple or white with ring of pale lavender violet.				7.8
	22	do	Coarse cottony.	Vinaceous purple and white.				7.4
	26	do	do	do				7.4
A	1	Appressed.	Absent[6]	Vinaceous purple			(5)	7.7
	6	do	do.[6]	do			(5)	7.9
	15	do	do	Vinaceous purple and light buff.				7.5
	18	do	do	Vinaceous purple				7.8
	24	do	do[6]	Light buff to vinaceous purple.			(5)	8.1
	27	do.[7]	Absent to coarse cottony.	Vinaceous purple with white.				7.7
	30	Raised	Woolly	Medium: dark vinaceous drab or dark purple drab with band of neutral gray. Sclerotia: storm gray. Discolored agar: maroon	+		+	8.7
	25	Intermediate raised.	Abundant fluffy cottony.	Morocco red and white with mottling of Morocco red.			+	9++

[1] Color nomenclature according to Ridgway (*16*).
[2] Average diameter of 4 colonies, 7 days old.
[3] Saltation very infrequent.
[4] With appressed tendency.
[5] Occasionally completely appressed.
[6] In a few cases very scanty hyphae of cobwebby appearance growing on surface of appressed mat.
[7] With intermediate tendency.

The macroscopic characters are given for each *Fusarium* selection in table 2. The range in mat character was from slimy and completely appressed to fluffy and quite raised aerial growth. It appeared that practically all the gradations between these two extremes were present. There were, however, the five general, rather clear-cut groups or types of cultures spoken of above (see fig. 1) and herewith described:

Completely appressed, A: Mat slimy, appressed to agar, no fluffy aerial mycelium, translucent, forming a tough to moderately tough film over agar; usually vinaceous purple but ranging from light vinaceous gray to slate purple, sometimes light vinaceous gray, light vinaceous drab, or light buff; abundant production of macrospores and microspores on the slimy pionnotial surface.

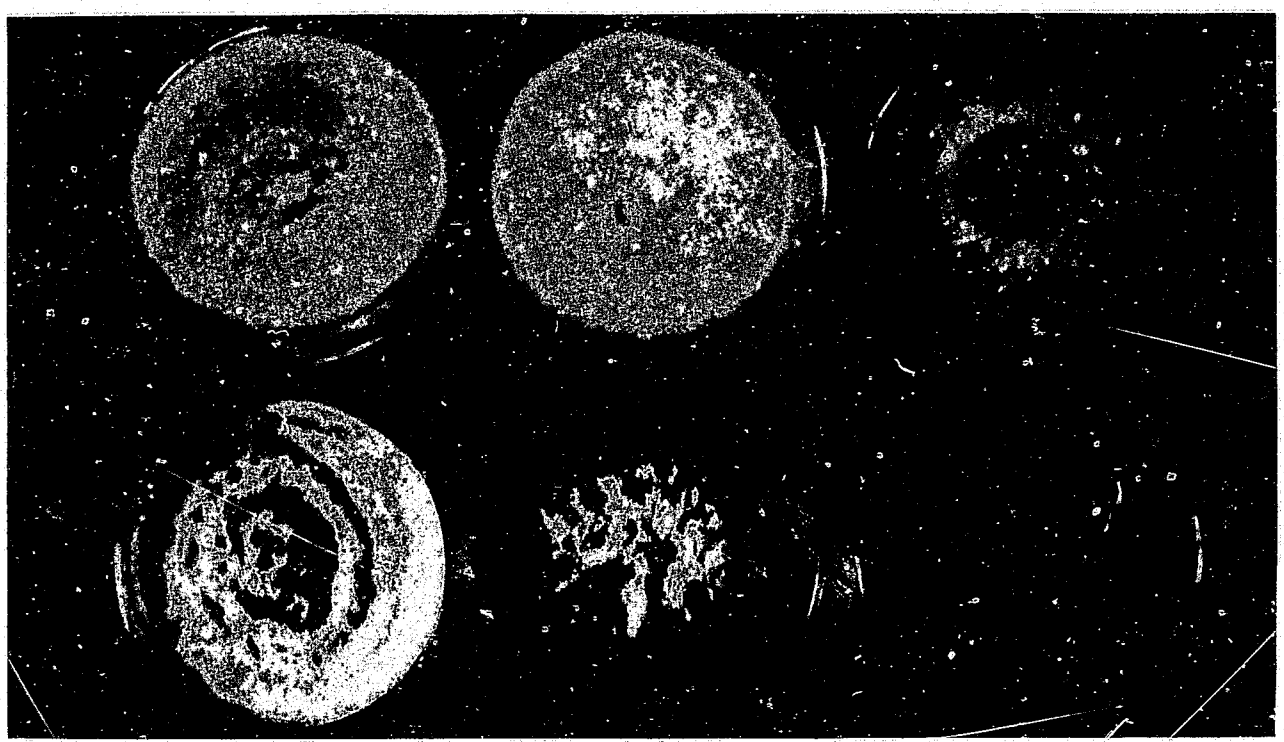

FIGURE 1.—Representative potato-dextrose agar cultures in Petri dishes (8.5 cm. inside diameter), of five types of *Fusarium bulbigenum* var. *lycopersici*. *A*, Fully raised (R), most virulent; *B*, *a*, and *B*, *b*, raised sclerotial (RS), next in virulence. *a*, Young culture showing sclerotial bodies; *b*, sclerotia less evident, buried in mycelium of older culture. *C*, Intermediate raised (IR). *D*, Intermediate appressed (IA). Both intermediate cultures of intermediate virulence, IA less pathogenic than IR. *E*, Completely appressed (A), least pathogenic. All grown at room temperature (24° C.) 14 days, except *B*, *b*, which was 7 days old.

Intermediate appressed, IA: Most of mat slimy, without aerial mycelium, translucent, forming tough to moderately tough film on agar, with only small amounts of aerial mycelium, appearing as fibrilous cottony tufts, narrow cottony zonate rings or patches or pulverulent areas; usually vinaceous purple but ranging in color about the same as the completely appressed cultures; raised growth white, pale vinaceous lilac or salmon; abundant production of macrospores and microspores.

Intermediate raised, IR: Mat next to agar mostly a tough appressed film, usually covered with matted aerial mycelium of coarse cottony, usually water-soaked, raised character; raised growth white, light vinaceous gray, or pale salmon, undermat usually light vinaceous gray but with considerable range of color as noted in the intermediate appressed class; abundant production of macrospores and microspores.

Raised sclerotial, RS: Undermat inconspicuous, mat mostly raised, woolly to coarse woolly, fragile, with sclerotialike clumps of mycelium and occasionally sporodochia occurring singly or in groups; raised mycelium white or with trace of light vinaceous purple, sclerotia cream color, sporodochia salmon, no cultures with any purples; sectoring of striking nature common in this group; spores not abundant except on sporodochia or in older cultures in which microspores appear on surface of woolly mat giving it a dusty or pulverulent appearance.

Fully raised, R: Mat raised, felty or compact cottony with slightly reticulate surface, margin always cottony; color, usually white with 1 or 2 cm. ring of pale lavender violet in central area of mat, sometimes pale lavender violet or light vinaceous gray often with small areas of white; abundant production of microspores on small pionnotial patches developing on surface of cultures 3 or more weeks old.

There are, of course, deviations from the characters described under the classes defined above. Of the 30 selections, 2 (table 2) were so markedly different from the other 28 that it was decided to treat them separately. One of them, No. 30, which was of the common tomato *Fusarium* wilt species, differed from all the rest in having a very dark-colored mat that was usually a dark vinaceous drab or dark purple drab with a wide central band of dark neutral gray. It produced storm-gray sclerotia and developed a maroon discoloration that diffused through the agar about the mycelium in the medium. The other anomalous selection, No. 25, was an isolate able to cause a wilt of tomato, but was not the common tomato *Fusarium* species. It produced a slimy appressed undermat with an abundance of rapidly growing, fluffy, loose cottony, aerial mycelium. The undermat was usually morocco red; the white aerial growth had a characteristic mottled appearance caused by patches of salmon, cinnamon, primrose yellow to etruscan red and begonia rose. This *Fusarium* grew about twice as rapidly as any of the other 29 isolates of *F. bulbigenum* var. *lycopersici*, and the aerial hyphae filled all the air space between agar and cover of the Petri dish within 5 days, whereas none of the 29 other selections developed nearly that much aerial growth.

When grown on cooked rice[6] the mat characters, with the exception of color, were, in nearly every case, the same as those produced on potato-dextrose agar. The colors were, however, more intense. Those isolates that produced vinaceous purple on potato-dextrose agar were livid brown or salmon on rice. Those which were white on the agar produced vinaceous rose to begonia rose on rice.

The character of growth of the isolates on certain cooked tissues, potato plugs, carrot plugs, twigs of locust, oat kernels, wheat heads, rye heads, and fresh tomato stems, was similar to that produced on the potato-dextrose agar. It was possible to distinguish appressed, raised, or intermediate types, or sclerotial development occurring on

[6] Uncoated rice kernels, by volume, 12 cc., water 37 cc.; water poured in bottom of Petri dish, rice distributed evenly over bottom, covered and placed level in autoclave, sterilized one-half hour at 15 pounds pressure. Rice flasks and tubes were prepared using same proportions of grain to water.

these media. Color, however, tended to be somewhat obscured, poorly developed, or even lacking in some cases.

Five liquid media [7] were used in this study of growth characters. All of the 30 selections were grown in all of these liquids, in parallel series under the laboratory conditions described on page 5. The relative differences in appressed and raised growth characterizing the isolates on agar were compared in these media and in all cases they were the same as described for potato-dextrose agar. Mat development, however, was poor in Brown's medium and in the soil extract. While mat development was fairly good in Richard's liquid, no colors were produced. In general, the relative amounts of fluffy aerial mycelium were somewhat less in liquids than on agar or other solid media. The most distinctive differences were obtained in Tochinai's liquid. Color and mat characters developed clearly and almost as well in this liquid as on potato-dextrose agar.

OCCURRENCE OF SALTATIONS IN CULTURES

A common character of *Fusarium* cultures is the spontaneous occurrence of areas, in otherwise apparently homogeneous colonies, that seem to be distinctly different in character from the growth of the original colony planted on the medium. These are most readily observed on agar or in liquid culture but are demonstrable to some extent on cultures on plant tissues as well. These areas are strikingly notable as sectors in an agar colony, although at times the saltations [8] take other forms, such as aberrant strands of wiry, aerial mycelium; water-soaked, oily spots of irregular shape toward the center of the mat; or an occasional fluffy mound of raised growth.

Saltations generally occurred in all classes of cultures of the tomato *Fusarium*, with what appeared to be varying degrees of frequency. Relatively speaking, the most stable forms were the so-called completely appressed (A) and fully raised (R) types. Saltation was active in the intermediate-appressed (IA) and intermediate-raised (IR) cultures, but was most striking in appearance and consistency of occurrence in the so-called raised-sclerotial (RS) class of *Fusarium* cultures.

In completely appressed cultures, visible saltations were often absent for a number of cultural generations. At times, however, what appeared as a fluffy mound of mycelium would be produced which, when transferred, resulted again in a slimy appressed mycelium deviating, however, in some ways from the original appressed culture. In distinctly raised cultures, a few saltations resulted that were of an intermediate-raised class. Intermediate-appressed and intermediate-raised types of cultures produced sectors of appressed or coarse fibrilose or cottony growth, which upon transferring developed colonies tending towards the appressed and low intermediate characters.

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[7] Brown's solution: Glucose, 2 gm.; asparagin, 2 gm.; tripotassium phosphate, 1.25 gm.; magnesium sulfate, 0.75 gm.; water, 1,000 cc. Leonian's solution: Proteose peptone, 5 gm.; dihydrogen potassium phosphate, 1 gm.; magnesium sulfate, 1 gm.; maltose, 25 gm.; and water, 1,000 cc. Richard's modified solution: Sucrose, 50 gm.; potassium nitrate, 10 gm.; dihydrogen potassium phosphate, 5 gm.; magnesium sulfate, 2.5 gm.; water, 1,000 cc. Soil-extract solution: 100 gm. air-dry sandy loam in which tomato *Fusarium* occurred naturally, 1,000 cc. cold water. Decanted clear liquid after 24 hours, added water to make up to 1,000 cc. Added 30 gm. of maltose. Tochinai's solution: Peptone, 10 gm.; monopotassium phosphate, 0.5 gm.; magnesium sulfate, 0.25 gm.; maltose, 20 gm.; water, 1,000 cc.

[8] The exact nature of the factors causing these variations is still a matter of conjecture. It is believed that the general term "saltation" or "saltant," as used by Stevens (*18*), is best to apply in this case where segregations are observed that may be due to mutations, nuclear fusions, or even from some cytological phenomenon as yet unknown.

The raised-sclerotial class of cultures appeared to be the most unstable. Sectors often appeared in the otherwise even, raised, woolly growth with its scattered clumps of sclerotial bodies. Growth in these sectors was, variously, more coarse and raised than the parent mat, more depressed with a water-soaked felty appearance, intermediate appressed and raised, or completely appressed and slimy with or without powdery aerial growth. Transfers from all these saltant types of growth yielded appressed forms or forms intermediate between the raised and completely appressed. In some cultures, transfers were made from random portions of what appeared to be a homogeneous mat, and intermediate and appressed forms of the *Fusarium* were cultured from these transfers. This sort of subculturing was done repeatedly, and in many cases variations were demonstrated which had not been visible in the original mat from which the transfers were made.

Intermediate types of saltants obtained from otherwise raised cultures often continued to produce variant sectors, always apparently of a more appressed sort than the mycelium from which they originated. In some cases by successive culturing and selection, completely appressed forms were derived from originally raised cultures, once saltation was found to occur.

Some care is necessary in transferring appressed saltants, since in certain cases small sectors of the original raised parental type of growth may still be present. This might give the impression that the appressed transfers can produce saltants returning the culture to its originally raised condition. This, however, was not observed in these studies. Once an appressed saltant is fully purified this more appressed character seems to remain nearly constant with any deviations tending toward a lower-growth type.

In these studies it was also found that great care must be exercised in transferring inocula of raised forms lest the intermediate or appressed saltants be carried on as the sole representatives of the selections originally known to be more raised. The resulting danger of erroneous interpretation of the behavior of successive cultures of some of the more unstable types of the tomato *Fusarium* is obvious.

VARIATIONS IN PATHOGENICITY OF FUSARIUM SELECTIONS

DIFFERENCES AMONG FUSARIUM CULTURES

Gross observations have been made on differences in virulence of cultures of the tomato *Fusarium* in such work as that of White (*26*) and Haymaker (*7*, *8*). Previous note of such variation also was suggested by Clayton (*4*). In the past, differences in extent of infection or degree of injury in tomato by *Fusarium* could be indicated only as rough approximations, because no suitable technique had been developed for insuring comparability among tests or even among reactions of different forms of *Fusarium* within a test. The methods described on page 4 were developed to reduce "error" to a level low enough to justify consideration of relatively small differences as significant.

It should be noted that the 30 *Fusarium* selections were grouped according to cultural characteristics incidental to preparations for determination of relative pathogenicity. The 5 basic groups were defined above. Of the 28 selections used most in these studies, 6

were classified as completely appressed cultures, 5 as intermediate appressed, 6 as intermediate raised, 7 as raised sclerotial, and 4 selections were considered of the fully raised type of growth.

Judging disease reactions on Bonny Best as a standard susceptible host, marked general differences occurred in severity of infection and injury caused by inoculation with 28 *Fusarium* cultures of the various described culture classes. Data were taken on inoculated plants after they had been grown about a week in sterilized soil held at 26° C. and at an air temperature of around 28°.

In the first series of pathogenicity tests, all cultures designated as raised had caused, except in a very few instances, the most severe and rapid disease development. This consisted of a darkening of all vascular elements the full length of the plant, and early wilting and collapse of these plants. The most marked contrast with the raised group was produced by cultures of the completely appressed type. Most of the plants inoculated with these latter cultures were very mildly diseased, although occasional plants were more severely affected. The intermediate-appressed and intermediate-raised types of cultures produced fairly consistent, mild disease symptoms that were in general somewhat more serious than those produced by the completely appressed cultures, and at the same time considerably less severe than those caused by the fully raised cultures. The so-called raised-sclerotial forms of *Fusarium* were generally of the more virulent sort, yet the disease effects were erratic, varying from very mild to severe among the seven selections in the group.

Such pathogenicity studies were repeated under the partly controlled greenhouse conditions 5 to 11 times, using all 30 selections, and results were obtained during 4 different seasons of a year. In all these tests, parallel series, inoculated with the 28 selections in 5 different cultural groups of *Fusarium*, showed group and selection differences in disease intensity comparable to those noted in the first series just discussed (fig. 2).

For a general comparison of the degrees of injury produced by each of 28 isolates of *Fusarium*, the averages of all individual plant-disease evaluations on three varieties of tomatoes inoculated with each selection were computed and are presented in table 3 and figure 3. It can be seen from these data that the average values for plant disease reaction to the several *Fusarium* selections can be grouped roughly according to the 5 culture classes with the exception of the raised-sclerotial group. The table is so arranged that the culture group with the most completely raised character of growth is listed at the top, the group with most fully appressed character at the bottom, and the intermediate types listed between in descending order, with regard to appressed condition of the culture growth. The magnitudes of the mean disease indexes of plant-disease evaluation for the groups are definitely larger, beginning with results from the most fully raised group and decreasing successively through to the most completely appressed.

It is of interest to first consider in some detail the disease indexes for the different culture groups from the most susceptible and sensitive tomato, Bonny Best.

The first group of cultures designated as fully raised produced an average disease evaluation of 10.39, indicating a plant that would be fully wilted, beyond the stage from which it could recover even if

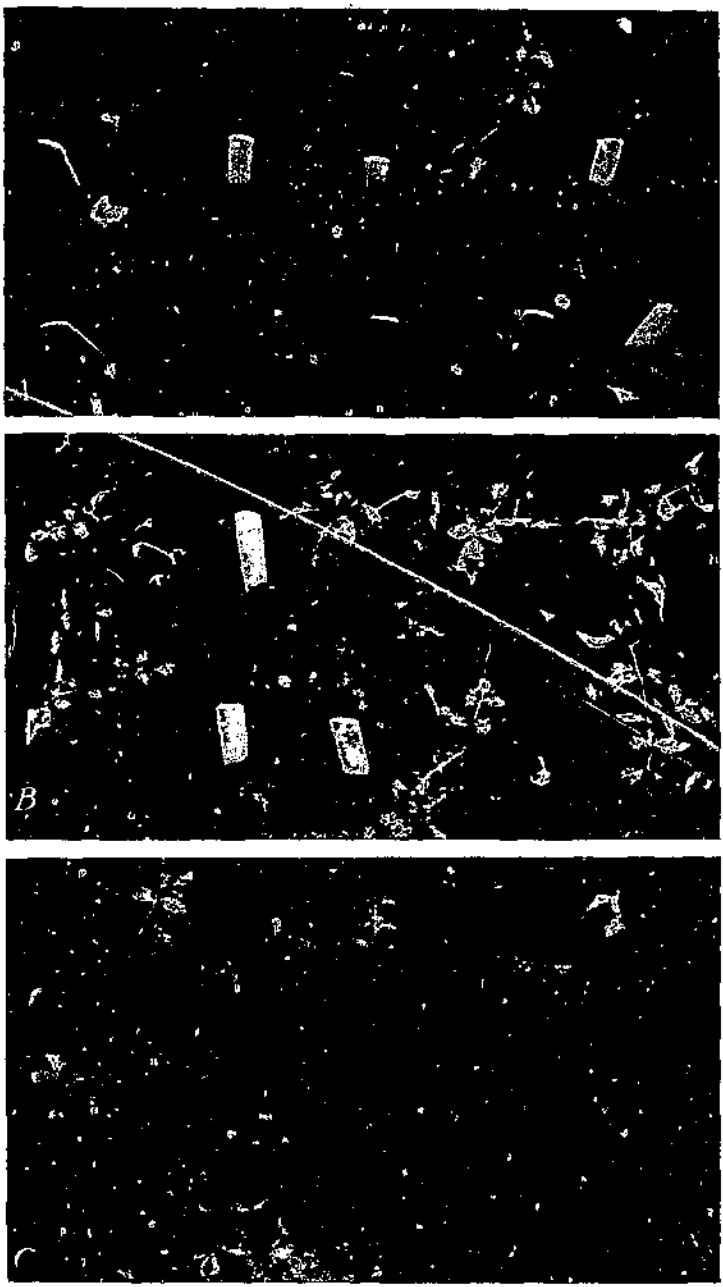

FIGURE 2.—Bonny Best tomato plants 6 days after inoculation with cultures of the fusarium-wilt organism, in sterilized soil held at 26° C., air temperature about 28°. *A*, Inoculated with fully raised (R) culture; 8 plants of 10 dead, remaining 2 wilted on seventh day. *B*, Inoculated with intermediate raised (IR) culture; 3 of 10 dead, 1 wilted, remaining 6 mild to serious infection on seventh day. *C*, Inoculated with completely appressed (A) culture; no plants dead or wilted, all mildly infected on seventh day.

TABLE 3.—*Variations in disease intensity as represented by average plant disease evaluation (pathogenicity) caused by selections of tomato-wilt Fusarium*

[For descriptions of culture groups, see pp. 6-8]

Culture group	Selection or culture No.	Bonny Best				Marglobe				Red Currant			
		Experiments	Total plants	Average pathogenicity evaluation		Experiments	Total plants	Average pathogenicity evaluation		Experiments	Total plants	Average pathogenicity evaluation	
				Per selection	Per class			Per selection	Per class			Per selection	Per class
		Number	*Number*			*Number*	*Number*			*Number*	*Number*		
R	5	11	133	11.32	10.39	6	81	9.12	7.50	4	46	1.74	1.42
	8	8	90	11.52		5	71	8.83		3	35	1.51	
	17	7	73	8.56		4	65	4.69		2	20	1.15	
	21	6	68	8.96		3	40	6.43		1	10	.10	
RS	2	11	131	7.49	8.67	6	81	6.10	6.25	4	46	.98	.92
	4	8	98	9.10		4	46	6.63		2	26	.23	
	7	9	116	10.81		4	46	6.94		2	26	.77	
	9	8	96	9.17		4	46	6.09		2	26	.69	
	12	9	100	8.40		5	71	5.01		3	37	1.32	
	16	8	84	8.27		4	65	7.97		2	20	1.35	
	29	7	73	7.00		4	65	5.32		2	20	1.00	
IR	11	9	101	8.72	8.34	5	68	5.09	4.45	3	36	.09	.48
	13	9	126	8.49		5	61	4.03		2	26	.12	
	14	8	96	7.83		4	46	4.74		2	26	.50	
	20	6	63	7.86		4	65	5.02		2	21	.86	
	23	5	58	8.47		3	40	4.15		1	10	.20	
	26	5	58	8.59		3	40	3.68		1	10	.10	
IA	3	9	111	7.10	6.31	6	81	4.93	4.09	4	45	.44	.45
	10	10	121	5.99		5	71	3.24		3	36	.61	
	19	7	88	5.65		3	40	2.50		1	10	.00	
	22	6	78	6.00		3	40	3.13		1	10	.10	
	28	7	83	6.72		4	65	5.54		2	20	.55	
A	1	9	106	5.02	4.69	5	55	3.31	2.99	3	35	.60	.52
	6	9	112	5.13		6	81	3.70		4	46	.50	
	15	10	121	3.84		5	71	1.80		3	36	.42	
	18	7	73	5.30		4	65	4.06		2	20	.75	
	24	8	92	3.90		4	65	2.11		2	20	.55	
	27	6	77	5.30		3	40	3.21		1	10	.10	

environmental conditions were very favorable for rapid growth, with darkened vessels extending from the roots of the plant to the apical bud. A plant of this character in the field would be designated as dying from wilt.

The raised-sclerotial group of cultures produced an average disease index of 8.67, which is very close to, although a shade higher than, the average secured from the group of the intermediate-raised cultures. This figure, however, indicates only roughly the true state of disease production by individidual selections within the raised-sclerotial group of cultures. At times many of these cultures were considerably more vigorous in disease production than any intermediate-raised

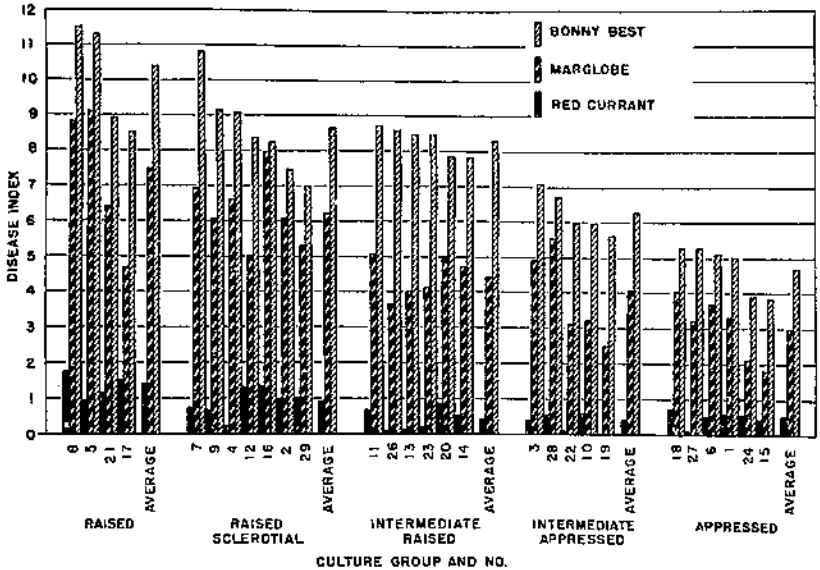

FIGURE 3.—Diagram of average plant disease evaluations (disease indexes) on three tomato varieties, produced by individual fusarium-wilt cultures grouped according to growth character in culture, in descending order of pathogenicity. Note that with the exception of certain individual pathogenicities in the raised sclerotial cultures, there is no "overlap" obtained from the cultural groups on the more sensitive Bonny Best tomato variety. The same general trend of pathogenicity, one group with another, is seen in the Marglobe, and to a lesser degree in the Red Currant data. Compare with table 3.

culture, and on the other hand, some produced at times considerably lower disease evaluations. The raised-sclerotial group was apparently the most unstable, or at least produced the most variable results among successive tests.

The intermediate-raised group of cultures caused severe disease resulting in average evaluation of 8.34, which may be characterized by a plant having a few tip leaves healthy, most of the lower leaves and those on the main body of the stem shrivelled, dropped, or chlorotic and wilted, and darkened vessels in the stem extending from the roots to within a few nodes of the tip. In the field such a plant would probably produce no marketable fruits, even when this degree of damage is shown after fruit setting.

The average disease evaluation produced by the intermediate appressed group of cultures was 6.31, representing a plant less seriously diseased than the last one mentioned. The lower leaves would be dropped or wilted and chlorotic, and the stem would have darkened vessels extending to about half way between the cotyledonary node and the growing tip. It is believed that a vigorously growing plant with fruit well set, and that is no more severely diseased than this description indicates, will probably produce ripe fruit in the field, under favorable conditions, although yield would be somewhat reduced with perhaps fruits of small size.

The mean disease evaluation resulting from the group of completely appressed cultures was 4.69, representing a mildly affected plant with the lowest leaves likely to be very slightly chlorotic, and with darkened vessels in the root and base of the stem. Standing in the field such a plant would generally pass as healthy. If this degree of infection did not become evident until after fruit setting, there is probability that it would bear a normal crop under favorable growth conditions.

A study of the data from the more wilt-tolerant Marglobe tomato reveals that it indicated a considerable range of virulence among culture groups. It is significant that average disease evaluations for the culture groups, although considerably lower than in Bonny Best plants, occur again in the same regular descending order of severity, ranging from the high produced by fully raised cultures to the low produced by completely appressed cultures.

No practically important degree of pathogenicity was demonstrable for any of the 30 *Fusarium* selections on Red Currant tomato after several tests. Major attention was therefore devoted to more thorough studies with varieties of tomato that were more evidently susceptible to *Fusarium* invasion and injury. For this reason the number of plants involved in studies on Red Currant tomato were not so extensive as with the other two varieties. The data show, however, that in Red Currant the fully raised cultures of *Fusarium* produced more disease symptoms than any of the other cultures, but even these were very mild and of no practical importance.

It has been pointed out that in pathogenicity tests certain plants of Bonny Best, Marglobe, and Red Currant appeared healthy. Roots and bases of stems of apparently healthy plants of all three varieties were cultured to determine whether they were infected. Many such reisolations were performed, and in every case if the apparently healthy tissues were taken from points low enough in the true root region, the pathogen was recovered. Moreover, this recovered organism, when cultured further, invariably proved to be the same in every characteristic as the culture that had been used originally for inoculum. With obviously diseased plants the culture types could be reisolated at will from the darkened tissues of stems or petioles of plants inoculated with cultures of any particular type.

Apparently, the healthy-appearing Bonny Best plants had become invaded a little later than other plants in the same test, and the disease had not had time to produce symptoms. It should be borne in mind that these tests were of short duration and a few more days would have made a marked difference in the appearance of such Bonny Best plants inoculated with a virulent form of wilt. The Marglobe, however, showed some true tolerance and Red Currant was

practically immune from damage, although the *Fusarium* could always be isolated from their tissues in the root region.

Table 4 and figures 4, 5, and 6 present distribution "curves" of percentages of plants in each disease evaluation class for Bonny Best,

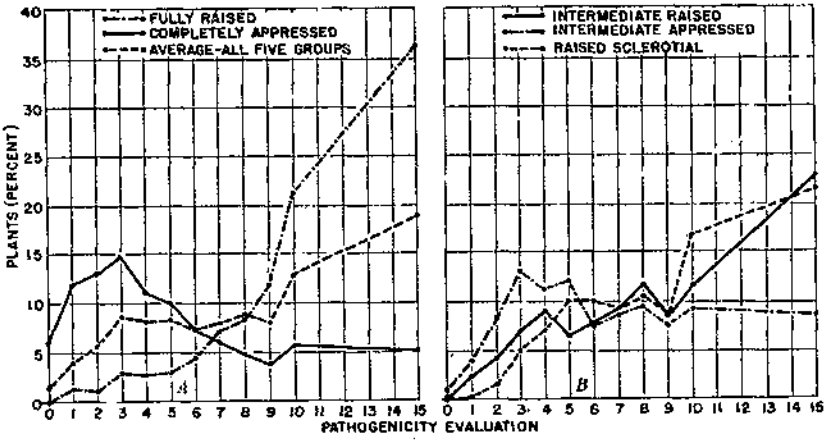

FIGURE 4.—*A*, Frequency distribution of pathogenicity (plant-disease) evaluations on Bonny Best, as the result of attack after inoculation with cultures of wilt *Fusarium* of fully raised and completely appressed culture groups, compared with the curve representing averages from all pathogenicity data from the five groups of the pathogen; *B*, frequency distributions of pathogenicity evaluations of raised-sclerotial, intermediate-raised, and intermediate-appressed culture groups. Compare with table 4.

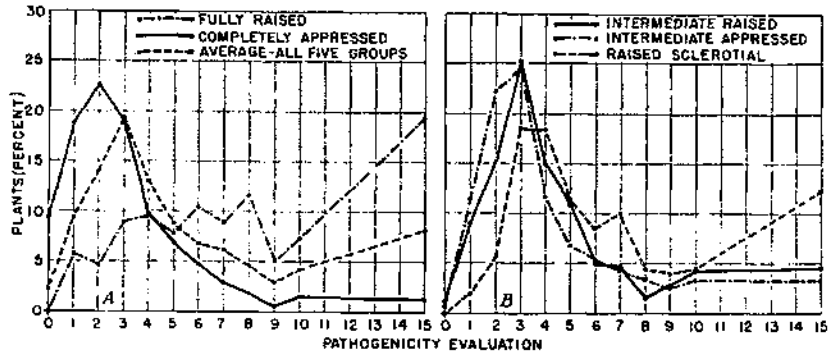

FIGURE 5.—*A*, Frequency distribution of pathogenicity (plant disease) evaluation on Marglobe, as the result of attack after inoculation with cultures of wilt *Fusarium* of fully raised and completely appressed culture groups, compared with the curve representing averages from all pathogenicity data from the five groups of the pathogen; *B*, frequency distributions of pathogenicity evaluations of raised-sclerotial, intermediate-raised, and intermediate-appressed culture groups. Compare with table 4.

Marglobe, and Red Currant tomato, resulting from inoculation by the *Fusarium* selections in the various groups, and also the averages for all groups. The data show consistently that the raised groups are more virulent than the appressed groups, and that this holds true

regardless of the general level of disease injury suffered among the three varieties.

The completely appressed and intermediate-appressed cultures, which are also perhaps the most stable in appearance of growth in culture, show the least variation among their pathogenicity-index distributions.

The greatest variations occur in the frequency distributions from the data from intermediate-raised and raised-sclerotial forms of cultures. These groups, it should be observed, were the ones in which the cultures were found to be most liable to saltations. It has been stated that the cultures in the fully raised group were about as stable as the cultures in the completely appressed group; figure 3 indicates a comparable constancy in pathogenicity.

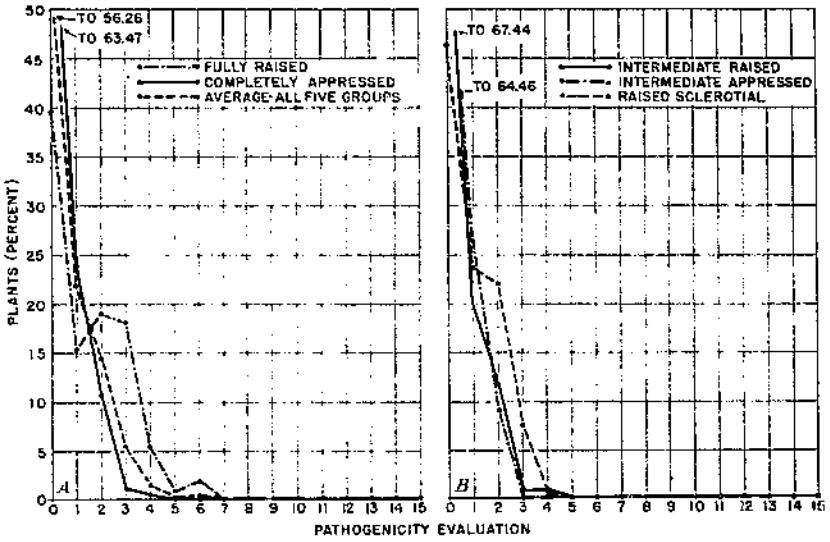

FIGURE 6. *A*, Frequency distribution of pathogenicity (plant disease) evaluations on Red Currant, as the result of attack after inoculation with cultures of wilt *Fusarium* of fully raised and completely appressed culture groups, compared with the curve representing averages from all pathogenicity data from the five groups of the pathogen; *B*, frequency distribution of pathogenicity evaluations of raised-sclerotial, intermediate-raised, and intermediate-appressed culture groups. Compare with table 4.

On account of the relatively small numbers of plants studied and with the fine distinctions between the rather numerous–12–pathogenicity classes, irregularities in the general trends are unavoidable. With much greater numbers of plants or a smaller number of classes, these irregularities would be less marked. However, the main features of the differences in pathogenicity as correlated with culture types are evident, and except for greater perfection of detail, more extensive experimentation with these 28 selections would not materially alter these results.

The Bonny Best data will be considered in greatest detail because this variety is the one most sensitive to infection and best shows differences among the milder disease-producing cultures. Figure 4, *A*,

TABLE 4.—*Averages of plant-disease evaluations on different tomato varieties arranged according to frequency of occurrence in each evaluation class under the five groups of cultures of Fusarium wilt related according to varying macroscopic growth characteristics*

[For definitions of pathogenicity evaluation, see p. 4; for descriptions of culture groups, see pp. 6–8]

Disease (pathogenicity) evaluation	Plant disease evaluations [1] on—																	
	Bonny Best (2,626 plants)						Marglobe (1,671 plants)						Red Currant (729 plants)					
	A	IA	IR	RS	R	Average	A	IA	IR	RS	R	Average	A	IA	IR	RS	R	Average
0	6.02	0.83	0.00	0.14	0.00	1.40	9.55	0.67	1.25	0.00	0.00	2.29	63.47	64.46	67.44	46.27	39.64	56.26
1	12.03	3.95	2.39	.28	1.37	4.01	18.83	11.54	9.06	1.91	5.84	9.44	23.95	26.45	19.38	23.88	15.32	21.80
2	13.08	8.32	4.18	1.43	1.10	5.62	22.81	22.22	15.31	5.72	4.67	14.15	10.78	9.09	11.62	21.89	18.92	14.46
3	14.80	13.10	6.97	5.01	3.02	8.58	19.10	24.58	25.31	18.57	8.95	19.30	1.20	.00	.78	7.46	18.02	5.49
4	11.19	11.23	8.96	7.02	2.75	8.23	9.81	11.78	15.00	18.33	9.73	12.93	.60	.00	.78	.50	5.40	1.46
5	10.15	12.06	6.37	10.03	3.02	8.33	6.90	6.73	10.94	11.43	7.78	8.76	.00	.00	.00	.00	.90	.18
6	7.40	7.48	7.77	10.03	4.40	7.42	4.77	5.39	5.00	8.57	10.51	6.85	.00	.00	.00	.00	1.80	.36
7	6.02	8.52	9.16	9.17	7.14	8.00	2.92	4.38	4.69	10.00	8.95	6.19	.00	.00	.00	.00	.00	.00
8	4.82	9.36	11.56	10.32	8.24	8.86	1.86	3.37	1.56	4.52	11.66	4.59	.00	.00	.00	.00	.00	.00
9	3.79	7.48	8.37	8.45	11.82	7.98	.53	2.69	2.81	4.05	5.06	3.03	.00	.00	.00	.00	.00	.00
10	5.68	9.15	11.55	16.77	21.15	12.86	1.59	3.37	4.38	4.52	7.39	4.25	.00	.00	.00	.00	.00	.00
15	4.99	8.52	22.72	21.35	35.99	18.71	1.33	3.37	4.69	12.38	19.46	8.25	.00	.00	.00	.00	.00	.00

[1] Expressed as percentages of plants inoculated with cultures within the group.

shows the marked difference in disease produced on Bonny Best by the completely appressed cultures and by cultures of the fully raised type. Of all the plants of Bonny Best inoculated with *Fusarium* selections of completely appressed cultural type, less than 10 percent were severely infected. Some of the remaining plants were somewhat seriously diseased, but the majority of the plants were comparatively mildly affected. Of all the Bonny Best plants inoculated with selections of fully raised types of *Fusarium*, only about 13 percent showed mild injury, while all the rest were seriously and severely diseased, and a large percent of them killed. A few days' delay in recording results would have found them all dead. The curves for the raised and the appressed cultures both show radical departures from the average evaluations produced by all the five culture classes.

The distribution curves in figure 4, *B*, show that the pathogenicity of the intermediate classes of cultures is intermediate between the appressed and the raised groups, and that the results produced by cultures of intermediate type lie in fairly close proximity to the average of all evaluations for all five culture classes. The distribution of disease severity produced by all cultures of the raised-sclerotial class tends to be similar to that produced by the fully raised group of cultures, although the percentages of plants in the severely diseased class produced by raised-sclerotial cultures is considerably less than that resulting from cultures of the fully raised group.

On examination of the distributions of disease evaluations on Marglobe (fig. 5), and on Red Currant (fig. 6), it will be seen that in these varieties also there is the least virulence in completely appressed cultures; a medium virulence in intermediate-appressed and intermediate-raised types of cultures, with the latter somewhat more pathogenic than the former; a severe disease effect in the raised-sclerotial forms; and the most consistent and greatest virulence exhibited by the fully raised culture types. In no case, however, was Red Currant damaged by any of the cultures to more than a very mild degree.

DIFFERENCES AMONG HOSTS

Figure 7 represents distribution curves of comparable average disease occurrence in Bonny Best, Marglobe, and Red Currant varieties of tomato and is based on the percentages of plants occurring in the different disease evaluation classes from the pathogenicity data of all the five culture types of *Fusarium*. (See table 4.) In Bonny Best, the most susceptible variety, the largest proportion of plants fall in the seriously and very severely diseased classes. In Marglobe, a more wilt-tolerant variety, the tolerance is clearly noted in the large percentage of plants in the slightly infected and mildly diseased classes. In Red Currant, a highly resistant variety, nearly all plants fall under the classification of apparently healthy or only very slightly diseased. Only a few plants of Red Currant were observed having even a mildly diseased condition, and their numbers were so low that they could be considered of no great importance. The very marked differences in disease reactions of these three tomato varieties were without any doubt due to hereditary differences in resistance or tolerance to the *Fusarium* wilt organism.

It should be remembered that the data from which these graphs have been constructed came from plant populations that were very

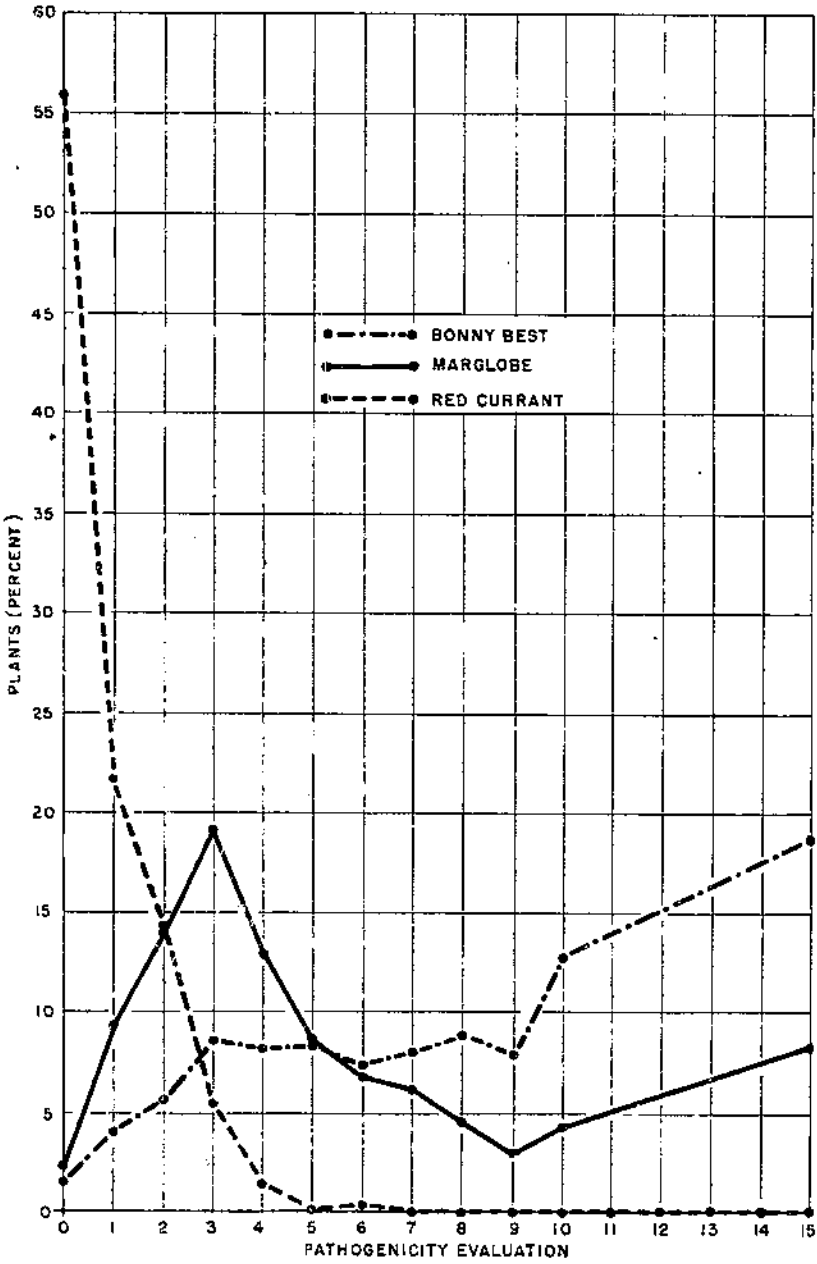

FIGURE 7.—Average frequency distribution of all pathogenicity (plant disease) evaluation data from disease produced from *Fusarium* wilt inoculations of all the cultures in all the five culture groups on Bonny Best (susceptible), Marglobe (tolerant), and Red Currant (resistant) tomato varieties. (Compare with table 4.)

heavily inoculated and grown for 5 to 9 days under ideal conditions for infection and progress of the disease (p. 4). In the field, fortunately, such favorable conditions for disease development are seldom, if ever, encountered because plants are further developed when planted, less inoculum is in the soil, the microbiological balance of the soil has not been disturbed by sterilization, and temperature and moisture are markedly variable.

TESTS OF SIGNIFICANCE

In order to determine the significance of differences in relative pathogenicity exhibited by the several selections and groups of selections of *Fusarium*, frequency distributions of percentages of plants in the several disease evaluation classes were subjected to the chi-square test. Differences among culture groups were tested by using the mean distribution of all groups as the theoretical or calculated value. Certain rather similar pairs of groups were tested, and chi-square values were also determined for the several selections within a group of cultures to test the uniformity of the behavior of the group. Calculations were first made using data from the 12 disease evaluation classes as originally secured. The sums were obtained for each class from comparable experimental series and from all of the separate culture selections in the 5 growth-character groups. It was found in these preliminary calculations that 12 classes were too many to give dependable frequency distributions, even with the data of cultures that were known through previous observations to be remarkably similar. For this reason the 12 original evaluation classes were combined into 4 classes as follows: Class 1, apparently healthy and very mild disease, 0 to 2; class 2, mild disease, 3 to 5; class 3, serious disease, 6 to 8; class 4, very severe disease and death, 9 to 15.

Data from all of the cultures making up the five groups were employed. The results, from which chi-square analyses were made, were from series of experiments in which approximately the same numbers of plants occurred, and which had been handled comparably in all ways, including dates of experiments. The numbers of plants from which data were secured varied slightly in the experiments as a result of unavoidable or unnoticed injuries to plants at inoculation and transplanting time, the occasional death of plants from water-borne damping-off fungi and insect damage during seasons when greenhouse ventilators were necessarily open. Because of these irregularities the original data were converted to percentages. For various reasons some cultures were used in pathogenicity studies more often than others, and the physical limitations of experimental procedures and space for the growing of sufficient plants for testing all cultures resulted in major variations in populations that were eliminated from consideration in these chi-square tests.

Table 5 shows the percentages of plants falling in each of four pathogenicity classes, the mean pathogenicity index, and the chi-square values for each of five cultural groups used to inoculate Bonny Best and Marglobe tomato. Upon Bonny Best the raised-sclerotial and fully raised groups and the completely appressed group produced results that deviated very significantly from the mean results of all groups. When inoculated on Marglobe, all groups except the intermediate raised produced results significantly different from the mean

of all five groups. The departures of the fully raised and fully appressed groups showed very high significance, while the others mentioned were near the 5-percent and 1-percent points. The differences in effects produced on the two varieties by the several groups of fusaria were also very highly significant (chi square 49.1; 1-percent point, 11.3).

TABLE 5.—*Mean pathogenicity effects produced by different cultural groups of Fusarium upon susceptible Bonny Best and wilt-tolerant Marglobe tomato varieties* [1]

[For descriptions of culture groups, see pp. 6-8; for definitions of pathogenicity evaluation class, see p. 21]

Culture group	Bonny Best			Marglobe		
	Mean percentage of plants in pathogenicity class shown		Chi square	Mean percentage of plants in pathogenicity class shown		Chi square
	Percentage	Class		Percentage	Class	
R	4.31	1	44.810	10.51	1	58.558
	13.36	2		26.46	2	
	17.67	3		31.13	3	
	64.66	4		31.90	4	
Weighted mean		3.49			2.84	
RS	1.48	1	14.597	18.92	1	7.831
	21.98	2		45.95	2	
	34.81	3		13.51	3	
	41.73	4		21.62	4	
Weighted mean		3.17			2.38	
IR	5.41	1	4.064	31.40	1	3.300
	28.21	2		46.90	2	
	31.05	3		8.91	3	
	35.33	4		12.79	4	
Weighted mean		2.96			2.03	
IA	16.61	1	5.440	38.97	1	13.352
	32.81	2		48.36	2	
	27.34	3		7.04	3	
	23.87	4		5.63	4	
Weighted mean		2.65			1.79	
A	30.55	1	57.629	60.86	1	44.983
	41.50	2		30.02	2	
	19.31	3		4.26	3	
	8.64	4		4.26	4	
Weighted mean		2.06			1.52	

[1] Chi-square values calculated on distribution of percentages of plants in 4 classes of pathogenicity index values; see p. 21.

In Marglobe the chi square of intermediate-raised and raised-sclerotial groups were fairly close together. Data from these two were tested one against the other resulting in a chi square of 106.8 (1-percent point 11.3). In Bonny Best intermediate-appressed and intermediate-raised gave chi squares of nearly the same size. Data from these two groups on Bonny Best were also tested one against the other, which gave a chi square of 27.9 (1-percent point 11.3). Since both of these chi squares are so significant, they give further proof of the fundamental difference between the less obviously diverse intermediate-appressed, intermediate-raised, and raised-sclerotial cultures.

PATHOGENICITY OF SALTANTS

Saltation has already been described in cultures of *Fusarium* selections (p. 9). It was in some of the unstable cultures in which saltations occurred most frequently and with the greatest variation from the original type that marked variation in virulence occurred. It remained then to determine whether saltants of intermediate or appressed types occurring in otherwise raised cultures differed in virulence from the cultures of their origin. It should be recalled in this connection that in the cultural studies of mat sectors and other saltation areas, no raised aberrant growths were ever encountered that, upon transfer and reculturing, were more raised in growth characteristics than the original cultures from which they were derived.

A number of cultures on potato-dextrose agar, which had obvious sectors or other saltation areas, were used in these studies. In some cases apparently homogeneous cultures were used that had a previous history of producing plentiful saltations. Transfers were made of saltants and original colony types and, in some cases, random portions of apparently homogeneous known saltant-producing cultures were recultured to observe whether saltants had occurred but escaped visual detection. After observations on such subcultures and recording of their growth characters, transfers were made from these varying subcultures to Tochinai's liquid medium and the resulting growth used as inoculum in exactly the same manner as in the previously described pathogenicity studies (p. 4). In addition, the pathogenicity of several spontaneous saltations, not recultured, arising directly in liquid medium was also studied.

The results of these studies are presented in table 6. From these data three points are evident: (1) Saltants of the kind found in these cultures were never more pathogenic than the original cultures and often much less virulent; (2) two cultural types of saltants often originating from the same source differed considerably in pathogenicity, the completely appressed types being less virulent than the intermediate types; (3) saltations, when they arose spontaneously in flasks of cultures used for inoculum, apparently caused a marked reduction in pathogenicity of the entire culture when compared with the homogeneous culture from which they originated.

Since the means shown in table 6 were based on small numbers of plants, most of their standard errors are relatively high. Nevertheless, many rather striking differences are evident, especially between the raised cultures showing no saltation and the appressed saltants derived therefrom. Comparisons should be made among cultures only within a single culture number, because many of the pathogenicity tests between culture numbers were made at different times.

DISCUSSION

The greatest importance of the results reported in this bulletin is the emphasis they place upon accurate knowledge of the type of the wilt organism used in breeding or selecting for *Fusarium* resistance in tomatoes. The Marglobe and practically all other so-called wilt-resistant varieties that belong in this class were markedly resistant to cultures of completely appressed, intermediate-appressed, and intermediate-raised type, yet when exposed to fully raised and nonsaltant

TABLE 6.—*Pathogenic differences noted in saltations of Fusarium bulbigenum var. lycopersici, as obtained from average plant-disease evaluation on 10 plants inoculated with cultures of saltants compared with nonvariant cultures of original selections*

Type of study	Selection or culture No.[1]	Saltant used	Growth character of inoculum	Average[2] disease evaluation on hosts	
				Bonny Best	Marglobe
Saltants purified and studied in culture before using as inoculum.	4	None	Raised sclerotial	10.3±0.54	5.9±0.62
		a	Intermediate raised	11.3±1.31	6.2± .96
		b	Appressed	7.0±1.00	4.3± .56
		c	do	5.6± .54	4.0± .37
	7	None	Raised sclerotial	13.5± .76	7.4± .37
		a	Intermediate raised	13.4± .82	6.8± .49
		b	Appressed	5.5± .50	3.6± .37
	9	None	Raised sclerotial	12.0± .82	6.0± .60
		a	Intermediate appressed	6.4± .50	5.1± .41
	12	None	Raised sclerotial	12.3± .91	6.7± .34
		a	Intermediate raised	9.4± .43	6.7± .42
		b	Intermediate appressed	8.4±1.14	4.7± .42
		c	Completely appressed	3.9± .43	3.1± .53
	16	None	Raised sclerotial	14.0± .67	7.7± .30
		a	Intermediate	9.5± .24	5.8± .55
		b	Completely appressed	5.1± .67	4.8± .33
	29	None	Raised sclerotial	[3] 13.7± .02	[3] 7.1± .55
		a	Intermediate raised	[3] 13.6± .56	[3] 4.9± .25
		b	Intermediate appressed	[3] 9.9± .73	[3] 6.0± .39
		c	Completely appressed	[3] 6.4± .45	[3] 4.6± .27
		d	do	[3] 6.1± .43	[3] 3.7± .30
Saltants occurring spontaneously in mats used as inoculum without reculturing and purification.	1	None	do	[4] 8.0±1.18	
		a	do	[4] 6.9± .90	
	3	None	Intermediate appressed	4.4± .45	
		a	Completely appressed	4.0± .56	
	4	None	Raised sclerotial	10.3± .54	5.9± .62
		a	Small raised portion, rest intermediate raised.	9.1±1.09	3.5± .34
		b	Small raised portion, rest completely appressed.	6.6± .93	6.4± .56
	6	None	Completely appressed	4.1± .43	
		a	do	3.6± .48	
	9	None	Raised sclerotial	13.5± .76	5.6± .39
		a	Intermediate raised	10.5± .81	5.2± .63
		b	Appressed with flecks of intermediate growth.	4.8± .66	2.3± .37
	11	None	Intermediate raised	13.5±1.00	
		a	Intermediate appressed	8.8±1.58	
	15	None	Completely appressed	1.2± .13	
		a	do	1.0± .00	
	16	None	Raised sclerotial	14.0± .67	9.9± .33
		a	Raised with small intermediate sector	11.2±1.09	7.0± .37
		b	Appressed with intermediate sectors	9.6± .31	7.2± .42
	29	None	Raised sclerotial	[3] 12.5± .57	[3] 5.8± .27
		a	Raised with large appressed region	[3] 8.2± .32	[3] 6.1± .34

[1] For description of original culture type designated by numbers shown, see table 2.
[2] Mean together with standard error of mean.
[3] Results from 20 plants.
[4] Results from 8 plants.

cultures of the raised-sclerotial type they showed serious injury and severe wilt.

The susceptible tomato, Bonny Best, on the other hand, was more or less seriously diseased, when inoculated, by all types of the wilt *Fusarium*. When infected by the completely appressed type, it gave evidence of the least rapid injury, amounting to what would almost appear as occasional resistance if the virulence of the *Fusarium* cultures were not known.

When the resistant Red Currant was inoculated, it did not succumb to any serious or severe disease even when the most virulent types of cultures were used for the tests. This plant was invariably infected by the organism regardless of virulence in the cultures, but its successful inhibition of serious subsequent injury demonstrated that true resistance is readily detectable in such studies as these reported herein.

Tomato varieties can be selected under field conditions and prove highly resistant to the particular *Fusarium* type occurring where the selections were made. Yet, when these varieties are sent to another locality large numbers of the plants may be severely wilted. The studies described herein indicate that this difference in susceptibility may occur because more virulent forms of the pathogen attack plants that were selected originally to resist less virulent organisms.

It is of interest to note in this connection that in 1921 Edgerton and Moreland (*5*) reported the cultures they isolated from wilted tomatoes to be of two types, one of which they considered to be the "typical" organism with abundant light-colored aerial mycelium and the other which they described as a low-growing, deeply colored species that they thought to be another organism and hence discarded. Edgerton's subsequent selections of wilt-resistant tomatoes for Louisiana were based on the use of soil inoculated with the "typical" tomato *Fusarium* cultures. It is to be observed that, as Boswell (*2*) has pointed out, one tomato variety selected by Edgerton has been known as generally the most wilt-resistant commercial variety available and has entered into the parentage of many other resistant varieties now on the market.

It would seem to behoove the plant pathologists and the geneticists, when working to develop wilt-resistant tomato varieties that can be grown under the most severe conditions, to insure that these varieties are subjected to the most virulent types of the common tomato *Fusarium*.

It appears that in some crops the variability of the *Fusarium* species involved is not so important with regard to the disease-resistance problem. One example of this may be seen in the several resistant cabbage varieties produced by J. C. Walker and his coworkers. These appear resistant wherever grown. The work of Blank (*1*) has shown that no great variations in virulence were involved in the *Fusarium* in question.

The practical effects of *Fusarium* variations have, however, been pointed out as a possibility in a few instances. For example, in the work of Nelson et al. (*11*) definite proof is given that, in the *Fusarium* infection of celery, differences in the organism are of importance in relation to the control problem.

It is notable that a wide range of crops is affected by *Fusarium* diseases, and the selection of disease-resistant varieties has been a profitable line of endeavor. There are, of course, other types of problems involved in the study of *Fusarium* diseases, such as dispersal of the organisms, infection and growth of the pathogen in the plant, distribution in the soil, different methods of control than disease resistance, host-range studies, and other matters (e. g. Jones and Gilman (*9*), Walker and Wellman (*21*), Pritchard (*14*), Porte (*13*), Wellman (*23*), Scott (*17*), Wade (*20*), Orton (*12*), Fahmy (*6*), Tisdale (*19*), Brandes (*3*), Wardlaw (*22*), and Reinking and Manns (*15*)). Yet in the majority of all such problems it is apparent that the investigators have not considered the variability of the organism to be of practical importance. Even in the case of the purely descriptive mycological phases of the *Fusarium* problem, at least in some instances, the range of variability of the organisms should be more fully investigated. Some workers have found such great instability in some species of Fusaria that critics have arisen to doubt certain generic and specific rankings

erected and defined by the taxonomists. It is believed that a fuller study and description of the variations to be expected in the species or subspecies will eventually clarify these points.

In conclusion, one matter of major importance deserves special mention. One of the cultures (see No. 25 in tables 1 and 2) of the 30 that were picked at random from a wilt-producing population was found to be a species other than *Fusarium bulbigenum* var. *lycopersici*. On using this organism for inoculation of Bonny Best, Marglobe, and Red Currant tomatoes, it attacked and caused severe wilting in a few plants of both Bonny Best and Marglobe. Moreover, the wilt symptoms produced were indistinguishable from those caused by the common wilt *Fusarium*. It appears from this experience that other species of *Fusarium* may enter into the tomato-wilt problem. This requires serious attention, as every new pathogenic species is potentially able to cause disease in tomato varieties that are otherwise developed for resistance to the *Fusarium* most commonly met. A new wilt-producing species may spread in the tomato-growing areas. Should this happen it may require the development of different new wilt-disease-resistant varieties.

SUMMARY

Studies were made on 30 random cultures from diverse regions of the United States. All produced tomato wilt and, except for one, were *Fusarium bulbigenum* var. *lycopersici* (Brushi) Wr. and R. Members of this subspecies, except one, were sharply separated on cultural appearance, and grouped into 5 types based on dissimilar growth characters, which were correlated with distinct variations in pathogenicity.

Virulence was tested on Bonny Best, Marglobe, and Red Currant tomatoes. Cultures characterized by raised light-colored mycelium were most virulent; raised types with sclerotialike bodies were erratic and on the whole slightly lower in pathogenicity; cultures of intermediate-raised type produced considerable disease but less than the two raised types; those of an intermediate-appressed type with scanty mycelium over a dark appressed growth were weaker in pathogenicity than the other groups; and the dark-colored, completely appressed cultures with no aerial mycelium were the least effective cause of disease.

Saltation occurred in all groups of *Fusarium* cultures, was least noticeable in fully raised and completely appressed cultures, most conspicuous and frequent in raised sclerotial, and intermediate in occurrence in the intermediate groups. Saltants tested were generally less virulent than the cultures of their origin.

Pathogenicity data on the three tomato varieties showed marked divergences due to differences in relative disease resistance. Red Currant, wilt resistant, was in most cases practically unaffected and only very mildly diseased even when infected with most virulent cultures; Marglobe, wilt tolerant, was severely diseased by the most virulent cultures but was distinctly resistant to less pathogenic types; and Bonny Best, wilt susceptible, was severely diseased by all cultures except the least virulent which produced a medium amount of injury.

LITERATURE CITED

(1) BLANK, L. M.
1934. UNIFORMITY IN PATHOGENICITY AND CULTURAL BEHAVIOR AMONG STRAINS OF THE CABBAGE-YELLOWS ORGANISM. Jour. Agr. Research 48: 401–409.

(2) BOSWELL, VICTOR R.
1937. IMPROVEMENT AND GENETICS OF TOMATOES, PEPPERS, AND EGGPLANTS. U. S. Dept. Agr. Yearbook 1937: 176–206, illus.

(3) BRANDES, E. W.
1919. BANANA WILT. Phytopathology 9: [339]–389, illus.

(4) CLAYTON, EDWARD E.
1923. THE RELATION OF TEMPERATURE TO THE FUSARIUM WILT OF THE TOMATO. Amer. Jour. Bot. 10: 71–88, illus.

(5) EDGERTON, C. W., and MORELAND, C. C.
1920. TOMATO WILT. La. Agr. Expt. Sta. Bull. 174, 54 pp., illus.

(6) FAHMY, TEWFIK.
1934. THE SELECTION OF WILT IMMUNE STRAINS OF LONG STAPLE COTTON (sakha 4 gidad). Min. Agr. Egypt, Tech. and Sci. Serv. (Mycol. Sect.) Bull. 130, 25 pp., illus.

(7) HAYMAKER, H. H.
1928. PATHOGENICITY OF TWO STRAINS OF THE TOMATO-WILT FUNGUS, FUSARIUM LYCOPERSICI SACC. Jour. Agr. Research 36: 675–695, illus.

(8) ———
1928. RELATION OF TOXIC EXCRETORY PRODUCTS FROM TWO STRAINS OF FUSARIUM LYCOPERSICI SACC. TO TOMATO WILT. Jour. Agr. Research 36: 697–719, illus.

(9) JONES, L. R., and GILMAN, J. C.
1915. THE CONTROL OF CABBAGE YELLOWS THROUGH DISEASE RESISTANCE. Wis. Agr. Expt. Sta. Research Bull. 38, 70 pp., illus.

(10) LEONIAN, LEON H.
1929. STUDIES ON THE VARIABILITY AND DISSOCIATIONS IN THE GENUS FUSARIUM. Phytopathology 19: 753–874, illus.

(11) NELSON, RAY, COONS, G. H., and COCHRAN, L. C.
1937. THE FUSARIUM YELLOWS DISEASE OF CELERY (APIUM GRAVEOLENS L. VAR. DULCE D. C.). Mich. Agr. Expt. Sta. Tech. Bull. 155, 74 pp., illus.

(12) ORTON, W. A.
1908. ON THE THEORY AND PRACTICE OF BREEDING DISEASE-RESISTANT PLANTS. Amer. Breeders Assoc. Proc. 4: 144–156.

(13) PORTE, WILLIAM S.
1932. THE PRITCHARD TOMATO. U. S. Dept. Agr. Cir. 243, 4 pp., illus.

(14) PRITCHARD, F. J.
1922. DEVELOPMENT OF WILT-RESISTANT TOMATOES. U. S. Dept. Agr. Bull. 1015, 18 pp., illus.

(15) REINKING, OTTO A., and MANNS, M. M.
1933. PARASITIC AND OTHER FUSARIA COUNTED IN TROPICAL SOILS. Ztschr. Parasitenk. 6: 23–75, illus.

(16) RIDGWAY, ROBERT.
1912. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C.

(17) SCOTT, IRL T.
1924. THE INFLUENCE OF HYDROGEN-ION CONCENTRATION ON THE GROWTH OF FUSARIUM LYCOPERSICI AND ON TOMATO WILT. Mo. Agr. Expt. Sta. Research Bull. 64, 32 pp., illus.

(18) STEVENS, F. L.
1922. THE HELMINTHOSPORIUM FOOT-ROT OF WHEAT, WITH OBSERVATIONS ON THE MORPHOLOGY OF HELMINTHOSPORIUM AND ON THE OCCURRENCE OF SALTATION IN THE GENUS. Ill. Nat. Hist. Survey Bull. 14: 77–185, illus.

(19) TISDALE, W. H.
1917. RELATION OF TEMPERATURE TO THE GROWTH AND INFECTION POWER OF FUSARIUM LINI. Phytopathology 7: 356–360, illus.

(20) WADE, B. L.
1929. INHERITANCE OF FUSARIUM WILT RESISTANCE IN CANNING PEAS. Wis. Agr. Expt. Sta. Research Bull. 97, 32 pp., illus.

(21) WALKER, J. C., and WELLMAN, F. L.
1928. A SURVEY OF THE RESISTANCE OF SUBSPECIES OF BRASSICA OLERACEA TO YELLOWS (FUSARIUM CONGLUTINANS). Jour. Agr. Research 37: 233–241, illus.

(22) WARDLAW, C. W.
1933. PANAMA DISEASE. A REVIEW OF THE OCCURRENCE OF PANAMA DISEASE ON THE CAVENDISH OR DWARF BANANA IN THE CANARY ISLANDS. Trop. Agr. [Trinidad] 10: 151–154.

(23) WELLMAN, F. L.
1931. PROGRESS OF FUSARIUM WILT INSIDE THE RHIZOMES OF BANANA PLANTS. (Abstract) Phytopathology 21:121.

(24) ———
1939. A TECHNIQUE FOR STUDYING HOST RESISTANCE AND PATHOGENICITY IN TOMATO FUSARIUM WILT. Phytopathology 29:945-956, illus.

(25) ——— and BLAISDELL, D. J.
1939. DIFFERENCES IN CULTURAL CHARACTERS AND PATHOGENICITY OF STRAINS OF TOMATO-WILT FUSARIUM. (Abstract) Phytopathology 29: 24.

(26) WHITE, RICHARD P.
1927. STUDIES ON TOMATO WILT CAUSED BY FUSARIUM LYCOPERSICI SACC. Jour. Agr. Research 34: 197-239, illus.

(27) WOLLENWEBER, H. W., and REINKING, O. A.
1935. DIE FUSARIEN, IHRE BESCHREIBUNG, SCHADWIRKUNG UND BEKÄMPFUNG. 355 pp., illus. Berlin.

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