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THE INFLUENCE OF HYDROGEN-ION CONCENTRATION ON THE GROWTH OF *FUSARIUM LYCOPERSICI* AND ON TOMATO WILT

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THE INFLUENCE OF HYDROGEN-ION CONCENTRATION ON THE GROWTH OF *FUSARIUM LYCOPERSICI* AND ON TOMATO WILT

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Abstract.—The influence of the reaction on the growth of the fungous parasite *Fusarium lycopersici* and its capacity to infect the host was the subject under investigation. In four experiments where the fungus was grown in mineral nutrient solutions of varying reactions it was found that there is a limited range of acidity where the growth is at a minimum within the normal growth range of the organism. Tomato plants were grown in a series of soils with varying reactions obtained artificially by the use of acid or alkali. The seeds were inoculated with a spore suspension of *Fusarium lycopersici* and allowed to germinate and grow under greenhouse conditions favorable for tomato wilt. A minimum of wilting was found to occur in soils with reactions more or less near those at which growth of the fungus was at a minimum in the cultural experiments. The results obtained suggest a possibility of adjusting soils to reactions unfavorable for wilt by proper soil treatments, or that by using soils with natural reactions unfavorable for infection a minimum of wilt would be obtained.

This investigation is concerned with the determination of the influence of hydrogen-ion concentration upon the growth of *Fusarium lycopersici* and its capacity for infecting the host plant, the cultivated tomato (*Lycopersicum esculentum*).

The disease resulting from the infection of the tomato plant by *Fusarium lycopersici* is variously known as tomato wilt, Fusarium wilt, and sleepy disease. It is a disease of considerable economic importance in various sections of the United States, as well as in most parts of the world where tomatoes are extensively grown. Pritchard[27] reports that the annual loss in the United States from tomato wilt is 115,000 tons, which is more than four times the total tomato production in Missouri for 1921. The Plant Disease Survey of the United States Department of Agriculture for 1921 and 1922 gives the estimated loss from tomato wilt in the several states where it is most prevalent as follows:

NOTE:—Also presented to the faculty of the Graduate School of the University of Missouri in 1923, in partial fulfillment of the requirements for the degree of Master of Arts.

State	Per cent loss 1921	1922	State	Per cent loss 1921	1922
Pennsylvania	1 to 2	1	Louisiana	25	20
Maryland	5	Present	Texas	5	8
Virginia	30	Slight	Arkansas	10	20
Kentucky	33	6	Missouri	5	20
North Carolina	6	Trace	Indiana	3	4
South Carolina	3	3	Kansas	5	7
Tennessee	Severe	10	Alabama	Moderate	--
Mississippi	15	15	Ohio	15	12
Illinois	Moderate	10	Florida	Moderate	Present
New York		2½			

The greatest losses in Missouri have been reported from St. Louis County and from Southwest Missouri. From all indications the disease is spreading in Missouri as well as in the other states where it is generally prevalent. Losses in some of the commercial fields in Missouri averaged from 5 to 10 per cent during the years 1921 and 1922, but losses as high as 75-100 have been reported. The tomato production in Missouri as reported by the United States Department of Agriculture[34] was 25,262 tons for the year 1921. A loss of only 5 per cent of the crop in Missouri would entail a decrease of 1,263 tons of tomatoes yearly, based upon the production of 1921. At the present time the acreage of tomatoes grown is on the increase. As will be subsequently shown the disease is one that is difficult to eradicate and when once established proves a serious menace to tomato growing for market and canning purposes.

Several investigators have shown that the hydrogen-ion concentration of the substratum is a factor of importance affecting the virulence of some soil-borne plant pathogenes. The experimental work reported herein was undertaken to determine the relation of this factor to *Fusarium lycopersici* and its virulence. If it can be demonstrated that a particular plant pathogene living in the soil and infecting the host from the soil has a definite range of hydrogen-ion concentration at which its capacity for infection is at a minimum, then we may have a basis for working out successful control measures based upon the true acidity of the soil.

The experimental work in the study of the relation of *Fusarium lycopersici* to the hydrogen-ion concentration of the substratum resolved itself into two phases: (1) The relation of the growth of the organism to the hydrogen-ion concentration of culture media. (2) The relation of the capacity of the organism for infection to the hydrogen-ion concentration of the soil.

TOMATO WILT: DESCRIPTION OF THE CAUSAL ORGANISM AND THE DISEASE

Fusarium lycopersici Sacc. is the causal organism of tomato wilt. It was briefly described by Saccardo[29], Vol. 4, p. 705, in 1886. Several other species of fungi, particularly of the genus *Fusarium,* have been reported as causing a wilt of tomato, but apparently are not the causal organism of "true" Tomato Wilt caused by *Fusarium lycopersici* Sacc. A species of *Verticillium* causing a wilting or "Sleepy Disease" of tomato has recently been studied by Bewley[4]. The Western Blight or Yellow Blight of the West and Northwest as noted by Pritchard[35] and Humphrey[21] are caused by other species of Fusaria, and possibly by other species of fungi such as Rhizoctonia. The summer blight reported by Smith[31] from California in 1906 and referred to by Stevens[32] is now quite certainly known to be caused by a species of *Fusarium* other than *F. Lycopersici.* It has been suggested[35] that there are at least three species of *Fusarium* causing a wilting or blighting of tomato, but that the "true" tomato wilt or Fusarium wilt of the southern, southeastern, and central United States is undoubtedly due to *F. lycopersici.* All isolations made from wilted tomato plants by the writer have been found to be identical with an authenticated culture of *F. lycopersici* which had been obtained from Dr. C. W. Edgerton, of the Louisiana Agricultural Experiment Station.

F. lycopersici Sacc. belongs to the sub-group *Elegans,* of the genus *Fusarium,* and is closely related morphologically and physiologically to several other Fusaria producing wilts of the higher plants, particularly to *F. oxysporum,* one causing the Fusarial wilt of potato.

The fungus has been found to grow well on various kinds of ordinary culture media. The writer has obtained profuse growth on plain nutrient agar, potato dextrose agar, oatmeal agar, boiled rice, several mineral nutrient agars, and in synthetic nutrient solutions. It grows well over a wide range of acidity on solid media though the rate of growth varies at different reactions. Spore production seems to be limited to a markedly acid reaction on potato dextrose agar and in several culture solutions employed. It is probable that the reaction would be found to be an important factor in spore production on a greater variety of media than that on which it was observed. Spore production is also quite strong in boiled rice cultures. Both microconidia and macroconida are produced.

Strong color production occurs on solid media, particularly in boiled rice cultures, where the shade varies from light pink to old

rose, sometimes with areas of reddish-purple. It is probable that the variation in color observed depends upon moisture relations, reaction of medium, oxygen supply, and other factors. However, in a number of like cultures the color appears to be fairly constant for the species. On potato dextrose agar the color may vary with the reaction.

Infection of the host occurs through the root system as stated by Edgerton and Moreland[9], and Humbert[20]. The root-hairs probably offer the most favorable point of entrance for the pathogene, but it is possible that the fungus may enter at other points in the root system. The plants do not immediately wilt as a result of infection. The lower leaves generally wilt first, often turning yellow and sooner or later dropping off, followed by a characteristic drooping of the entire plant. However, young plants have been observed to wilt when only 3 to 4 inches high if grown in highly infected soil under greenhouse conditions. The organism seems to be confined to the vascular system of the host, and is found penetrating the vessels. The bundles in infected roots and stems become yellow to dark brown in color and show up quite distinctly to the naked eye when viewed in cross or longitudinal section. The fungus penetrates well up into the stem and sometimes into the bundles of the leaves in nearly mature plants. Two explanations have been suggested to account for the wilting: (1) The fungus interferes with the water supply of the plant through the gradual plugging of the vessels; or (2) the fungus produces certain toxic materials which in some way interfere with the proper functioning of the host tissues and bring about improper water relations with consequent wilting[15], [17]. The plant slowly dies after pronounced wilting occurs.

There is no external appearance of the fungus on the living host. There is no evidence that the host can become infected through the aerial parts, so that the spores produced externally on dead stems spread the disease only by being carried to uncontaminated soils where they lodge and infect seedling tomato plants. The chief modes of dissemination are probably through seedling plants grown in infected seed beds, and by infected seed. McClintock[22] particularly calls attention to the fact that seedlings shipped from the South to the North for use by market gardeners are likely to be infected unless care is taken to grow them in wilt-free soil. Edgerton and Moreland[9] state that dissemination is chiefly through diseased seedlings and probably by the seed. Elliott and Crawford[11] found *F. lycopersici* to be carried externally on tomato seed, and probably internally although their results are not very conclusive as to the latter.

When once established in the soil the organism is capable of living over the winter as a saprophyte on and in the dead stems of wilted tomato plants and on other organic matter in the soil. It is capable of living in the soil for several years and of retaining its virulence although the host plant has not been grown. Edgerton and Moreland[9] state that it decreases year by year after the growing of tomatoes ceases and that heavy infection is not likely to occur after three years. Humbert[20] states that a four-year rotation is not long enough to eliminate the fungus. In general, it appears to be quite persistent in the soil when once established there.

Few data are available as to what types of soil show the greatest amount of infection by *F. lycopersici.* The disease becomes quite a serious problem in greenhouse seed beds. Edgerton and Moreland[9] especially note that it is serious on the bluff, prairie, and sandy soils of Louisiana, but is of little importance on the heavy alluvial soils. McClintock[22] reports it to be most severe on the coastal plain and the heavier soils of the Piedmont region of Georgia. The various reports of the Plant Disease Survey show that it is quite widely distributed so that there is little doubt but that it is found on a large number of soil types. The writer has found the wilt serious on the Putnam silt loam in Central Missouri, the infection being as high as 100 per cent in some cases. The reaction of this soil type in a limited number of samples determined was between pH 5.1 and 5.5.

Various factors affect the severity of the disease. The optimum temperature for the growth of *F. lycopersici* as well as for the development of the wilt has been found by Clayton[6] to be about 28° C., although good growth of the fungus is secured from 18° to 31° C. In rather extensive soil experiments at the Wisconsin Agricultural Experiment Station in which tomato plants were grown at different soil temperatures, Clayton[6] found the most pronounced and rapid wilting occurring at a soil temperature of either 27° or 33° C. Hence, he concludes that a soil temperature and an air temperature around 27° C. are most favorable for pronounced wilting of the tomato plant. He likewise found the disease more active on clear days or partly clear days than on cloudy days. At soil temperatures lower than near the optimum the disease was confined to the lower part of the host and the characteristic drooping wilt did not occur, a fact that has been verified by the writer in greenhouse experiments. Edgerton and Moreland[9] also state that the optimum temperature is about 28° C., and Edgerton[35] maintains that no wilt occurs at temperatures of 50°

to 60° F., the temperature relations being due to an effect on the fungus rather than on the host.

Clayton[7] has recently studied the relation of soil moisture to Fusarium wilt of tomato. He found that when tomato plants were grown in crocks containing sterilized soil inoculated with a spore suspension of *F. lycopersici,* they showed considerable resistance to wilt in a soil with a moisture content of 13 to 19 per cent. Thirty-five per cent moisture content represented complete saturation in the soils used. In soils that were kept saturated he found the plants immune. Any moisture shortage sufficient to check vegetative vigor of the plant checked the disease to a greater or less extent. Plants in water-logged or saturated soils showed high infection in the experimental work reported in this paper.

Aside from the practice of long-time rotations, soil disinfection in the seed bed, or complete abandonment of tomato growing for quite a long period, the principal method of control of this disease suggested at the present time is by the use of resistant varieties. Norton[26], Durst[8], McClintock[22], Edgerton[10], Edgerton and Moreland[9], Humbert[20], and others have worked on the development of resistant varieties or strains. Recently Pritchard[27] has succeeded in finding varieties which he records as producing excellent yields on heavily infected fields. He states that these varieties are well suited to parts of the country where the wilt is prevalent.

Edgerton[10] attempted to control infection in seed beds by the use of lime and other soil treatments. He succeeded in obtaining considerable decrease in number of diseased plants by treating the soil with 5 to 10 tons of lime per acre but doubts the economic practicability of using such large amounts. Treatments with formaldehyde and corrosive sublimate in sufficient quantities to check infection proved toxic for the host plant.

None of the earlier work on control by cultural methods has taken the reaction of the soil into consideration, except from a more or less qualitative standpoint. That this is a factor of considerable importance has been shown in connection with the study of other soil-borne plant pathogenes as will be pointed out.

THE RELATION OF THE HYDROGEN-ION CONCENTRATION TO INFECTION BY PLANT PATHOGENES.

Gillespie and Hurst[16], [15] first pointed out definitely the relation between hydrogen-ion concentration of the soil and infection by plant pathogenes. They observed that potatoes grown on the Washburn

loam soil in Maine were more susceptible to scab than potatoes grown on the Caribou loam. Electrometric determinations of the hydrogen-ion concentration of a large number of samples of potato soils of northern Maine revealed a good correlation between the hydrogen-ion concentration and the prevalence of potato scab. This fact accounted for the variation in the amount of scab in the two types of soil as observed. Potato scab was rarely found on soils with a pH of 5.2 or less, while those with less acidity generally produced scabby potatoes. Gillespie[13] found that the causal organism grown in culture media developed more slowly and less vigorously at pH 5.2 than at values higher than this. Those strains started at initial values from pH 4. 8 to 5.2 later grew better, but in all cases the growth was accompanied by a decrease in acidity of the medium. Gillespie states that as a general rule a pH of 4.8 to 5.0 gives little growth. It would be expected that soils having comparatively low exponents (pH 5.2 or less) would result in a marked decrease in infection, while those with a reaction approaching neutrality and upwards would show heavy infection. This is actually found to be the fact in most cases.

Martin[23] attempted to control infection of the potato by the scab organism by the application of varying amounts of sulphur with the intention of increasing the hydrogen-ion concentration of the soil. He found that when the amounts of sulphur applied were increased the hydrogen-ion concentration of the soil extracts increased, resulting in a corresponding decrease in the number of scabby potatoes. The increase in acidity, was, in most cases, proportional to the amount of sulphur applied. When inoculated sulphur was used the greatest number of clean tubers was obtained. The New Jersey Agricultural Experiment Station is now recommending the application of sulphur for the control of potato scab[24].

Hopkins[18] found that *Gibberella saubinetii* would grow over a wide range of hydrogen-ion concentration in culture solutions and on potato dextrose agar. He used three series of media, the first being a mineral nutrient solution adjusted to varying reactions by means of sulfuric acid and sodium hydroxide; the second a mineral nutrient solution adjusted to varying reactions by means of primary and secondary potassium phosphates and potassium hydroxide; and the third, potato dextrose agar adjusted by means of lactic acid. In most cases his curves show growth beginning weakly near an average pH of 3.0 and increasing to a more or less marked maximum between an average pH of 3.9 to 5.5, followed by a minimum between an average pH of 5.5 and 6.0, with a secondary maximum at about pH 7.0. In a series

of soil experiments under greenhouse conditions where the reaction of the soil was adjusted with sulfuric acid and sodium hydroxide, and hydrochloric acid and sodium hydroxide it was found that a minimum seedling infection of the wheat plant by *Giberella saubinetii* occurred near pH 5.5.

Arrhenius[1, 2] found *Bact. solanacerarum* causing serious infection in acid soils, but in neutral and well buffered alkaline soils the host plant (tobacco) was seldom diseased. He found further that when the organism was grown on agar the growth was strongly inhibited by alkalinity, maximum growth occurring at pH 6.0 and decreasing above or below this point.

From a comparatively early date liming of the soil was recommended for the control of the Club-root or Finger-and-Toe disease of Cruciferous plants. Tubeuf[33] states that "Massee points out that development of the fungus is favored by acids and checked by alkalies." Practically all recent references to the control of this disease recommend the application of materials to the soil that will render it more alkaline. It is probable that the causal organism (*Plasmodiophora brassicae* Wor.) has a definite range of hydrogen-ion concentration at which its capacity for infection is reduced. Atkins[3] has quite recently pointed out that this organism is favored or inhibited at certain reactions of the soil, but the difference in the hydrogen-ion concentration of the two soils he studied was not marked thus making the evidence rather inconclusive.

EXPERIMENTAL WORK

Source, Isolation, Identification and Pathogenicity of the Organism.—The organism used in all the experimental work reported in this paper was isolated in September, 1921, from the stem of a completely wilted tomato plant grown on the experimental grounds of the Horticulture Department of the Missouri Agricultural Experiment Station. Microscopic examination of this organism grown on potato dextrose agar revealed characters belonging to the sub-group *Elegans* as described by Wollenweber[38] and Sherbakoff[30], and also the characters ascribed by Wollenweber[39] to *Fusarium lycopersici* Sacc. The potato dextrose agar used throughout the work reported herein was made in accordance with the formula and procedure given by Hopkins[19].

In order to obtain a single-spore strain a single macroconidium was isolated and germinated. From the colony thus obtained stock cultures were prepared and used as a source for all cultural work that followed.

Tests of the pathogenicity of the single-spore strain were made and positive results were obtained in all cases. This was accomplished by either inoculating healthy plants by puncturing the stem and inserting a needle bearing the fungus, or by spraying the seed bed of uninfected soil with a spore suspension of the organism. Sterile soil was not used but soil was obtained which was assumed not to be infected with wilt. This assumption was verified by the fact that none of the control plants showed any symptoms of wilt, and no Fusarium was isolated from them.

On the basis of source, morphological and physiological characters, comparison with an authentic culture of *Fusarium lycopersici,* and pathogenicity it seems quite certain that the organism used in these experiments was *Fusarium lycopersici* Sacc.

Growth of *Fusarium lycopersici* in Culture Solutions of Varying Hydrogen-ion Concentration.*—Four experiments were carried out in which *Fusarium lycopersici* was grown in nutrient solutions of varying acidities. The dry weight of the mycelium produced and the changes in reaction resulting from the growth of the fungus in the solutions were determined. The general procedure was the same in each of the four experiments, variations consisting of changes in the nutrient medium or in the length of the growth period. The procedure can be made clear by describing the first experiment in detail.

EXPERIMENT ONE

In this experiment the nutrient medium used consisted of:

Potassium nitrate (KNO_3)	3 gms.
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	.75 gms.
Potassium dihydrogen phosphate (KH_2PO_4)	1.82 gms.
Dipotassium hydrogen phosphate (K_2HPO_4)	3.48 gms.
Ferric chloride ($FeCl_3$)	trace
Dextrose	10 gms.
Distilled water to make	1000 cc.

The amounts of potassium dihydrogen phosphate and dipotassium hydrogen phosphate were so chosen as to give a 2 to 3 ratio of an M/30 concentration of each. This gave a buffered solution with a reaction slightly below pH 7.0. The mineral salts used were Baker's "Analyzed", and the dextrose was prepared by the Eastman Kodak Company. Normal H_2SO_4 was used to obtain the acid reactions and

*A progress note of the cultural experiments was published in Bulletin 197, page 49, Missouri Agr. Exp. Sta. 1922.

normal KOH to adjust the alkaline reactions. A titration curve (Fig. 1) of this medium using the normal acid and alkali was prepared. The hydrogen-ion concentration was determined colorimetrically using Gillespie's method[14] which had been checked against standard buffer

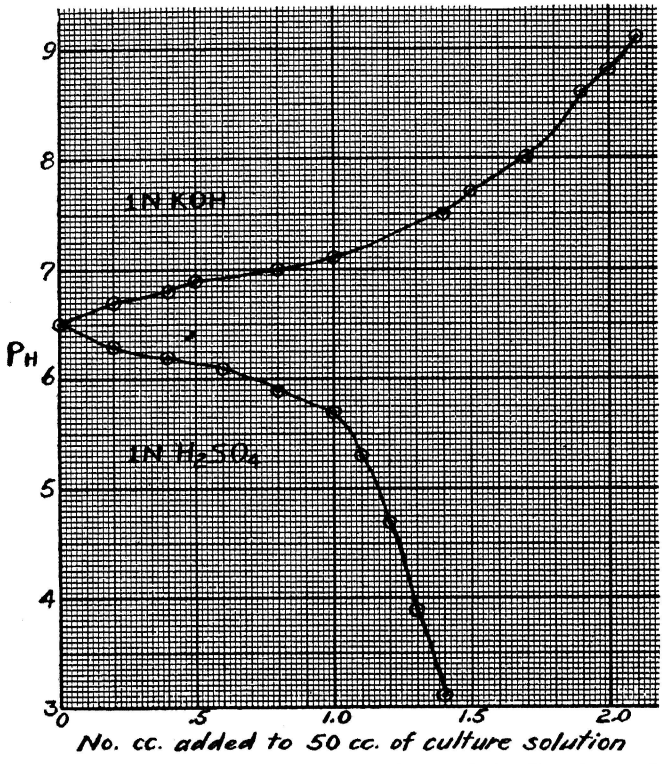

Fig. 1.—Titration curve of culture solution used in Experiment One with 1N H_2SO_4 and 1N KOH.

solutions the hydrogen-ion concentration of which had been verified with the hydrogen electrode. Using the titration curve to determine the necessary quantity of normal H_2SO_4 and normal KOH the reaction of aliquots of the basic nutrient solution were adjusted to vary by steps of approximately 0.5 Sorensen units from pH 3.0 to pH 9.0.

The mineral nutrients and the dextrose were prepared separately in double strength and sterilized separately to prevent caramelization of the sugar, particularly in the alkaline range. Twenty-five c.c. of the double-strength sterilized dextrose solution were then added aseptically to 25 c.c. of the double-strength sterilized mineral solution, thus giving 50 c.c. of the single-strength sterilized solution. As culture ves-

sels 125-cc. Pyrex flasks were used. Since there was likely to be some change in reaction due to sterilization the reaction of all culture solutions was determined colorimetrically after sterilization by removing aseptically 5 c.c. of solution from each flask. This left 45 c.c. of culture solution in each flask in which the organism was grown.

The culture solutions were inoculated by cutting out blocks of mycelium about 5 mm. square from the edge of a colony of *F. lycopersici* growing on a thin plate of potato dextrose agar. These blocks floated on top of the solution in all of the flasks. The cultures were then placed in an incubator at 28° C. One series of 15 cultures was allowed to grow 5 days; a second, for 7 days; and a third, for 15 days. At the end of each period of growth the cultures were removed from the incubator and a sufficient amount of the culture solution withdrawn to determine the hydrogen-ion concentration, it then being rinsed back into the flask so as to avoid any loss of mycelium. Ten c.c. of concentrated HCl was then added to each flask to prevent further growth. Within 48 hours the cultures were filtered through Gooch crucibles. By adding approximately an equal volume of 95 per cent alcohol to each flask the filtration was greatly facilitated. Each mycelial mat was thoroughly rinsed four or five times with 50 per cent alcohol, placed in the oven and dried until a constant weight was obtained.

TABLE 1.—DRY WEIGHT OF MYCELIUM IN MILLIGRAMS AT VARYING HYDROGEN-ION CONCENTRATIONS OBTAINED IN NUTRIENT SOLUTIONS IN EXPERIMENT ONE. TEMPERATURE 28° C.

	5-day Series			7-day Series			15-day Series		
Culture No.	Initial pH	Average pH	Dry Wt. mycelium. Mgs.	Initial pH	Average pH	Dry Wt. mycelium. Mgs.	Initial pH	Average pH	Dry Wt. mycelium. Mgs.
1	3.3	3.6	13.1	3.3	4.25	23.2	3.3	4.7	88.1
2	3.6	4.2	17.0	3.5	4.25	21.6	3.6	4.9	128.0
3	3.8	4.3	20.8	3.8	4.4	17.4	3.7	4.85	106.4
4	4.2	4.75	33.2	4.3	5.0	48.0	3.3	4.65	95.7
5	5.0	5.1	16.3	5.1	5.4	23.4	4.9	5.55	84.8
6	5.4	5.45	14.6	3.1	4.05	14.9	5.5	5.7	102.2
7	5.9	5.85	18.0	6.0	5.8	18.1	6.0	6.0	190.3
8	6.5	6.35	14.2	6.4	6.35	20.8	6.7	6.65	94.0
9	6.9	6.8	10.2	6.9	6.7	17.0	6.9	6.75	79.1
10	7.4	7.25	8.2	7.5	8.35	15.3	7.5	7.25	42.7
11	8.1	7.8	6.9	8.0	7.67	18.5	8.0	7.4	48.5
12	8.6	8.1	lost	8.7	7.8	20.2	8.6	8.45	66.5
13	8.85	8.2	8.9	8.85	8.17	22.1	8.8	8.25	48.8
14	8.85	8.27	5.8	8.8	8.15	13.6	9.0	8.35	39.6
15	9.0	8.35	7.3	9.0	8.25	21.1	9.3	8.45	34.1

The results of this experiment are recorded in Table 1. Fig. 2 shows curves with the average pH plotted on the abscissa and the dry weight of the mycelial mats in milligrams plotted on the ordinate.

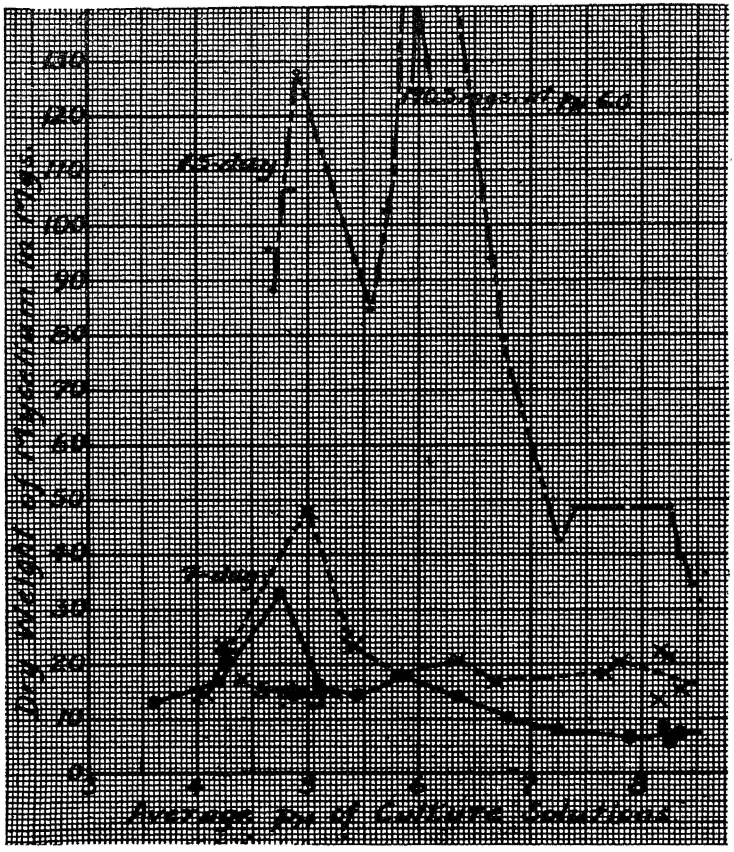

Fig. 2.—Influence of hydrogen-ion concentration on the growth of mycelium in 5-, 7-, and 15-day cultures in Experiment One.

As we proceed from the most acid culture solutions the dry weight increased to a maximum at average pH 4.75 to 5.0. It then decreased rapidly to a minimum at average pH 5.4 to 5.8. In the 15-day series a marked secondary maximum occurred at pH 6.0. This second maximum was very slight in the 5-day and 7-day series.

The growth of the fungus in the culture solutions in the 5-day series changed the solutions of pH 5.4 and less to greater alkalinity, and of pH 5.9 or more to greater acidity. In the 7-day cultures the solutions of pH 5.1 or less became more alkaline and those of pH 6.0

or greater became more acid with the exception of No. 9 (pH 7.5) which changed to greater alkalinity. In the 15-day series all solutions of pH 5.5 or less became more alkaline and of pH 6.7 or greater became more acid. Culture No. 7 remained unchanged at pH 6.0. The solutions at the extremes showed the greatest change in reaction.

No color production was evident in any of the mycelial mats and was observed only in the cultures where some of the mycelium had crept up the sides of the flask above the surface of the culture solution. Here faint shades of light to dark pink were observed. Apparently the mycelium of this organism does not develop color in liquid cultures but only on solid media. The growth in the acid solutions was more floccose than in the alkaline solutions where the mats appeared to be quite gelatinous, especially as the concentration of the hydrogen-ion decreased.

EXPERIMENT TWO

In the second experiment the culture solution was changed to the extent of increasing the concentration of the two phosphates to 3.64 grams of KH_2PO_4 and 6.96 grams of K_2HPO_4 per liter of solution. This gave a 2 to 3 ratio of an M/15 concentration of the two salts. The increase in concentration of the phosphates was for the

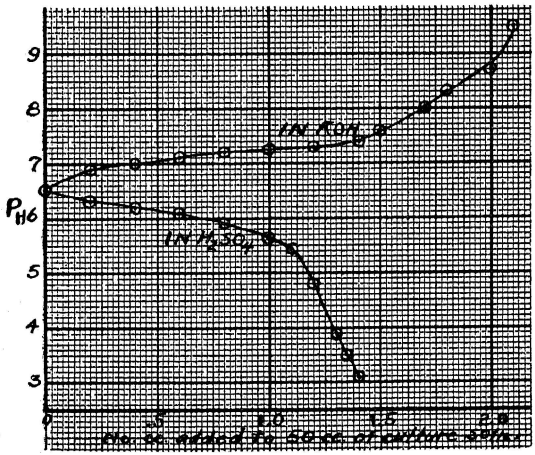

Fig. 3.—Titration curve of culture solution used in Experiment Two with 1N H_2SO_4 and 1N KOH.

purpose of obtaining a more strongly buffered solution, inasmuch as considerable shifting of the reaction had occurred in the first experiment. However, the results show that this had little effect on the

shifting of the reaction. The acid range was adjusted with normal H_2SO_4 and the alkaline range with normal KOH as in Experiment 1. The titration curve for this medium is shown in Fig. 3. Three series

TABLE 2.—DRY WEIGHT OF MYCELIUM IN MILLIGRAMS AT VARYING HYDROGEN-ION CONCENTRATIONS OBTAINED IN NUTRIENT SOLUTIONS IN EXPERIMENT TWO. TEMPERATURE 28° C.

	5-day Series			7-day Series			15-day Series		
Culture No.	Initial pH	Average pH	Dry Wt. mycelium. Mgs.	Initial pH	Average pH	Dry Wt. mycelium. Mgs.	Initial pH	Average pH	Dry Wt. mycelium. Mgs.
1	3.0	3.9	35.1	3.0	4.3	46.3	3.0	4.55	67.8
2	3.4	4.5	85.4	3.5	4.6	87.7	3.3	4.8	96.9
3	4.3	5.02	70.1	4.2	5.05	101.1	4.3	5.35	100.0
4	4.7	5.05	38.5	4.5	5.2	73.8	4.6	5.45	86.7
5	5.1	5.35	37.7	5.2	5.55	60.6	5.2	5.8	84.1
6	5.6	5.67	40.9	5.6	5.75	51.3	5.5	6.0	95.5
7	6.1	6.0	49.9	6.1	6.15	64.2	6.1	6.35	88.4
8	6.5	6.4	44.4	6.5	6.55	59.0	6.5	6.75	81.0
9	6.7	6.6	38.7	6.7	6.75	63.1	6.7	7.0	75.5
10	7.0	6.85	34.4	7.0	6.92	50.1	7.0	7.25	79.2
11	7.4	7.0	23.6	7.4	7.2	43.9	7.4	7.6	63.1
12	7.9	7.25	24.0	7.9	7.5	45.8	7.9	7.9	65.9
13	8.3	7.85	21.1	8.4	7.87	48.8	8.4	8.25	64.2
14	8.6	7.95	24.3	8.7	7.95	48.8	8.6	8.4	60.4
15	8.8	8.05	25.2	8.8	7.97	45.5	8.8	8.55	61.8

of cultures were used in this experiment for growth-periods of 5, 7, and 15 days, respectively. The results are recorded in Table 2, and the growth curves are plotted as before in Fig. 4.

In this experiment a marked maximum occurred between an average pH 4.5 to 5.35, followed by a secondary maximum occurring near pH 6.0. The minimum between these two maxima was located at pH 5.35-5.8. The changes in reaction in the culture solutions due to the growth of the fungus were very similar to those noted in Experiment One, with one exception. In the 5-day series culture solutions of pH 5.6 or less changed toward greater alkalinity while culture solutions of pH 6.1 or greater shifted toward greater acidity. In the 7-day series the solutions of pH 6.7 or less changed toward greater alkalinity and solutions of pH 7.0 or greater changed toward greater acidity. In the 15-day series solutions of pH 7.4 or less changed toward greater alkalinity and solutions of pH 8.4 or greater shifted toward greater acidity. Solution No. 12(pH 7.9) did not change.

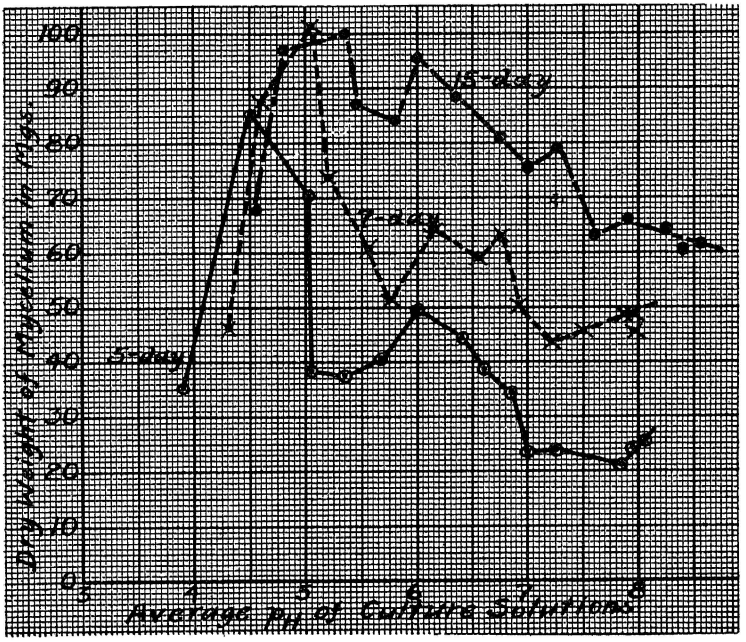

Fig. 4.—Influence of hydrogen-ion concentration on the growth of mycelium in 5-, 7-, and 15-day cultures in Experiment Two.

EXPERIMENT THREE

In the third experiment the same basic nutrient solution as in Experiment Two was used but the acid reactions were adjusted with normal H_3PO_4 and 3N H_3PO_4, the first being used to obtain values from pH 4.5 to 6.5; the latter, for values below 4.5. The alkaline reactions were adjusted with normal KOH. The 3N H_3PO_4 was used for the greater acidities because too great dilution would result if the normal solution had been used throughout as may be seen from an examination of the titration curve obtained. Eighteen flasks were used in each series, only two series being prepared. The titration curve of this medium is shown in Fig. 5. The results of the growth obtained in the two series, incubated for growth-periods, of 5 and 7 days, respectively, are given in Table 3, with growth curves plotted in Fig. 6.

By an examination of the data and curves of the 5-day cultures it is seen that slight growth occurred at an average pH as low as 2.5. It increased to a maximum at average pH 4.55, followed by a minimum at pH 5.5, and a very slight second maximum at 6.1. In the 7-day cultures we have a similar curve except for the absence of the second maximum. In this experiment a marked increase in the production

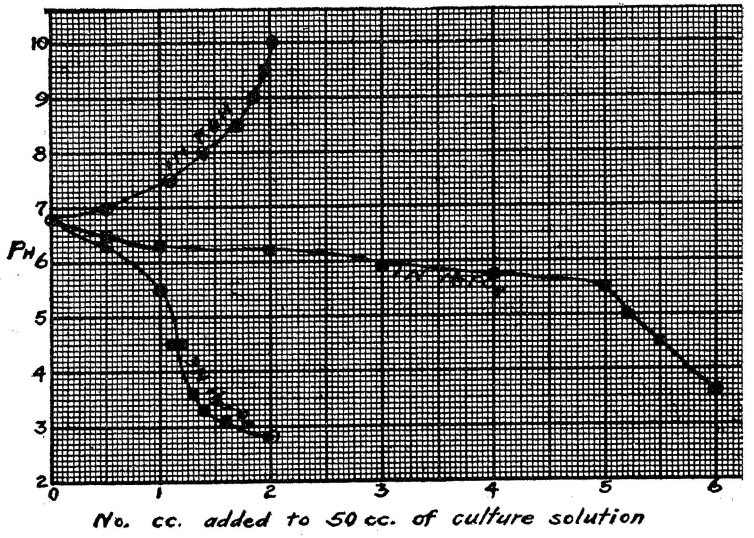

Fig. 5.—Titration curve of culture solution used in Experiment Three with 1N H_3PO_4, 3N H_3PO_4, and 1N KOH.

of dry matter occurred in the alkaline solutions with average pH of 7.5 to 8.0. This same phenomenon is evident in the curves obtained by Hopkins[18] for *Gibberella saubinetii*.

TABLE 3.—DRY WEIGHT OF MYCELIUM IN MILLIGRAMS AT VARYING HYDROGEN-ION CONCENTRATIONS OBTAINED IN NUTRIENT SOLUTIONS IN EXPERIMENT THREE. TEMPERATURE 28° C.

	5-day Series			7-day Series		
Culture No.	Initial pH	Average pH	Dry Wt. mycelium. Mgs.	Initial pH	Average pH	Dry Wt. mycelium. Mgs.
1	2.3	2.4	7.4	2.4	2.7	21.7
2	2.6	2.8	12.6	2.6	3.0	25.5
3	3.0	3.55	29.3	3.0	3.95	60.8
4	3.7	4.35	46.8	3.7	4.35	69.3
5	3.9	4.55	53.7	3.9	4.85	68.7
6	4.3	4.7	37.9	4.3	5.1	53.4
7	4.8	4.8	36.6	4.8	----	lost
8	5.3	5.3	19.4	5.2	5.25	24.2
9	6.1	5.5	12.8	6.1	----	lost
10	6.5	6.1	16.7	6.5	6.65	24.8
11	6.7	6.5	12.5	6.7	6.6	30.4
12	6.9	6.7	13.4	6.9	6.85	28.2
13	7.6	7.25	15.8	7.7	7.45	30.1
14	8.1	7.4	27.2	8.2	7.65	47.4
15	8.8	7.7	30.7	8.8	7.9	44.5
16	8.7	7.8	29.5	8.8	7.85	62.4
17	9.0	8.0	26.0	9.0	7.9	55.6
18	9.4	8.2	23.3	9.2	8.0	66.2

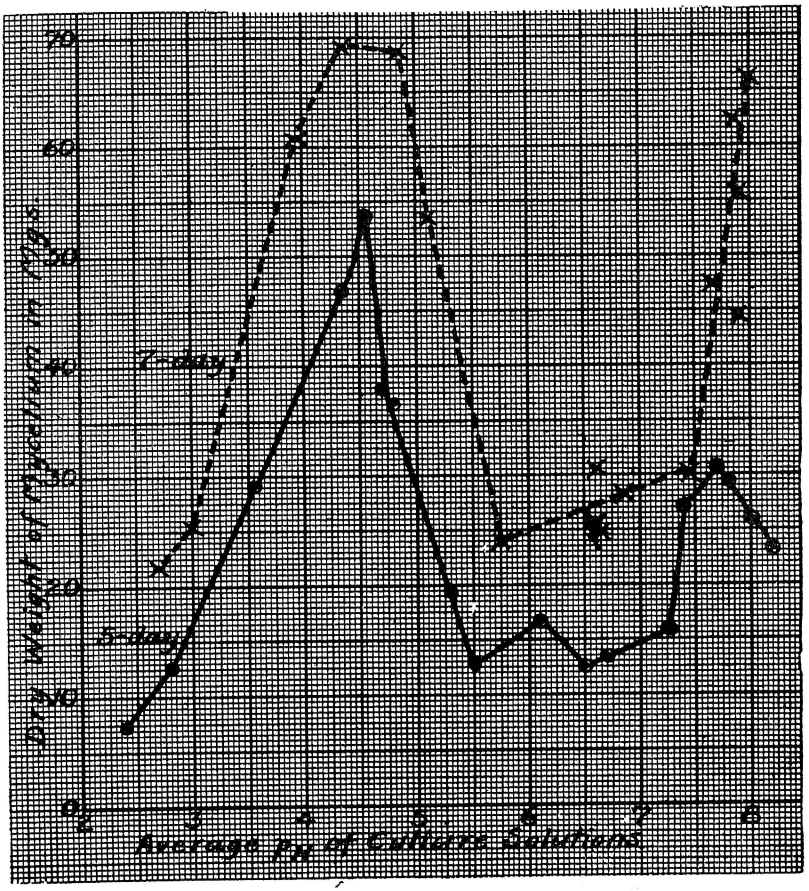

Fig. 6.—Influence of hydrogen-ion concentration on the growth of mycelium in 5-day and 7-day cultures in Experiment Three.

In Experiment Three the growth of the fungus changed the reaction of solutions of pH 4.3 or less toward greater alkalinity in the 5-day series and made solutions of pH 6.1 or greater more acid. Solutions 7 and 8(pH 4.8 and 5.3 respectively) remained unchanged. In the 7-day series all solutions of pH 6.5 or less became more alkaline and of pH 6.7 or greater more acid.

EXPERIMENT FOUR

In Experiment Four the same basic nutrient solution as in Experiment Two was used but the acid reactions were adjusted with normal HCl and the alkaline reactions with normal NaOH. The titration curve of this medium is shown in Fig. 7. The data are recorded in Table 4. The curves produced when the dry weight of the my-

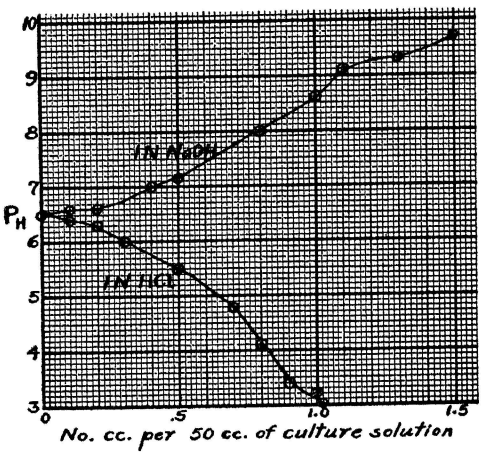

Fig. 7.—Titration curve of culture solution used in Experiment Four with 1N HCl and 1N NaOH.

celium is plotted against the average hydrogen-ion concentration of the solution expressed in Sorensen units showed, in general, a double maximum (Fig. 8). The first maximum was located at average pH 4.95 in the 5-day series and at 4.6 in the 8-day series. The second maximum occurred at average pH 6.05 and 6.1. The minimum between the two maxima was at average pH 5.55 for the 5-day series and 5.7 for the 8-day series. In the 5-day series in this experiment the growth of the fungus made all solutions of pH 5.3 or less more alkaline and all solutions of pH 6.1 or greater more acid. In the 7-day series all solutions of pH 5.5 or less became more alkaline and all of pH 6.1 or greater were made more acid by the growth of fungus.

TABLE 4.—DRY WEIGHT OF MYCELIUM IN MILLIGRAMS AT VARYING HYDROGEN-ION CONCENTRATIONS OBTAINED IN NUTRIENT SOLUTIONS IN EXPERIMENT FOUR. TEMPERATURE 28° C.

	5-day Series			8-day Series		
Culture No.	Initial pH	Average pH	Dry Wt. mycelium. Mgs.	Initial pH	Average pH	Dry Wt. mycelium. Mgs.
1	3.3	4.15	22.4	3.4	4.3	31.2
2	3.9	4.7	65.1	3.8	4.45	94.8
3	4.2	4.95	68.0	4.25	4.6	126.7
4	4.5	5.12	57.5	4.8	5.1	80.1
5	4.9	5.35	52.9	5.0	5.4	52.0
6	5.3	5.55	20.0	5.5	5.7	38.5
7	6.1	6.05	33.6	6.1	6.0	48.6
8	6.3	6.2	28.5	6.3	6.1	53.9
9	6.8	6.4	29.1	6.7	6.3	40.3
10	7.2	6.9	20.3	7.2	6.8	44.2
11	7.6	7.17	16.7	7.5	7.2	45.3
12	8.2	7.9	15.2	8.2	7.4	39.8

GENERAL SUMMARY AND DISCUSSION OF THE RESULTS OF SOLUTION CULTURES

The two most noteworthy facts in the results of the experiments in culture solutions are the changes in reaction of the culture solu-

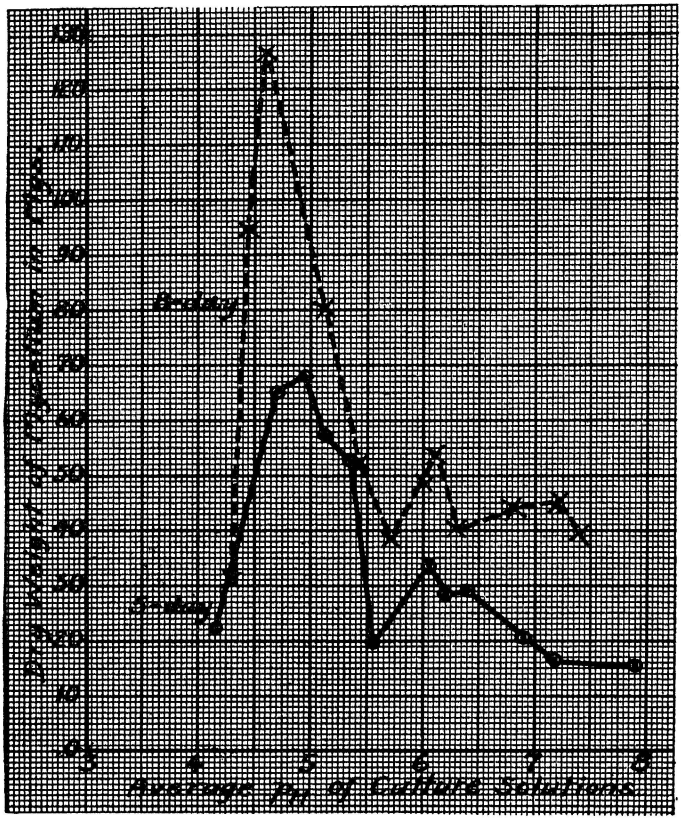

Fig. 8.—Influence of hydrogen-ion concentration on the growth of mycelium in 5-day and 8-day cultures in Experiment Four.

tions produced by the growth of the fungus and the relation of the hydrogen-ion concentration to the dry weight produced.

In the discussion of each experiment it was mentioned that the acid solutions became more alkaline and the alkaline solutions more acid due to the growth of the fungus. This effect of the fungus upon the reaction of the culture solution has been summarized in Table 6.

The changes in reaction may be due to the production and excretion by the fungus in the acid solutions of materials which reduce acidity and of acid materials such as carbonic acid and organic acids in the alkaline media. The changes might also be due to the selective absorption of ions. An excess of anions absorbed in the acid solutions and cations in the alkaline solutions would produce an effect such as was observed. In fact if we consider the 5-day series in each experiment the effect of the fungus on the reaction is analogous to

that of a colloidal ampholyte with an isoelectric point at between pH 5.6 and 6.1, as would be suggested by a consideration of the work of Michaelis[25], p. 58; and Robbins[28]. The fact that solutions of greater pH than 6.1 changed to greater alkalinity in the 7- and 15-day series of Experiment Two, and 7-day series of Experiment Three would indicate either a higher isoelectric point or a stronger absorptive power for anions than cations.

The curves of growth show that *Fusarium lycopersici* will grow over a wide range of acidities. Growth occurred in these experiments in the most acid solutions used, pH 2.4, and in the most alkaline, pH 9.4.

Considerable variations in the amount of growth in the different series is evident. The greater amount of growth obtained in any of the experiments was in the 15-day series of Experiment One, amounting to 190.3 milligrams of dry matter. The smallest growth in the 5- or 7-day series was obtained in Experiment One where the more dilute buffer mixture was used. Evidently the doubling of the concentration of the buffer salts in the basic nutrient solution for Experiments Two, Three and Four, did not injuriously affect the early growth of the fungus. Variations in the amount of dry matter in the various series were probably due to differences in the amount of inoculum and differences in the composition of the nutrient solutions.

Maximum growth occurred with one exception at average pH 4.35 to about 5.35. In the 15-day series of Experiment One maximum growth was found at pH 6.0. The primary maximum was followed by a secondary maximum between average pH 5.85 to 6.35 in many cases. The minimum between the two maxima was located at pH 5.25 to 5.9. In Table 5 the locations of the maxima and intermediate minimum for each experiment are summarized. It appears rather significant that the location of this minimum corresponds quite closely with the point for the 5-day cultures below which a change in the reaction of the culture solutions was noted.

The same basic nutrient solution was used throughout these experiments, the only variations being in the lower concentration of phosphates in Experiment One, and the acid and basic radicals in the acids and alkalies used in adjusting the reactions of the solutions. The only constantly variable factor throughout was the concentration of the hydrogen-ion. There is a general agreement between the curves in all experiments with the exceptions noted. This would seem to indicate that the differences in growth were wholly or largely due to variations in the hydrogen-ion concentration. If the fungus acts as a

TABLE 5.—SUMMARY OF CULTURAL EXPERIMENTS GIVING pH VALUES AT WHICH MAXIMA AND MINIMUM OF GROWTH (IN TERMS OF DRY WEIGHT OF MYCELIUM) WERE OBTAINED.

Series	pH values		
	1st Max.	Minimum	2nd Max.
Exp. I:			
5- day	4.75	5.45	5.85
7-day	5.00	5.80	6.35
15-day	4.90	5.55	6.00
Exp. II:			
5-day	4.50	5.05–5.40	6.00
7-day	5.05	5.75	6.15
15-day	5.35	5.80	6.00
Exp. III:			
5-day	4.55	5.50	6.10
7-day	4.35–4.85	5.25	
Exp. IV:			
5-day	4.7 –5.0	5.55	6.05
8-day	4.60	5.70	6.10

colloidal ampholyte with an isoelectric point as previously suggested, then the minimum growth in culture solutions with varying hydrogen-ion concentration would occur in those solutions at or near this isoelectric point.

The double maximum curve when the dry matter produced by *Fusarium lycopersici* in solutions of different hydrogen-ion concentration was plotted against the average pH of the solution is similar to that found by Hopkins[18] for *Gibberella saubinetii.*

Hopkins also found that seedling infection of wheat by *Gibber-*

TABLE 6.—SUMMARY OF CHANGES IN pH VALUES IN THE DIFFERENT SERIES OF CULTURES DUE TO SHIFTING OF REACTION. EXPERIMENTS ONE, TWO, THREE AND FOUR.

Series	Initial pH value at and below which reaction shifted toward alkalinity	Initial pH value at and above which reaction shifted toward acidity	Initial pH value at which there was no shifting of reaction
Exp. I:			
5-day	5.4	5.9	
7-day	5.1	6.0	
15-day	5.5	6.7	6.0
Exp. II:			
5-day	5.6	6.1	
7-day	6.7	7.0	
15-day	7.4	8.4	7.9
Exp. III:			
5-day	4.3	6.1	4.8 and 5.3
7-day	6.5	6.7	
Exp. IV:			
5-day	5.3	6.1	
8-day	5.5	6.1	

ella saubinetii in soil cultures where reactions were adjusted artificially was least at a point which corresponded rather closely with the minimum between the two maxima of growth of the organism in solution cultures of varying reaction. Two greenhouse experiments were therefore undertaken during the fall and winter of 1922-1923 with the object of determining the effect of soil reaction on the infection of tomato by *Fusarium lycopersici.*

THE RELATION OF INFECTION OF THE HOST BY *FUSARIUM LYCOPERSICI* TO THE HYDROGEN-ION CONCENTRATION OF THE SOIL

The first experiment was a preliminary one in which tomato plants were grown in inoculated flats of soil which had been used the previous year by Hopkins[18] working with seedling infection of wheat by *Gibberella saubinetii* and which had been adjusted to varying reactions. Owing to the installation of a new central heating plant temperature conditions became so unsatisfactory during the course of the experiment that no reliance could be placed in the results obtained. Therefore, in January, 1923, after the heating arrangements had become satisfactory a second experiment was begun and conducted under more favorable conditions of temperature and moisture.

For this experiment soil was obtained from a plot on the horticultural experimental grounds at the Missouri Agricultural Experiment Station at Columbia, Missouri, which had been used in growing tomatoes for studies of wilt resistance and was known to be highly infected with *Fusarium lycopersici,* as high as 100 per cent infection occurring on parts of the plot the previous season (1922). The soil was taken from the field during mild weather in January and removed to the greenhouse where it was thoroughly air-dried. A representative sample was then taken and a titration curve obtained by treating 100-gram samples with varying amounts of normal H_2SO_4 and normal NaOH, allowing it to become air-dry, and then obtaining the soil extract for hydrogen-ion determination as recommended by Gillespie[12, 14]. The electrometric method was used in obtaining the hydrogen-ion concentration of the soil extracts, using a Leeds & Northrup type K potentiometer, type R galvanometer, and the Clark calomel and hydrogen electrode vessels. Hydrogen-ion determinations were also obtained by using Gillespie's colorimetric method[14] and were found to check to within 0.1 Sorensen exponent with the electrometric method in all cases where the soil extracts were clear enough to permit the use of the indicators. The titration curve obtained is shown in Fig. 9.

TABLE 7.—RESULTS OF INFECTION EXPERIMENTS WITH *Fusarium lycopersici* IN SOILS WITH VARYING HYDROGEN-ION CONCENTRATION.

Pot No.	Initial pH	Final pH	Average pH	Total No. plants	Total No. wilted plants	Percentage of wilt
1	3.43	4.0	3.7	142	72	50.7
2	3.84	4.5	4.17	92	41	44.6
3	4.22	5.1	4.66	72	31	43.1
4	4.78	5.5	5.14	79	28	35.4
6	4.84	5.4	5.12	68	31	45.0
7	5.1	5.9	5.5	76	33	44.7
8	5.44	6.8	6.12	79	24	30.5
9	5.9	7.0	6.45	89	10	11.2
10	6.1	7.1	6.6	81	5	6.2
11	6.62	7.2	6.91	83	18	21.5
12	6.64	7.5	7.07	73	11	15.1
13	7.54	7.9	7.72	53	29	54.7
14	8.5	8.4	8.45	51	35	68.6

From this were calculated the amounts of acid and alkali required to produce a range of soil reactions varying by 0.3 to 0.5 Sorensen units. The pH of the soil extract from the untreated soil was 5.1.

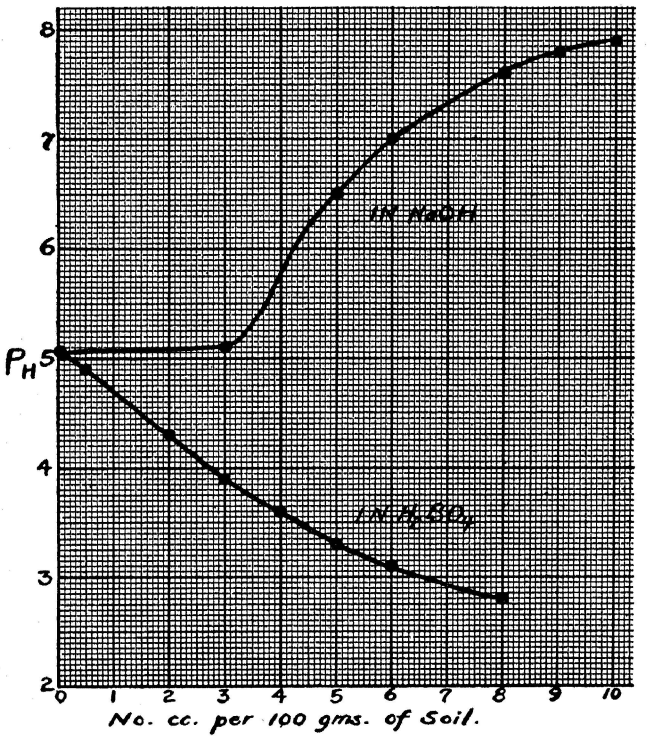

Fig. 9.—Titration curve of soil used in Exepriment Five with 1N H_2SO_4 and 1N NaOH.

The calculated amount of acid and alkali was diluted with a sufficient amount of redistilled water to give a volume of 1900 c.c. of solution, and then thoroughly mixed with 12 kilograms of the air-dried soil. Each 12-kilogram sample prepared was placed in a No. 4 glazed earthenware pot. The soils were allowed to stand for about six days when representative samples were withdrawn from each pot with a cork borer, air-dried, and the hydrogen-ion concentration determined using the same methods as employed before. The results are recorded in Table 7.

Eight days after the soils had been treated 100 to 140 tomato seeds of the Livingston's Early Stone variety were planted in each pot. The seeds and the surface of the soil in each pot were sprayed thoroughly with a heavy spore suspension of *Fusarium lycopersici,* excepting Pot 7, which was left as a check. Pot 6 was inoculated with the spore suspension and treated with 1900 c.c. of redistilled water. Pot 7 was not artificially inoculated and was not treated with water or solution.

These pots were at first placed under too low temperature conditions for the development of wilt, but after ten days were removed to a bench with bottom heat where the temperature conditions were quite favorable for wilt development and where they remained until the end of the experiment. Thermometers were inserted in several of the soils and readings taken and recorded three times daily, morning, noon, and late afternoon, respectively. The temperature of the soil remained around 25° C. to 30° C. throughout the experiment. A record of the air temperature was obtained throughout the experiment by using a thermograph. The air temperature fluctuated considerably but averaged around 27° C. for the period of the experiment.

Germination of the tomato seed was apparently greatly influenced by the reaction of the soil. It was much slower in the extremely acid pots and very poor at pH 8.5, the reaction of the most alkaline soil used. Germination was slow at pH 7.54 but showed a fairly high percentage. Conditions were very poor for growth of the plants in Pots 13 and 14. Many of the plants in Pot 14 died from other causes than wilt before they were 4-5 cm. high. From a rough estimate, less than 50 per cent of the plants in Pots 13 and 14 grew more than 4-5 cm. high before dying. These plants apparently died because of the toxic conditions existing in these alkaline soils. Therefore they were discarded and were not considered in calculating the final percentage of wilt. Only those were considered that remained comparatively normal. The plants in Pot 1 grew slowly and never reached over 15-18 cm. in height. For this reason the plants were not thinned out at the

end of 4 weeks as was done in the other pots. In spite of their small size and lack of vegetative vigor these plants in the extremely acid soils showed considerable wilt as an examination of the data indicates. Marked wilting first appeared in the various pots after the sixth week.

The plants were watered daily with tap water using a fine spray from a garden hose. Each soil was watered sufficiently to leave a small amount of water on the surface at each watering. This soon penetrated into the soil insuring a fairly high moisture content throughout the entire soil mass. The soils in Pot 13 and particularly in Pot 14 were heavy and water-logged at all times. This was due to the physical conditions resulting from the treatment of these soils with the amount of alkali used. Pot 14 had a moisture content at or near saturation throughout the entire experiment, while Pot 13 was not far from sat-

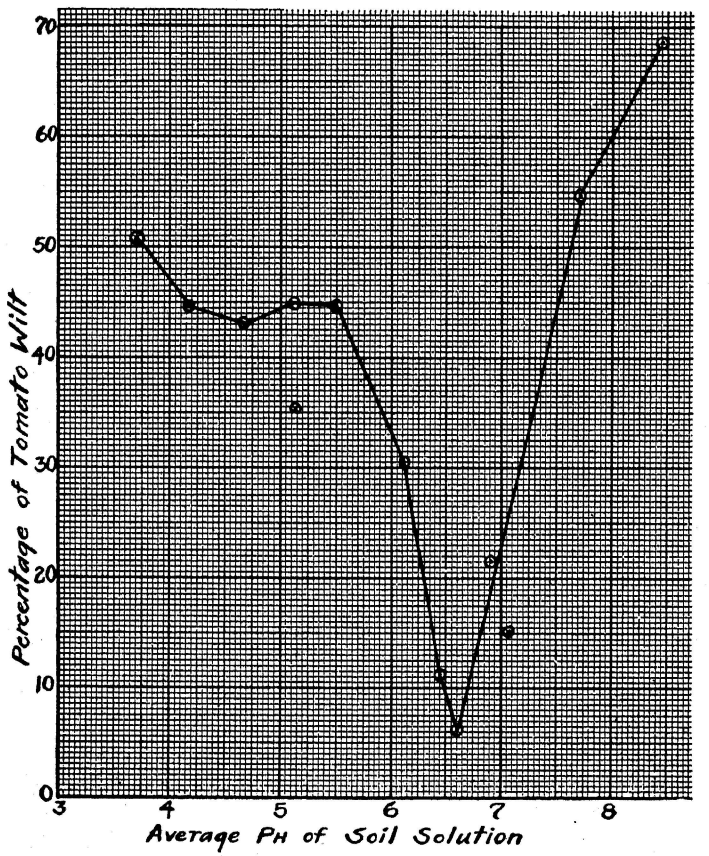

Fig. 10.—Influence of the hydrogen-ion concentration of the soil upon percentage of wilt. Experiment Five.

uration throughout the entire experiment. The plants in these pots after once beginning to grow were apparently quite thrifty until attacked by the wilt. However, they were never of the same vegetative vigor as in the soils with better physical conditions (Pots 2 to 12, inclusive) and at apparently more favorable reactions.

The results including the initial pH, the final pH, the average pH and the percentage of wilt after 14 weeks, when the experiment was ended, are given in Table 7. Fig. 10 shows the percentage of wilt plotted against the average pH of the soil. Those plants which showed marked browning and discoloration of the vascular bundles in the stem with slight to pronounced symptoms of wilting were used as a standard of wilting.

As can be observed in Table 7 the initial reaction of the soils was not maintained. All save the most alkaline one became less acid during the experiment. This decrease in acidity was probably due to the fact that equilibrium had not been reached in the pots when the first samples were taken and also because the pots were watered with tap water which had a high calcium content. The average pH was used in constructing the curve since it represents more closely the prevailing hydrogen-ion concentration during the course of the period of infection.

The curve in Fig. 10 shows that between 40 and 50 per cent of the tomato plants wilted from infection by *Fusarium lycopersici* in soils of average pH 3.7 to 5.5, with the exception of the plants in Pot 4 (average pH 5.14) in which the amount of infection was 35.4 per cent. In soils of pH 5.5 to 6.6 there was a gradual decrease in percentage of infection to 6.2 at pH 6.6. The amount of infection then increased in the more alkaline soils to a maximum of 68.6 per cent in the soil with an average pH of 8.45.

DISCUSSION OF RESULTS OF THE SOIL EXPERIMENT

The soil experiment indicates that there is probably a reaction of the soil at which the infection of tomato by *Fusarium lycopersici* is decidedly decreased. In the experiment performed the reaction at which the infection was least was in a range of average pH 6.4-7.0.

It is of course impossible to affirm from this single experiment that the decrease in infection is due to the concentration of hydrogen-ions alone. The adjustment of the reaction of the soil by means of sulfuric acid and sodium hydroxide necessarily causes the concentration of sulfate and sodium ions to vary also. Without doubt the solubility of various soil constituents changes with the soil reaction.

It seems probable however from the similarity of these results to those secured by Hopkins[18] in seedling infection of wheat by *Gibberella saubinetii* that the hydrogen-ion concentration was the controlling factor in determining infection here. It is also impossible from this experiment to fix definitely the value of the hydrogen-ion concentration at which the wilt is at a minimum since only a single experiment was performed and the soil reaction did not remain unchanged throughout the experiment. Additional experiments should be performed in which other acids and alkalies are used for adjusting the soil reaction and in which soils of varying natural reactions are used.

Hopkins[18] found that the minimum between the two maxima of growth of *Gibberella saubinetii* in culture solutions was located at pH 5.5 to 6.0. He also found that the minimum of infection in soil cultures was located at approximately the same value. In the case of *Fusarium lycopersici* the hydrogen-ion concentration of the soil at which minimum infection took place, pH 6.4 to 7.0, was distinctly higher than the hydrogen-ion concentration for the low point of growth between the two maxima found in culture solutions, i. e., average pH 5.6 to 6.1. Hence, there is a tendency for the percentage of wilt to be high at points where the growth is approaching a minimum in the cultural experiments, between pH 5.0 to 6.0. However, when the initial pH values of the soil are taken into consideration the curves of the soil experiment and cultural experiments come into somewhat closer agreement. The infection of the tomato plants probably occurred under conditions of soil reactions at or near the initial reactions. This supposition is based upon the fact that infection may occur quite early in seedling plants as was found to be the case in this experiment. Pronounced wilting of the plant may not occur until several weeks after infection has occurred. Therefore the reaction of the soil that was probably present at the time of actual infection was nearer the initial than the average.

As noted in the presentation of the experimental data, the soils in Pots 13 and 14 were heavy and water-logged throughout the experiment. After once beginning to grow the plants in these pots were moderately thrifty although tending to remain spindly and somewhat abnormal. However, in spite of the saturated condition of the soil, the percentage of wilt was high. This does not agree with the findings of Clayton[7] for saturated soils. However, in the writer's experiments the soils were not only saturated with water but were also quite alkaline.

Two possibilities for applying the results of this study to the con-

trol of tomato wilt suggest themselves. One is the artificial adjustment of the soil reaction to a point unfavorable for infection by *Fusarium lycopersici;* the other is the use of soils which naturally have a reaction unfavorable to tomato wilt. An attempt to adjust the soil of small field plots to a reaction unfavorable to wilt by the use of lime in the summer of 1923 was unsuccessful, apparently because the reaction of the entire mass of soil in which the tomato roots developed was not affected by the application of the lime. Whether it is possible under field conditions to change the reaction of the soil deeply enough and completely enough to be of practical importance remains to be seen. It would appear possible from the curve of infection in the soil experiment that the soil could be made too alkaline for successful control. The discovery of soils naturally unfavorable to tomato wilt because of their reaction would appear possible and might offer the most feasible method of attack.

SUMMARY

1. A single-spore strain of *Fusarium lycopersici* was grown in a mineral nutrient solution plus dextrose at varying hydrogen-ion concentrations. Variations in acid reactions were obtained by using H_2SO_4, H_3PO_4, and HCl; variations in alkaline reactions by using KOH and NaOH.

2. Three series of cultures at varying hydrogen-ion concentrations were used in two experiments for periods of 5, 7, and 15 days, respectively. In two other experiments the 15-day series was not used.

3. The dry weight of the mycelium was obtained upon the completion of growth in each series and data obtained showing the effect of the reaction upon growth.

4. The results indicate that in the culture media used a maximum of growth was obtained at an average pH of 4.5 to 5.3, followed by a minimum at average pH 5.25 to 5.8, with a second maximum in most cases at average pH 5.85 to 6.85.

5. A soil experiment was conducted in which tomato plants were grown in pots of soil which had been adjusted to varying hydrogen-ion concentrations by using normal H_2SO_4 and normal NaOH and artificially inoculated with a spore suspension of the single-spore strain of *Fusarium lycopersici.* This was done under greenhouse conditions.

6. In the soil experiment a minimum of tomato wilt occurred at a range of hydrogen-ion concentration from average pH 6.4 to 7.0, there being a maximum of wilt on either side of this range.

7. The results of the soil infection experiment indicate that the Fusarium wilt of tomato might be controlled by selecting soils having

a certain natural reaction, or by properly adjusting the reaction of the soil so as to produce a reaction unfavorable for infection. Further experimental data are required to determine definitely the range at which the infection is at a minimum and to determine the economy and efficiency of adjusting the reaction of the soil by the application of suitable substances.

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