STUDIES IN THE PHYSIOLOGY, GENETICS AND PATHOLOGY OF *COLLETOTRICHUM PHOMOIDES* (SACC.) CHESTER, THE CAUSE OF TOMATO ANTHRACNOSE

by

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STUDIES IN THE PHYSIOLOGY, GENETICS AND PATHOLOGY OF *COLLETOTRICHUM PHOMOIDES* (SACC.) CHESTER THE CAUSE OF TOMATO ANTHRACNOSE

INTRODUCTION

For over fifty years growers of canning tomatoes have suffered heavy losses due to anthracnose or ripe rot which, as the latter name indicates, is of importance only on mature tomato fruits. On green fruits, the anthracnose organism, *Colletotrichum phomoides*, produces only minor infections and therefore is of no importance to growers of market tomatoes who generally harvest green fruits; but on ripe fruits "an infection of only five or ten per cent will double even treble the cost of picking. Every fruit picked in an infected field has to be carefully examined on all sides ..... A half dozen severely infected fruit in a bushel basket may render the entire lot unfit for human consumption according to the rigid government regulations now in effect" (21).

Present researches are dealing more and more with the genetics of phytopathogenic fungi, for it is realized that hybridization and mutation of fungi may result in the production of new strains of organisms which are highly virulent and which do not respond readily to established control measures. Hence, it is well to know whether there are various strains of a fungus present and whether there is any likelihood

of the formation of new strains. Distinction of strains of organisms may be based on morphologic, physiologic or pathologic differences. A portion of this paper is devoted to determining whether there are physiologic strains of C. phomoides.

Other facts about C. phomoides which would prove helpful in the control of anthracnose are also sought. Among these are the environmental factors favoring growth of the organism in vitro, the method by which the fungus penetrates the host, the relative resistance of tomato varieties to infection and environmental factors most favorable to infection.

Colletotrichum phomoides is one of the Fungi Imperfecti of which certain members have shown a peculiar instability of mycelial characteristics; that is, they have shown the "dual phenomenon" as expressed by Hansen (16). Attempts to demonstrate this phenomenon in monosporous cultures are of interest to geneticists but have little bearing on the pathologic problem unless new morphologic forms are shown to appear. Occurrence of sectors may also be included in the genetic studies of the organism. Sectors arise usually as a result of mutations within single cells of the mycelium and offer an explanation of the appearance of new strains in fungi not possessing a perfect stage.

HISTORY

Nomenclature

Colletotrichum phomoides, the organism causing ripe rot or anthracnose of tomatoes, was first described by Saccardo (28), in 1882, as Gloeosporium phomoides. In 1891, Chester (1) claimed to have discovered a new organism Colletotrichum lycopersici, causing anthracnose, which later proved to be identical with the organism described by Saccardo. Since the two genera are distinguished by the presence of setae at the margin of the acervuli of Colletotrichum and the absence of them in Gloeosporium (32) and since Chester's first isolates possessed setae, the organism was called C. lycopersici. However, in 1892, Chester (2) found anthracnose was being caused by an organism without setae and renamed it G. phomoides. In 1893 and 1894 setae were again observed and the organism fitted the description Saccardo had made and was then called C. phomoides (Sacc.) Chester (3). Since that time, various workers have indicated that the presence or absence of setae may vary as the host or the culture medium of the organism is changed. For example, in 1895, Cobb (6) reported an organism causing pimply rot of tomatoes. Its description agreed with that of C. lycopersici Ches. except in spore measurements. Halstead (12, 13), in 1895 and 1896, referred to the ripe rot organism as G. phomoides Sacc. but in 1905 Rolfs (27) called it C. lycopersici Ches. Stevens (31), in

1913, placed the anthracnose organism in the genus Colletotrichum and states: "Work by Gueguin throws doubt on the American form on tomato being identical with the European form known as Gloeosporium phomoides." Since then only Tisdale (34), in 1915, has used the genus Gloeosporium, other American workers having accepted Colletotrichum as the genus of the anthracnose organism.

Etiology

Although the anthracnose organism was described by Saccardo in 1882, the disease caused by it was not mentioned in the literature until Chester (1) mentioned its economic importance in 1891. On the other hand, the tomato was a comparatively new crop in the United States and it is probable that he found the first significant outbreak to occur here. He reported that the lesions occurred only on ripened fruit. They were described as sunken spots with dark centers becoming black. The black centers bore acervuli which possessed numerous setae. Chester gave the following botanical description: "Colletotrichum lycopersici, n. sp. — Circular depressed spots slightly discolored, center black, becoming irregular and confluent. Acervuli abundant becoming densely gregarious, rusty brown to black, applanate 95-150 microns in diameter. Setae abundant, fuliginous, curved, undulate or straight, often geniculate, in places tapering, septate,

65-112 microns long. Spores oblong, 16-22 x 4 microns (av. 19 x 4 microns). Ends subacute, hyaline, containing oil drops staining brown with osmic acid. Basidia short, slender 30-40 microns arising from a well developed stroma."

Saccardo (28) gave a briefer description: "Gloeosporium phomoides — Acervuli innate — erumpent, fuscous, pulvinate; conidia oblong, clavate abruptly attenuate, apices rounded, 2.5 to 3 x 10 to 12 microns, biguttulate, hyaline; basidia bacillary fasciculate, 1.5 x 20 to 21 microns, hyaline arising from a dark, cellulose proligerous base."

In 1906, Hasselbring (18) showed that the germinating spores of the anthracnose organisms produced appressoria.

Harvey (17), in 1893, recommended K_2S for the control of the disease but Halstead (12), in 1895, found K_2S to be worthless, while plots sprayed with Bordeaux mixture were practically free from infection. Pool (24), in 1907, expanded the knowledge of the morphology of the organism paying particular attention to its development on artificial media. In 1912, Taubenhaus (33) reported that G. phomoides and other anthracnose fungi could also cause bitter rot of apples. Tisdale (34) worked out different physiologic aspects including the effects of temperature and pH and declared further that seed-borne spores caused a damping-off of seedlings.

After 1915, little was published on tomato anthracnose until 1930 when Hutchinson and Ashton (20) reported the effect of radiant energy on the growth and sporulation of

C. phomoides. Using light from a mercury arc they found that its spectrum could be divided into three groups of lines, each group having a different effect on spore production and rate of mycelial growth. In 1933, Hunter (19) recorded a high percentage of infection in tomato fields defoliated early by storms, and in 1937, Chupp (5) reported that high percentages of infection were favored by high humidity and high temperatures. Samson (29), in 1940, recommended good drainage, proper fertilization and copper sprays as insurance against severe tomato anthracnose. More recently, McNew (21, 22) and Wilson (35, 36) have shown that fermate gives nearly perfect control of the disease.

THE INVESTIGATION

A. Materials

In order to make physiologic and genetic studies of C. phomoides, five isolates of the fungus were used and designated as follows:

#6 - from tomato variety test plots of the Virginia Truck Experiment Station, Norfolk, Virginia.

#8 - from a victory garden at Blacksburg, Virginia.

#9 - from a commercial tomato field in Northhampton County, Virginia.

NJ - from the New Jersey Agricultural Experiment Station, New Brunswick.

S - from a commercial tomato field in New Jersey.

With the exception of S, all isolations were made in the summer of 1943; isolate S was collected in the summer of 1941 and had been stored in refrigeration as a stock culture.

Because of its stability and ease of preparation, potato dextrose agar (Difco) was used as the medium of cultivation unless otherwise stated.

B. Methods

For studying growth of the fungus in culture media, the smallest amount of mycelium obtainable was transferred from tube cultures three to five days old. Plates were then incubated until the fastest growing cultures had reached nearly to the edge of the plate and the diameters of the colonies were measured in two directions which were perpendicular to each other. The average of these two measurements was taken as the diameter of the colony. This compensated for minor irregularities in growth.

Spore suspensions were necessary for the isolation of single spores and for inoculating tomatoes. Suspensions were readily prepared by adding sterile distilled water to 4-day-old cultures on agar and agitating the surface with a sterile

wire loop. From this suspension monosporous isolations were made by the method described by Dickinson (7). With similar preparations, tomatoes were inoculated by the use of an atomizer.

In the preparation of the media for pH studies, the various H-ion concentrations were adjusted with 0.2 normal hydrochloric acid or .2N sodium hydroxide by use of glass electrodes and a Beckman potentiometer.

In making a study of the physiology of an organism it seems reasonable to begin with those factors which can be most easily controlled and which manifest the greatest effects on the growth of the organism, since an understanding of these factors is a prerequisite to the investigation of more complex factors and because it is necessary to understand these relatively simple factors in order to provide the organism with the most favorable conditions for growth when the more complex factors are being studied.

All colors mentioned are those shown in Ridgeway's color plates (26).

1. Temperature Study

Procedure.—Temperature relations were studied in a chamber consisting of five compartments with two copper coils serving as the temperature regulator. These coils were inserted in the freezing unit of an electric refrigerator at one end and in a compartment containing an electric light

bulb at the other. The arrangement was such that one coil was at the front of the chamber in an oval. Half of the coil passed through the bottom of the chamber and the other half through the top of it, as shown in figure 1. The second coil was arranged in a like manner at the rear of the chamber.

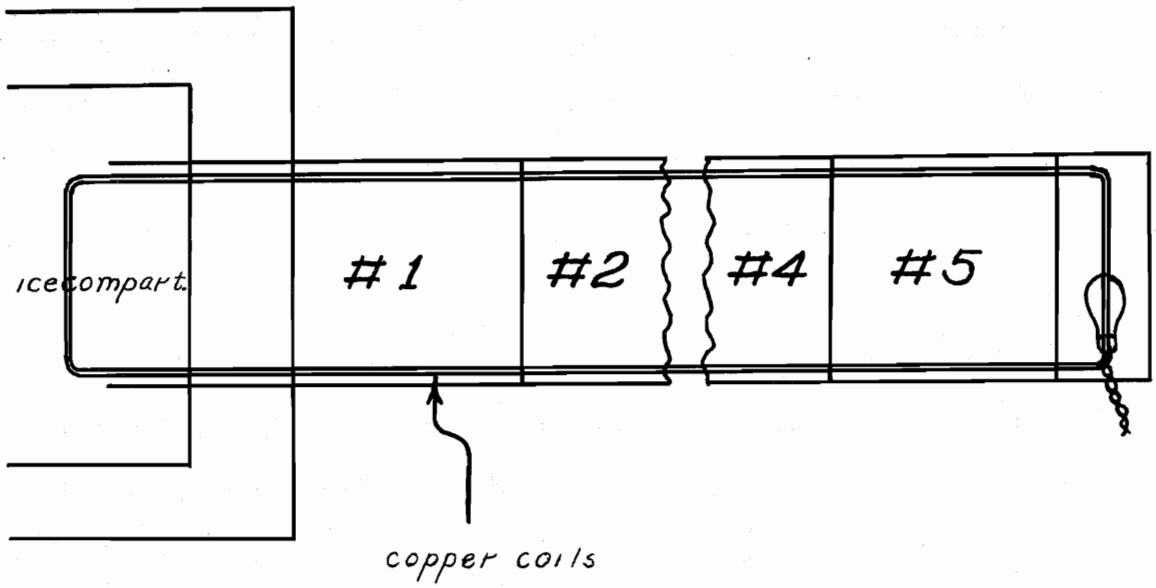

Fig. 1.—Front view of temperature chamber showing arrangement of copper coils (diagrammatic).

The chamber gave a temperature range of 10.5° to 47° C. The arrangement brought about a narrower temperature range within each compartment; hence it was necessary to determine the temperature maintained at the level of the agar in each layer of culture plates.

Results.—The diameter of the colonies was measured at the end of five days. After five days, interference from antibiotic contamination made measurements difficult. Results are shown in table I and curves plotted from these data are shown in plate IA. Since 28.5° C. was the approximate average optimum for the growth of the isolates used, it was selected as the incubation temperature for subsequent studies.

2. pH Study

Procedure.—Studies of the effects of various hydrogen-ion concentrations on the growth of C. phomoides were made in two series. The first series covered a pH range of 3.15 to 9.95. In this series eleven hydrogen-ion concentrations were spaced at as uniform intervals as it was possible to obtain. The second series covers a pH range of 1.6 to 11.1 with nine intervals unequally distributed but more numerous at the upper and lower limits of the pH scale.

Results.—The data in tables II and IV show the effect of pH on growth of the organism, and plate II A & B contains growth curves plotted from these data. Plates III, IV, and V, are photographs of some of the cultures obtained.

Table III shows the measurements of spores and sclerotia in the cultures of series one. This is to indicate the effect of pH on the size of these structures. In recording these results it was observed that #8 produced no spores above pH 7.25, #9 produced none above pH 9.3, NJ produced none below

Table I.—Diameters of C. phomoides colonies after five days growth on potato dextrose agar at various temperatures

Compartment	Level*	Temperature	Culture			
			#6	#8	#9	NJ
		Centigrade	Millimeters			
1	1	10.5	0.0	9.0	11.0	5.5
	2	11.5	0.0	10.0	10.0	6.0
	3	13.5	0.0	8.0	6.5	6.5
	4	14.5	4.0	7.5	9.0	10.5
2	1	17.5	28.5	17.0	15.5	16.0
	2	18.5	34.5	18.0	17.0	17.0
	3	20.0	36.5	18.0	20.5	20.0
	4	21.0	43.0	20.5	26.0	25.0
3	1	24.0	43.0	23.0	35.0	35.0
	2	25.5	47.0	24.5	38.0	37.5
	3	26.5	51.0	28.0	34.0	23.5
	4	27.8	47.0	28.0	32.0	37.0
4	1	31.0	68.0	28.0	31.0	41.0
	2	32.5	76.0	26.0	27.0	41.0
	3	34.0	77.0	22.5	19.0	34.0
	4	35.5	68.0	15.0	13.0	18.5
5	1	39.5	13.0	4.5	14.0	16.0
	2	40.7	8.5	4.0	11.0	9.0
	3	44.5	6.0	5.0	7.0	7.0
	4	47.0	5.5	5.0	7.5	5.5

*Level indicates the position of the plate in a stack of four, starting at the bottom where temperature was lowest.

Table II.—Diameters of C. phomoides colonies after eight days incubation at different hydrogen-ion concentrations (series 1)

pH	Culture				
	#6	#8	#9	NJ	S
	Millimeters				
3.15	27.0	17.5	16.5	27.0	14.0
3.15	26.0	18.0	19.0	25.5	16.0
3.64	----	29.0	32.0	49.5	45.0
3.67	68.5	30.0	33.0	49.0	44.5
4.35	86.0	39.5	44.5	58.0	65.5
4.35	86.0	42.5	37.5	59.0	65.0
5.00	86.0	50.0	54.0	63.0	72.0
5.02	86.0	53.5	54.0	63.0	72.0
5.82	86.0	57.0	57.0	65.0	73.5
5.82	86.0	57.5	58.0	63.5	72.0
7.1	86.0	59.0	56.5	64.5	----
7.13	86.0	----	59.0	63.0	70.0
7.25	86.0	59.5	59.0	64.5	72.0
7.25	86.0	57.0	61.0	66.5	70.0
7.95	86.0	55.5	60.0	66.0	70.0
7.96	86.0	57.0	61.5	66.5	69.0
8.6	86.0	57.0	62.0	66.0	69.0
8.63	----	56.0	39.0	65.0	67.5
9.27	86.0	55.0	65.5	65.0	72.0
9.3	----	56.0	62.0	65.5	71.5
9.93	86.0	50.5	64.5	61.5	71.0
9.95	86.0	53.5	62.0	63.0	71.0

Table III.—Effect of hydrogen-ion concentration on size of spores and sclerotia. pH series #1. Spore dimensions in microns*

pH	Culture #6	#8	#9	NJ	S
3.61	No spores	14.7 x 4.8	12.8 x 5.6	No spores	13.4 x 4.4
5.00	No spores	11.6 x 4.9	12.8 x 5.9	14.3 x 4.2	12.5 x 4.6
9.93	No spores	No spores	No spores	12.7 x 4.3	12.7 x 4.4

Sclerotium dimensions in microns*

pH	Culture #6	#8	#9	NJ	S
3.61	308 x 308	720 x 565	694 x 605	542 x 433	910 x 640
5.00	283 x 258	1023 x 810	559 x 481	Sclerotia coalescing	707 x 603
9.93	327 x 264	727 x 570	623 x 420	Sclerotia coalescing	620 x 442

*Averages for six measurements.

Table IV.—Diameters of C. phomoides colonies after five days of incubation at different hydrogen-ion concentrations (series 2)

pH	Culture				
	#6	#8	#9	NJ	S
	Millimeters				
1.6	0	0	∕	0	0
1.6	0	0	0	0	0
2.0	0	0	0	0	0
2.0	0	0	0	0	0
2.5	∕	∕	—	∕	4
2.5	∕	∕	∕	∕	6
3.0	13	10	12	13	12
3.0	14	8	—	13	7
4.25	65	30	24	42	45
4.25	65	30	32	—	43
7.05	73	39	43	46	48
7.05	74	37	42	45	49
9.8	57	37	43	42	47
9.8	58	37	41	43	47
10.4	38	22	27	31	31
10.4	35	26	26	34	34
11.1	9	0	0	0	0
11.1	0	0	0	0	0

(—) = contaminants causing antibiosis.

(∕) = trace of growth but immeasurable.

pH 3.64, and #6 produced none at all. Sclerotial measurements appeared to be uniform for each isolate, but wide variations were found among the five of them. For all isolates there was a slight increase in the amount of aerial mycelium as the pH approached 9.95. The mycelium between the area of sclerotia formation and the margin of the colony was salmon colored in isolates #8, #9, and S but was white in #6 and NJ. Change of pH had no effect on pigmentation.

3. Effects of Light

Procedure, part I.—Plates of the five isolates were incubated in total darkness, diffused daylight, and direct sunlight. All plates were necessarily incubated at room temperature because temperature of those plates in diffuse sunlight and in direct sunlight could not be controlled.

Results.—Both sets of cultures subjected to light showed concentric rings formed by sclerotia. Those cultures incubated in darkness showed the sclerotia to be evenly distributed; i.e., no rings were apparent (Plate IB). No other effects were manifested.

Procedure, part II.—Whether daylight inhibited or stimulated sclerotium production was not determined from the above experiment. Therefore, plate cultures of all isolates were incubated in total darkness for three days. On the morning of the fourth day these were placed in direct sunlight and the margin of the colony was indicated by marking

on the glass with a wax crayon. At the end of the day the margin of the colony was again marked and the plates were incubated for three more days in darkness. This allowed sclerotia to develop in the area where sunlight had shone on the growing mycelium.

Results.—In the zone which grew while exposed to sunlight fewer sclerotia were formed and a clear ring was discernable, but during the period of darkness immediately following this exposure sclerotia were produced in greater numbers than were normally produced in darkness. This resulted in a darkened ring (Plate IB).

4. Effects of Different Media on Growth of *Colletotrichum phomoides*

Procedure.—Tisdale (34) grew *C. phomoides* on several different culture media but did not mention the types of compounds most favorable to the growth of the organism. In these studies the organism was grown on four standard (Difco) media; corn meal, nutrient, potato dextrose and malt agars, and on media prepared with infusion from green and ripe tomato fruits.

Results.—Plate IIIA shows isolate #6 on the seven test media. Growth was greatest on potato dextrose and least on green tomato agar. The nitrogenous nutrient agar favored production of aerial mycelium but all others favored production of surface and subsurface mycelium.

Plate VIB shows isolate #8 on the same test media. In this case, greatest diameter was attained on potato dextrose and the smallest diameter on malt agar. Aerial mycelium was abundant only on potato dextrose, nutrient, and malt agars. Sclerotia were very small on nutrient agar, medium in size and uniformly distributed on agars made from green tomatoes and yellow and red ripe tomatoes, large and clumping on malt agar, scattered on corn meal agar, and large on potato dextrose. Marginal mycelium was pale salmon in color on malt and potato dextrose agars, white or gray on all others.

Plate VIIA shows isolate #9 which was similar to #8 in that greatest diametric growth was attained on potato dextrose and least on malt agar. Aerial mycelium was abundant on potato dextrose, nutrient, and malt agars; sclerotia were small and scattered on nutrient, yellow and green tomato agars, aggregated on corn meal agar, irregularly distributed on red tomato agar, medium in size and dense on malt and potato dextrose agars. Marginal mycelium was colored pale salmon on malt, seashell pink on nutrient, and gray to white on all other media.

Plate VIIB shows isolate NJ on six of the media. Growth was greatest on potato dextrose and least on nutrient agar. Aerial mycelium was dense on potato dextrose and malt agars; mycelium was subsurface on corn meal agar. Sclerotia were small and scattered on nutrient and green agars; irregular, large, deeply sunken on malt and potato dextrose; medium in

size, regularly distributed on yellow agar; few and deep on corn meal agar. Marginal mycelium was pale salmon on potato dextrose and malt agars, but white on others.

Plate VIIIA shows growth of isolate S. Growth was greatest on corn meal agar, and least on nutrient agar. Aerial mycelium was abundant on potato dextrose and malt agars. Sclerotia were very small on nutrient agar; evenly distributed in center and medium in size on corn meal agar; medium in size and scattered on green tomato agar; large and scattered on red and yellow tomato agars; and dense on potato dextrose agar. Marginal mycelium was pale salmon on potato dextrose; seashell pink on malt; and gray or white on all agars.

In only two cases, isolates #6 and NJ, did the growth on agar made from green tomatoes exceed the growth on agar made from ripe tomatoes, and in both these cases it exceeded the growth on agar made from ripe yellow tomatoes, but not that growth on agar made from red tomatoes.

5. Effects of Osmotic Pressure

Procedure.—To study the effects of osmotic pressure on C. phomoides, a medium consisting of the following formula was used:

H_2O	1000 cc
K_2HPO_4	1.8 gms.
$Fe_2(SO_4)_3$	trace
$MgSO_4$	.5 gm.
$CaNO_3$	.2 gm.

peptone (Difco)	.9 gm.
agar	1.7 gms.

This base medium was divided into 300 ml. aliquots and either glucose or sodium chloride was added to these aliquots in the following proportions per hundred ml.: 0 gms., 1 gm., 5 gms., 10 gms.; and 30 gms. From these concentrations the following osmotic pressures were calculated:

Concentration gms./100 cc.	Osmotic pressure in atmospheres	
	Glucose	NaCl
0	0	0
1	0.5	2.8
5	2.3	14.0
10	4.6	28.0
30	13.8	84.0

On these media the five isolates of C. phomoides were incubated for eight days.

Results.—Table V shows that the medium containing no salt or sugar did not support the growth of the organism very well; but with any increase in osmotic pressure, either by addition of glucose or sodium chloride, all isolates grew well. Osmotic pressure of 4.6 atmospheres was the most favorable for growth and above 28 atmospheres no growth occurred.

With glucose and osmotic pressure at 0.5 and 2.3 atmospheres, spore masses were produced within a gelatinous matrix. These spore masses were so large that they appeared to be

Table V.—Diameters of C. phomoides colonies after eight days growth at different osmotic pressures

Osmotic pressure in atmospheres - with glucose	Isolates				
	#6	#8	#9	NJ	S
	Millimeters				
0	4	1	1	1	3
0.5	77	46	51	60	53
2.3	82	61	63	74	63
4.6	88	41	66	73	79
13.8	71	44	44	41	47
- with NaCl					
2.8	73	68	65	63	58
14.0	58	53	53	55	45
28.0	39	20	26	20	37
84.0	0	0	0	0	0

bacterial contaminants. On isolates #8 and NJ these masses were salmon colored but on #9 and S they were lucid.

Aerial mycelium was very dense at pressures from 0.5 to 13.8 atmospheres, whereas sclerotia were sparse though large at first. As the age of the cultures increased the colonies became heavily blackened with sclerotia. Mycelial growth on the sodium chloride media was always sparser than that on the glucose media and there were fewer sclerotia.

6. Survival in Soil

Procedure.—To determine the length of time that C. phomoides could survive in soil, 500-gram samples of three types of soil were placed in flasks. These soils included (1) garden soil, (2) field soil as it occurs at Blacksburg, and (3) a mixture of manure and garden soil in a 1:2 ratio. One hundred milliliters of water was added to each of the flasks and they were then treated in duplicate as shown in the following scheme:

Soil treatment	Storage temperature conditions	Inoculated with culture
Sterilized 2 hours at 15 pounds pressure	28° C.	6
		9
	outdoor weather	6
		9
Not sterilized	28° C	6
		9
	outdoor weather	6
		9

Inoculations were made February 25, 1944 and those out-of-doors were subjected to a week-long freeze at first with occasional freezes following in March and in April. To determine the time of survival of the organism under these conditions 10-gram samples of soil were removed aseptically from

the flasks and plated out at 1:10,000 dilutions using sodium caseinate agar (10) as the medium of growth. Microscopic examination determined whether the fungus grew on agar.

Results.—After three weeks the organism was isolated from all flasks containing sterilized soil and stored at 28.5° C. It was never isolated from those containing unsterile soil. After five months, it was isolated from only two flasks. Both of these had been sterilized and stored at 28.5° C. Both contained the 1:2 mixture of manure and soil.

7. Genetic Studies

Hansen (16), in commenting on the instability of some fungi, particularly those belonging to the Fungi Imperfecti, attributed their instability to the possession of multiple nuclei. Thus, in a culture obtained from a single conidium, three types of cultures are possible; those which are predominantly mycelial but produce few conidia, those which produce large quantities of conidia but little mycelium, and combinations which produce both abundant mycelium and abundant conidia. This is referred to as the "dual phenomenon".

Procedure.—Twenty monoconidial isolates were made from three of the five original isolates. Cultures resulting from the germinated conidia were transferred to agar slants and observed for differences in mycelial characters which would indicate the occurrence of the "dual phenomenon". After several weeks these monosporous isolates were subcultured for further observation.

As a part of the study of monosporous isolates, observations were made to determine the frequency of sectoring. Comparisons were made with the frequency of sectors when the five original isolates were subcultured.

Results.--Monosporous isolates of #8 and #9 showed no evidence that the "dual phenomenon" occurs in this species. Sectors did not occur until the isolates were subcultured and these occurred only in two of the twenty monosporous cultures of isolate #8. During other experiments it was noticed that isolate #6 sectored once in 14 transfers; #8 sectored once in 23 transfers; #9 sectored once in 31 transfers; S sectored once in 40 transfers and NJ sectored once in 27 transfers. For the entire five isolates, sectors could be expected to occur one time in 23 transfers.

Plate VIIIB shows some typical sectors. In most cases the growth rate of the sector was greater than that of the parent mycelium; the amount of aerial mycelium increased but the number of sclerotia usually decreased. Sectors of isolate #6, however, showed a decrease in growth rate. (Plate IXA.) All isolates became more unstable; i.e., sectoring became more frequent as their age increased.

DISCUSSION

Experiments show that differences are consistently occurring among the five isolates of Colletotrichum phomoides. A very remarkable correlation can be seen in the response of the isolates to temperature, if the mean temperatures at the geographical origins of the isolates are compared with the optimum temperatures for growth of the isolates in vitro. This is shown below.

Isolate	Origin	Mean temperatures, 1943		
		July	August	Optimum
6	Diamond Springs Princess Anne Co.	26.5° C.	26.0° C.	33.8° C.
9	Cheriton Northhampton Co.	26.0° C.	25.5° C.	24.5° C.
NJ	New Jersey	23.5° C.	23.0° C.	32.0° C.
8	Blacksburg, Va.	21.0° C.	23.0° C.	28.0° C.

From the above, and with the aid of plate IA, it can be seen that the response to temperature was greatest as the mean temperature of the geographical origin increased with the exception of isolate #9. On the other hand, the correlations may be caused by chance arrangement of the isolates and hence may be meaningless. It might be of interest to study the effect of natural environmental conditions on the behavior of these and other isolates of the anthracnose fungus in vitro.

The effects of different hydrogen-ion concentrations are variable for each isolate but the range for growth was found to be from pH 2.4 to pH 11.1. In most cases the rate of growth decreases sharply between pH 4.0 and 5.0 at the acid end and between 9.5 and 10.5 at the alkaline end. The organism grows readily at any pH between 5.0 and 9.5. Plate IIA and B shows that the growth rate of #6 was much greater than that of the other isolates; in fact, it was so much greater that above pH 4.35 it reached the edges of the petri dish in six days, while the other isolates still occupied only a part of the surface of the agar. This necessitated the straight broken line plotted for #6 in series one, and the reduction of the period of incubation in series two from eight days to five. Whether growth is influenced more by the concentration of hydrogen- and hydroxyl-ions or by the toxicity effects of sodium and chlorine ions is not understood from these experiments.

Increase in pH seemed to have no effect on spore and sclerotium sizes (Table III) but did influence the production of spores. Microscopic examination revealed that isolate #6 did not produce spores at any pH, #8 sporulated at all hydrogen-ion concentrations below and including pH 7.25 but not at any pH above that figure, while #9 sporulated where the pH rose as high as 9.3. Isolate NJ failed to sporulate at any pH below 3.64. Only isolate S produced spores throughout the entire pH range. No explanation can be given for these phenomena but they suggest physiologic specialization.

All isolates produced increasing amounts of aerial mycelium from pH 4.0 to pH 9.0. Beyond either of these extremes they produced only surface or subsurface mycelium.

In considering the importance of pH to the development of anthracnose, it has been reported (30) that the pH of tomato fruit changes but little during ripening (from between 4.1 to 4.0 ±; a very slight increase in acidity). This change in acidity would result in less growth but we know that green fruits are seldom attacked by C. phomoides and ripening would further reduce their susceptibility to infection if pH is a major factor for anthracnose development in fruits.

The growths on the seven different media show that carbohydrates favor the development of C. phomoides more than do proteins. The growth on nutrient and malt agars were always less than on potato dextrose and corn meal agars. For the most part the organism grew better on ripe tomato agar than on green tomato agar. This indicates that during the ripening process some change takes place, other than change of pH, which makes the plant sap a more favorable medium for the growth of the fungus. It has been reported (30) that during the ripening process of tomatoes the sugar content changes from 25 per cent of the total carbohydratesto 48 per cent. Starch decreases from 15 per cent to 2.5 per cent of the total carbohydrate content. Although these changes appear large they are really quite small for the total mass of the fruit. The total sugar content changes from 1.7 per cent to 2.6 per

cent of the total mass and starch changes from 1.1 per cent to 0.18 per cent of the total mass. Perhaps it is these changes that make the ripe fruit agars more favorable for growth of the fungus than is the green fruit agar and similarly makes anthracnose more severe on ripe fruit than on green ones.

On all the agars made from the tomato extracts there was a tendency to produce subsurface mycelium, while on those of non-tomato origin there was a tendency to produce surface and aerial mycelium. Aerial mycelium was most abundant on potato dextrose and malt agars which were highest in sugar content. Likewise on these same two media sclerotium production was greatest indicating that sugar favored their formation.

Osmotic pressure seems to be of no significance to the occurrence of anthracnose on ripe fruits. The results show that at zero atmospheres there is no growth on artificial media but above 0.5 atmosphere growth is rapid and rich. Obviously the osmotic pressure in tomatoes never approaches 0 because the average sugar content, even in green tomatoes when it is lowest, is never below 1.7 per cent. The presence of sodium chloride which modified the osmotic pressure but did not materially affect the nutritive value of the media, shows that the presence of sodium or chlorine ions does not create a toxicity factor and hence causes no harmful effects when sodium hydroxide and hydrochloric acid are used to adjust media for pH studies.

The studies in different media indicated that sugar favors sclerotium formation more than do other types of compounds. The glucose media used in the osmotic pressure experiment help to confirm this, but it appears that sclerotium formation is delayed as long as there is an abundance of material with which the organism can carry on active metabolism.

The formation of black rings in the anthracnose lesions on fruit has often been reported (1, 4, 9, 24, 25, 34). These rings were produced by the organism *in vitro*, and it was shown that they were caused by alternating daylight and darkness, that after exposure to sunlight sclerotium production was inhibited temporarily but the inhibiting effects of sunlight were overcome during the ensuing darkness, resulting finally in uniform sclerotium distribution. Plate IB shows the effect wherein both cultures 6B and 8B show uniform distribution of sclerotia at their centers, the rings that were produced have been almost obliterated by sclerotium production. Likewise, the rings that were produced in cultures 6C and 8C by exposing them to sunlight during the fourth day of incubation have been rendered indistinct. This phenomenon explains the occurrence of blackened centers in old anthracnose spots.

The survival of *C. phomoides* in soil appeared to be favored largely by reduction of other microorganisms in soil and by high content of organic matter. However, it may be that abundance of faster growing organisms which overran the anthracnose fungus made it difficult to recover by ordinary

dilution plate methods. The fact that the organism was recovered after five months from soil high in organic matter suggests the possibility of the fungus surviving in crop residue over a considerable period of time.

The twenty monoconidial isolates each made from isolates #8 and #9 showed no indication of the "dual phenomenon" (see procedure) but it must be remembered that Hansen (16) reported only fungi which possessed multinucleate conidia show this phenomenon. Before monosporous isolations were made from cultures #8 and #9 they sectored very frequently when transfers were made from old cultures and sclerotia were used to inoculate fresh agar slants, but when young hyphae were transferred very few sectors appeared. However, after monosporous cultures were established sectoring appeared to be very rare even when sclerotia were used for the inoculum. This indicates that the nuclei in isolates that were subcultured many times were very unstable and mutations took place there more readily. This evidence is confirmed by the fact that nearly all sectors originated at the center of colonies (Plate VIIIB). The morphological characters of the five isolates are of two distinct types based on sizes of sclerotia; those with sclerotia having an average diameter less than 450 microns, #6 and NJ; and those with an average diameter greater than 500 microns, #8, #9, and S. By careful examination any isolate can readily be placed in one of these catagories. Further, the consistently different rates of growth found in both series of pH studies points toward

the existence of physiologic strains. It is of interest to note that sectors arising from the same isolate are morphologically similar to each other but are quite different from the parent mycelium in rate of growth and in size and distribution of sclerotia. It appears that these sectors are entirely new strains, a fact which supports the idea that physiologic and morphologic strains of C. phomoides exist.

Before there can be any further distinction between strains a larger number of isolates should be studied.

CONCLUSIONS

After considering the foregoing evidence the following conclusions may be drawn:

1. Isolates of Colletotrichum phomoides of different geographical origins show growth at temperatures ranging from 10° C. to 42° C.; optimums range from 24.5° C. to 33.8° C.

2. The isolates tolerate hydrogen-ion concentrations ranging from pH 2.2 to pH 11.0; variations in pH between 4.5 and 9.75 have little effect on their growth, making selection of optimum pH for growth impractical.

3. Daylight inhibits the speed of formation of sclerotia but does not prevent their formation. It has no effect on the rate of growth.

4. Carbohydrates are more favorable to the growth of C. phomoides than are proteins. Abundance of sugar seems to favor sclerotium production but large quantities of it delay the formation of sclerotia.

5. The organism tolerated osmotic pressures between 0.5 and 28 atmospheres.

6. A change in the carbohydrate system of tomatoes during their ripening yields more sugar making ripe fruits a better medium for growth of the anthracnose fungus.

7. The organism seems able to survive in warm soils which contain large amounts of organic matter but it is destroyed by prolonged freezing and lack of organic matter.

8. Monoconidal cultures fail to show the "dual phenomenon". Sectors occur more frequently in old cultures. Cultures may be temporarily stabilized by making monosporous isolations.

9. Differences in morphologic and physiologic characters reveal the existence of strains of C. phomoides. New strains may arise by sectoring.

SUMMARY

Anthracnose of tomato was first described by Chester in 1891. It has been of economic importance to the growers of canning tomatoes nearly ever since. Anthracnose is caused by Colletotrichum phomoides (Sacc.) Chester which appears to have different physiologic and morphologic forms. There is evidence that it is a soil organism but this remains to be definitely proved. The organism has a broad pH range and different isolates show different temperature optimums but about

the same temperature range for growth. The organism does not show the "dual phenomenon" but does sector frequently. Sectors are morphologically different from the parent mycelium and offer an explanation as to the origin of different strains of the organism.

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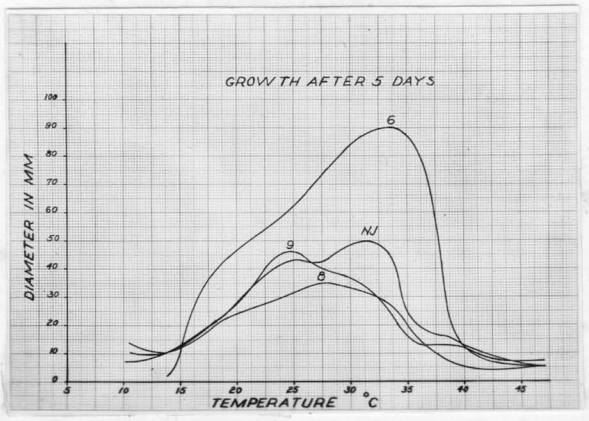
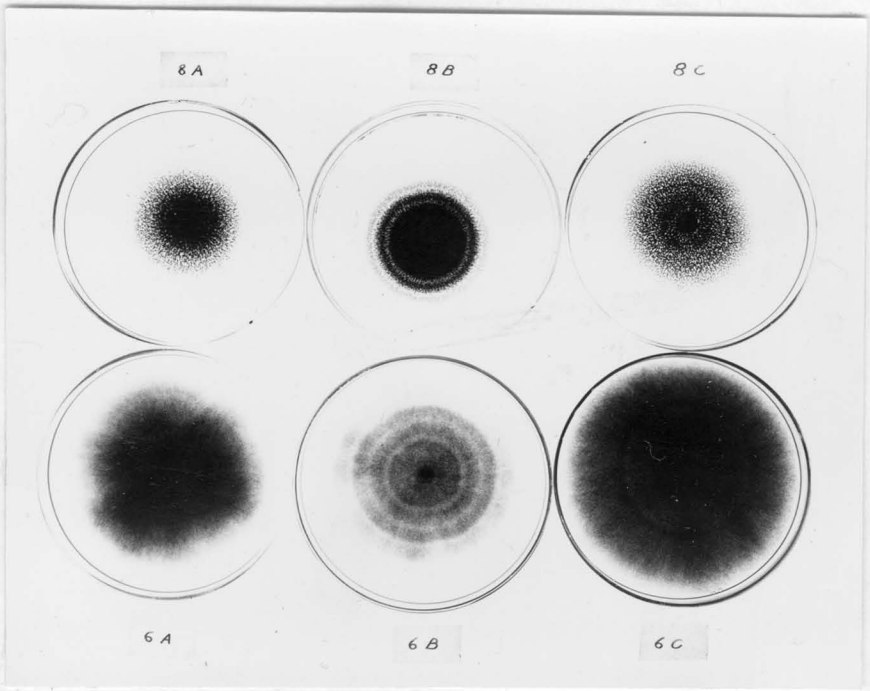
Plate I

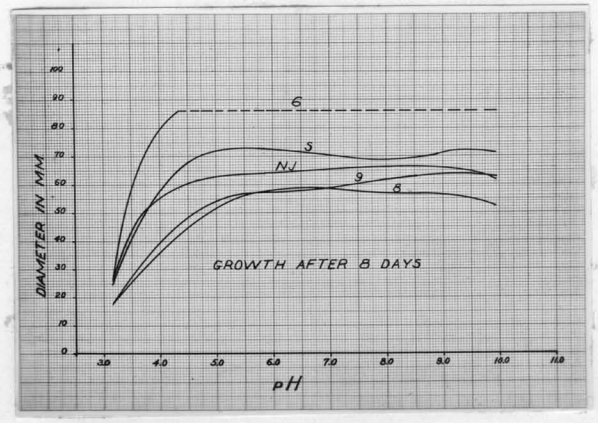


A.—Growth curves showing the effect of temperature on four isolates of Colletotrichum phomoides.

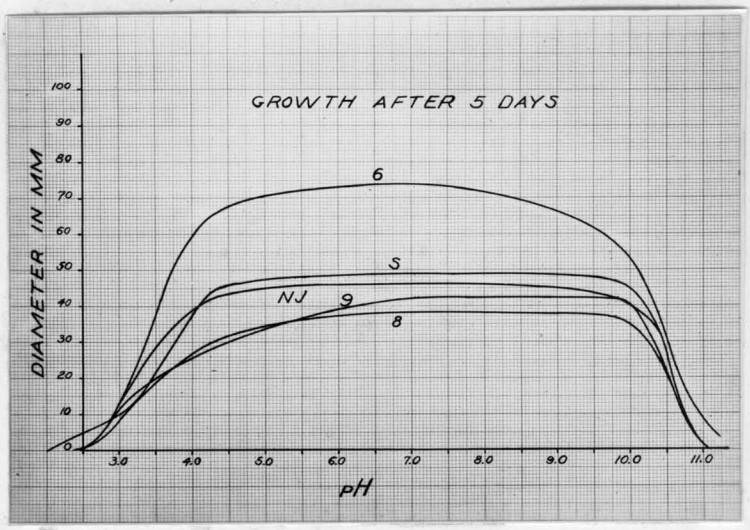


B.—Response of isolates #6 and #8 to light; A, grown in darkness; B, alternate day and night; C, grown three days in darkness, one day in light and three more days in darkness.

Plate II

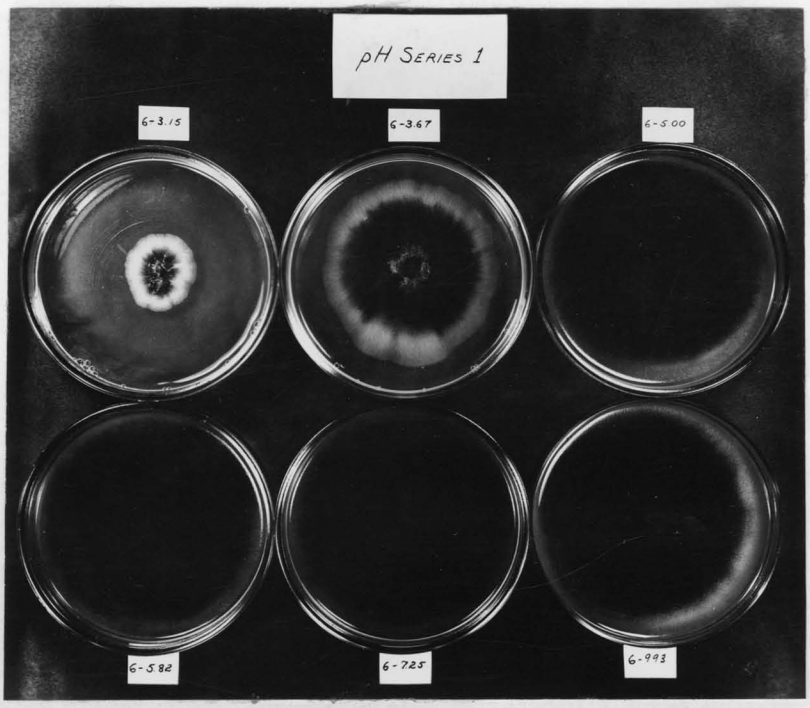


A.—Growth curves showing the effects of various pH values on five isolates of Colletotrichum phomoides, series 1.

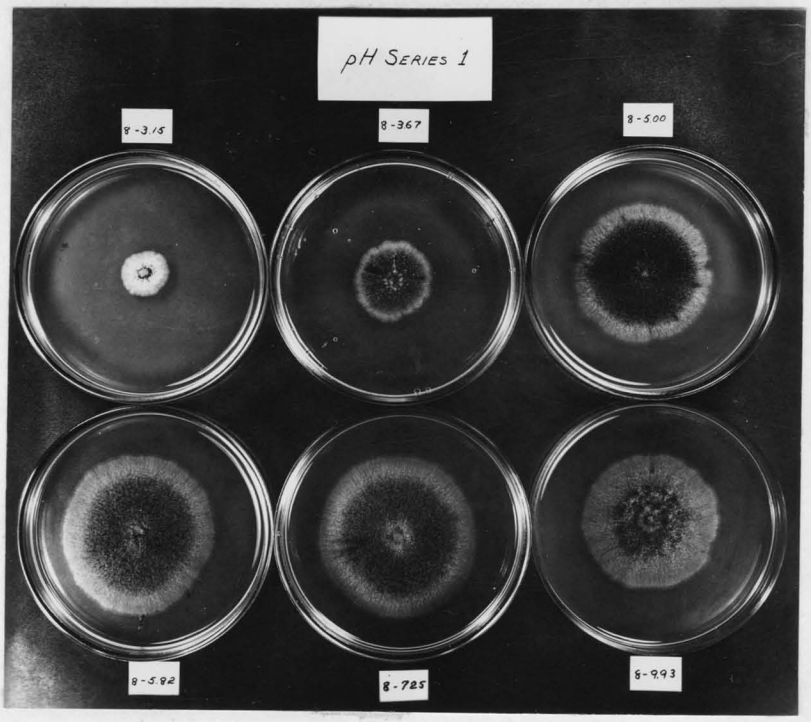


B.—Same as A, series 2.

Plate III

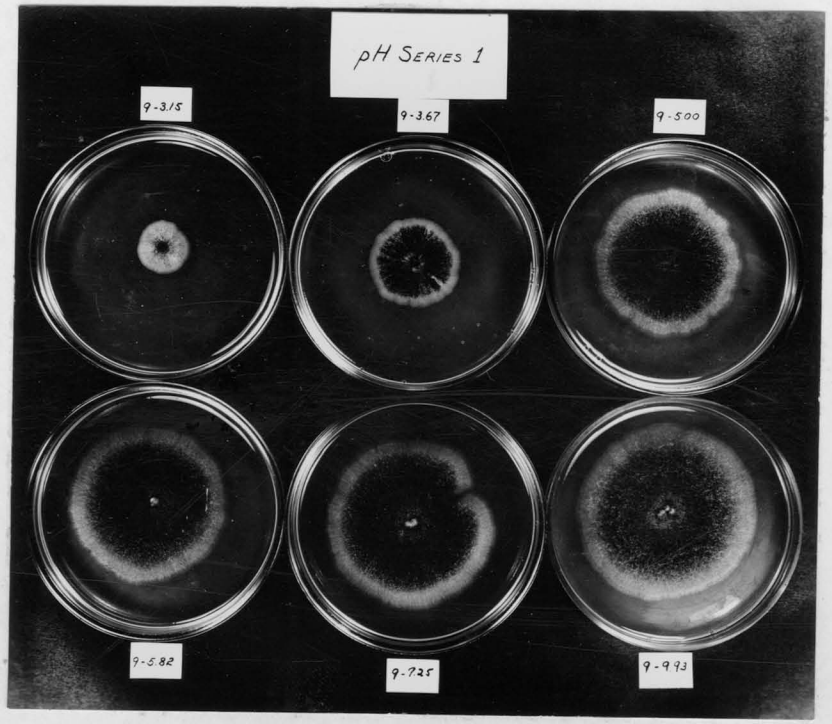


A.—Response of isolate #6 to various pH values in series 1.

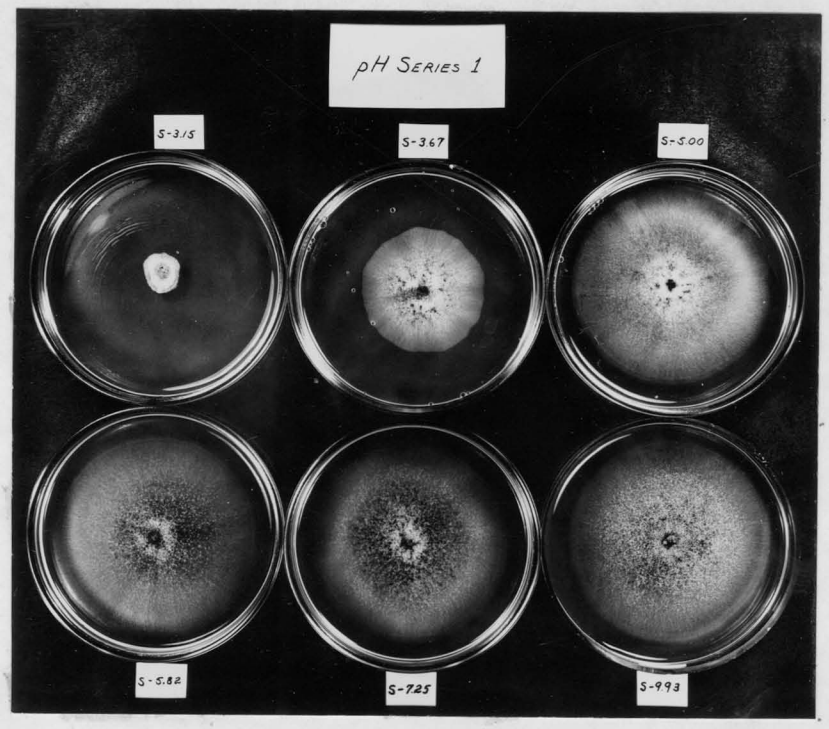


B.—Response of isolate #8 to various pH values in series 1.

Plate IV

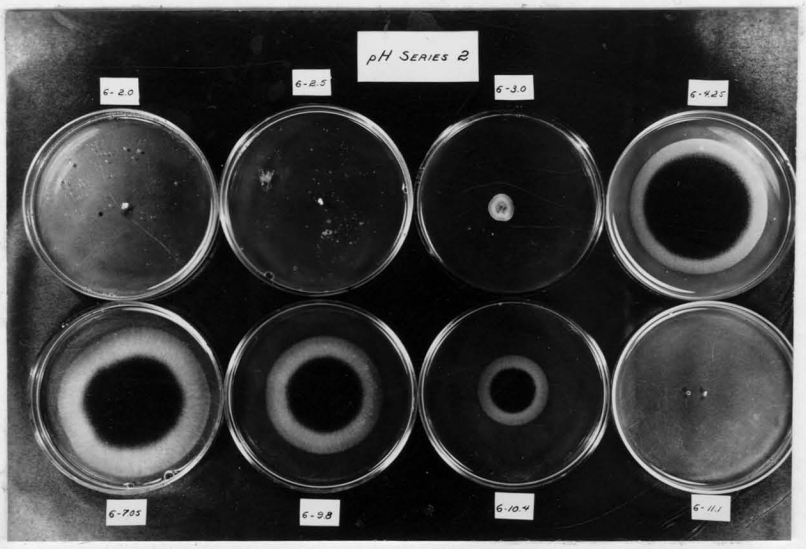


A.—Response of isolate #9 to various pH values in series 1.

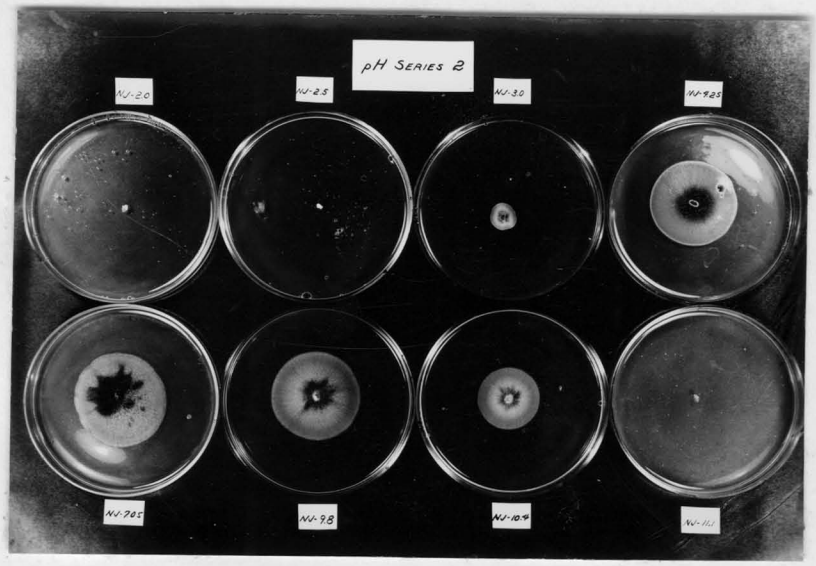


B.—Response of isolate S to various pH values in series 1.

Plate V

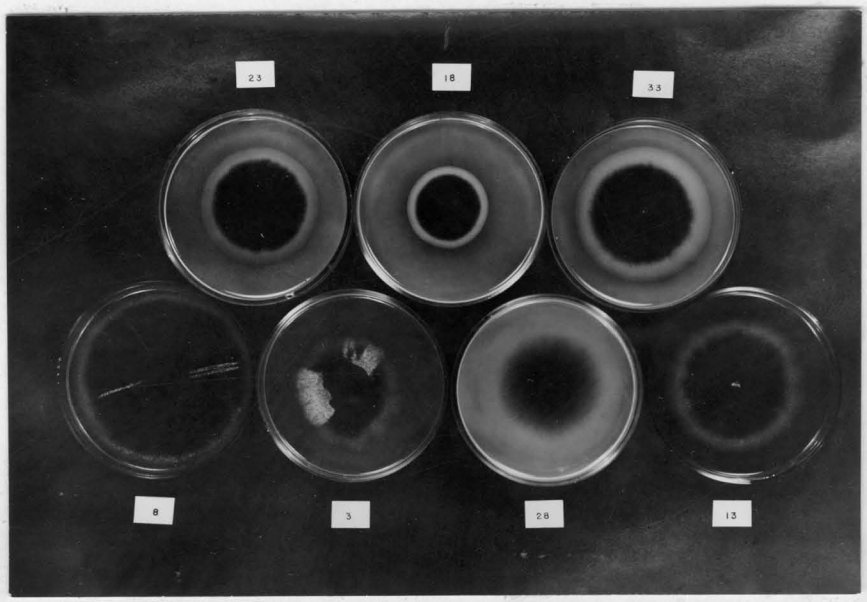


A.—Response of isolate #6 to various pH values in series two.

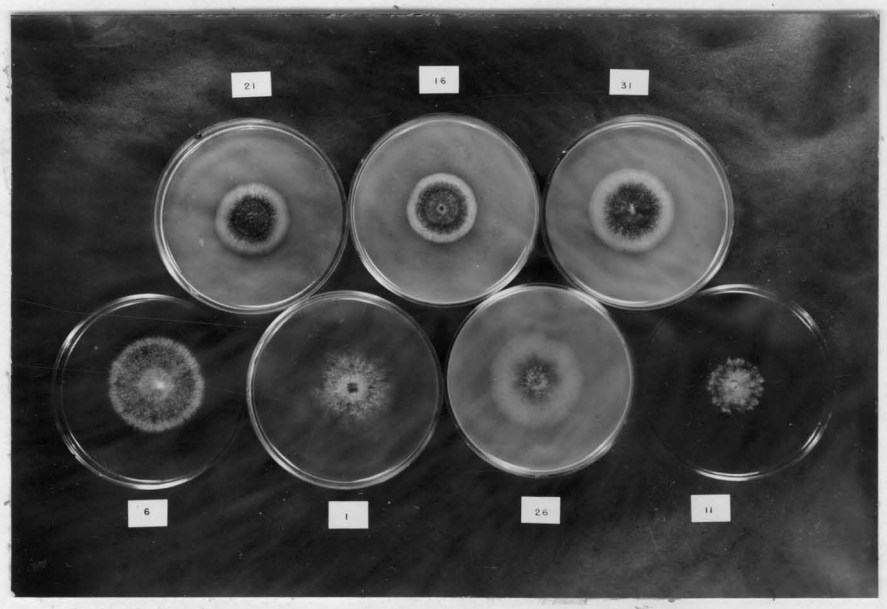


B.—Response of isolate NJ to various pH values in series two.

Plate VI



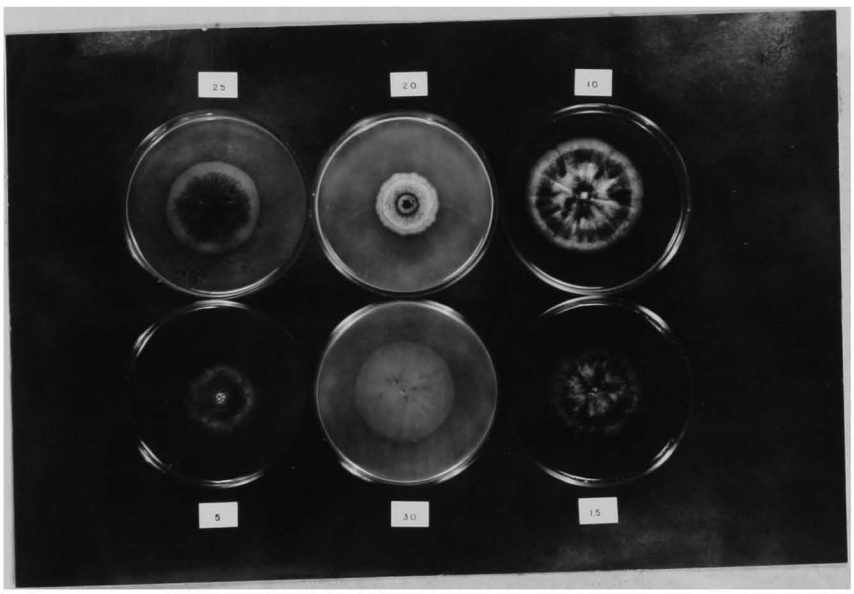
A.—Isolate #6 shown on various agars: 23, yellow tomato; 18, green tomato; 33, red tomato; 8, potato dextrose; 3, nutrient; 28, corn meal; 13, malt.



B.—Isolate #8 shown on various agars: 21, yellow tomato; 16, green tomato; 31, red tomato; 6, potato dextrose; 1, nutrient; 26, corn meal; 11, malt.

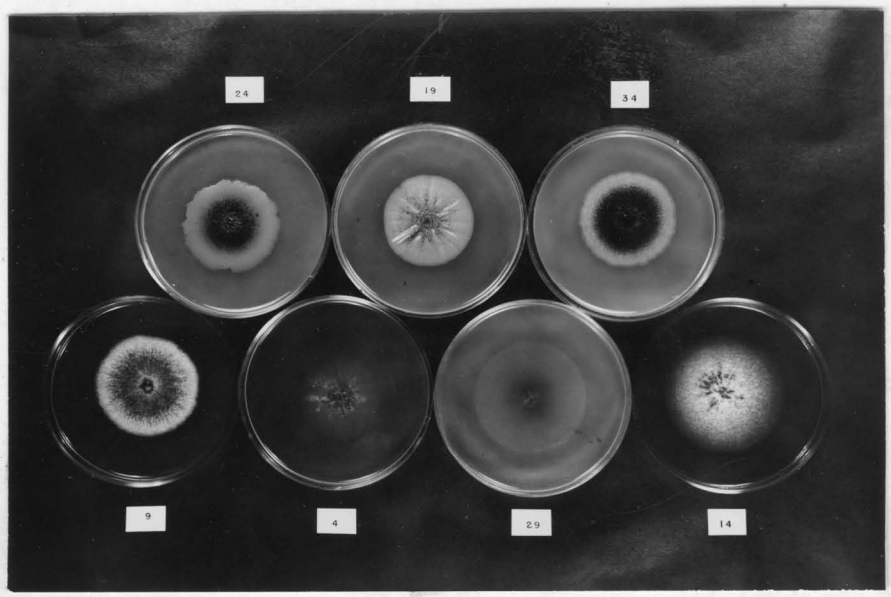
Plate VII

A.—Isolate #9 shown on various agars: 22, yellow tomato; 17, green tomato; 32, red tomato; 7, potato dextrose; 2, nutrient; 27, corn meal; 12, malt.

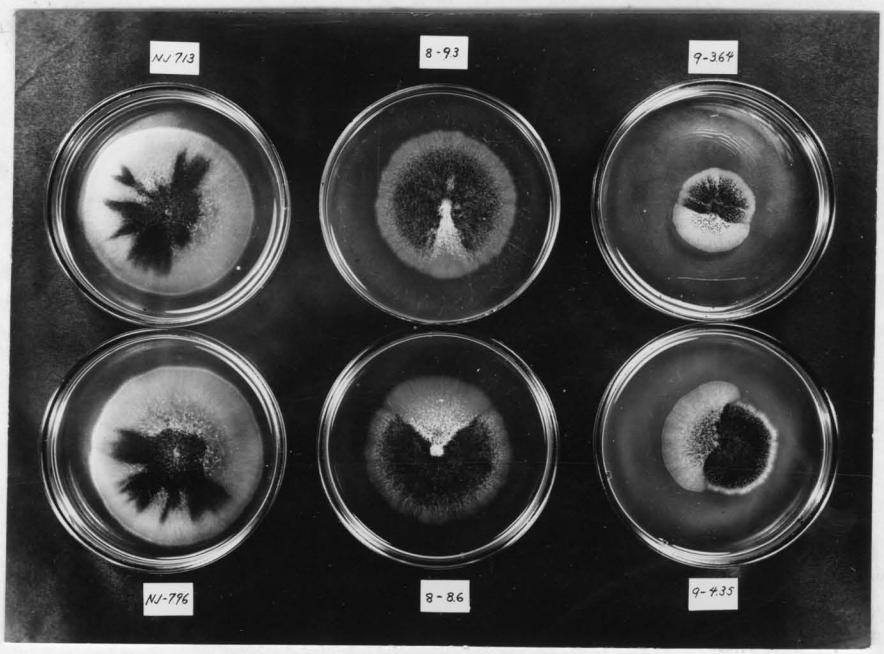


B.—Isolate NJ shown on various agars: 25, yellow tomato; 20, green tomato; 10, potato dextrose; 5, nutrient; 30, corn meal; 15, malt.

Plate VIII

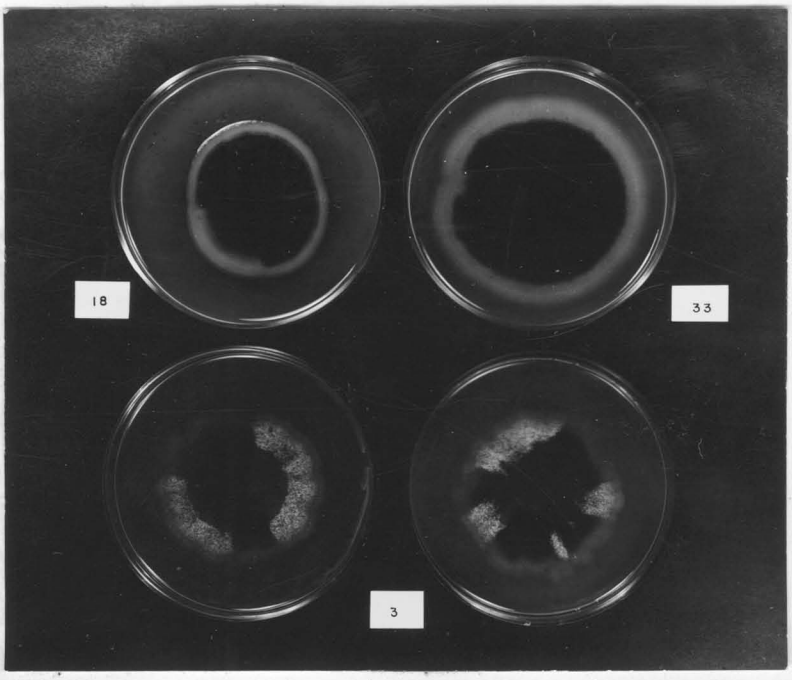


A.—Isolate S shown on various agars: 24, yellow tomato; 19, green tomato; 34, red tomato; 9, potato dextrose; 4, nutrient; 29, corn meal; 14, malt.



B.—Cultures of three isolates showing typical sectors. Note change of rate of growth in isolates #8 and #9.

Plate IX



A.--Cultures of isolate #6 showing two types of sectors; those in which the rate of growth of the sectored mycelium was less than that of the parent myceliums (18 and 33) and those in which the sectored mycelium was predominantly aerial, whereas the parent mycelium was surface or subsurface (3).