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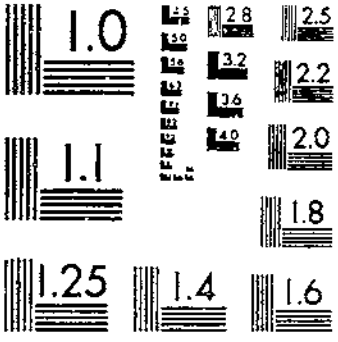
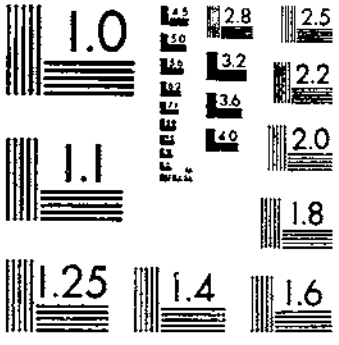


MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A



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TEMPERATURE STUDIES OF SOME TOMATO PATHOGENS

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CONTENTS

INTRODUCTION

Among the many factors influencing the carrying qualities of "green wrap" tomatoes none is more important than the maturity of the fruit and the temperatures to which the tomatoes are subjected during transit and while in storage for ripening on the receiving markets. As harvested commercially, all tomatoes have numerous fungi and bacteria on their surfaces, and many of these are capable of producing serious decay if conditions favor their entrance and development within the fruit. It has long been recognized that wounds greatly favor the development of tomato pathogens and that in some instances the presence or absence of wounds constitutes the limiting factor for infection and decay. Maturity of the fruit or temperatures encountered during transportation and storage like-

[1] Formerly Alice Allen Bailey.

wise may be limiting factors, some organisms growing better on more mature fruit while others seem to be more sensitive to temperature changes.

Although there had been previous investigation of the temperature relationships of some tomato pathogens, the temperature data available were not always applicable to transit and storage conditions, and little work had been done upon other organisms which cause serious transit and storage diseases of tomatoes. There seemed need also of further data directly applicable to the numerous questions arising relative to the influence of maturity on development of decay after the tomatoes leave the packing sheds. This study was begun, therefore, with the purpose of determining more accurately the influence of temperature and maturity of fruits upon development of decay during transit and during storage on the receiving markets.

Nine important tomato pathogens were selected for study, and each organism was checked in two ways as to its response to temperature changes and to changes in the maturity of the tomato fruits. Green, turning, and ripe tomatoes were carefully selected, sterilized, and inoculated for temperature studies. In addition to this work all organisms were carefully checked for rate of growth on potato-dextrose agar plates held at 5° intervals of temperature from 30° to 95° F.

METHODS

AGAR PLATE CULTURES

The agar plates were run in two sets for each experiment with each organism. One set contained potato-dextrose agar having a hydrogen-ion concentration corresponding to the average mean acidity (pH 4.7) of the mature-green tomatoes used in the inoculation tests; the other contained agar identical in formula[2] and made from the same lot of decoction but adjusted to pH 6.01, this degree of acidity being comparable to that found in ripe tomatoes of the variety used. The reaction of the medium was adjusted by adding hydrochloric acid to the hot agar for the medium of pH 4.7 and sodium hydroxide for the less acid medium. Subsequent sterilizations were made by steaming without pressure, but to be sure the pH had not varied with sterilization, samples of the agar were checked with the Youden hydrogen-ion apparatus before the agar was poured into Petri dishes. So little acid or alkali was needed to change the pH of the potato decoction that it was believed that the chemical elements thus added were negligible in comparing the two sets of plates. In fact, occasionally the potato-dextrose agar was already pH 6 without the addition of the alkali, and in one set of experiments it was necessary to add a little acid to reach this pH, yet the same contrasts held between the two lots of the two acidities.

Sufficient agar was poured to prevent undue drying even at high temperatures before the conclusion of the tests. The air was kept comparatively moist at all times by keeping an open pan of water in each chamber. Inoculations were made with mycelium or with spores and mycelium, depending on the organism. The fungus in each test was allowed to develop for 24 hours at room temperature, after which the colony was measured along a marked diameter and placed in the temperature-control chamber for a week. The tem-

[2] 1 l of potato decoction (200 g potato to 1 l of distilled water), 20 g agar; 20 g dextrose.

perature variations of the chambers from the chosen mean were 1° to 2° F., but variations within the Petri dishes were not so great, since all the extremes recorded are averages of those given by a maximum-minimum thermometer set daily, whereas the accompanying thermograph records showed that the fluctuations were too frequent and the duration of time at the extremes too short to influence greatly the temperature inside the Petri dishes where the temperature changes were found to lag several hours behind those outside.

The same diameter of each colony was measured in centimeters daily, but in computing the average daily growth rate the plates were allowed to stand for the first 24 hours in the temperature-control box to permit the culture plate to reach the temperature of the chamber, and the average daily growth rate was computed from the subsequent 6 days' growth. For agar of each hydrogen-ion concentration the data here recorded were obtained by averaging the growth of 20 colonies of each organism at each of the noncritical temperatures, although the growth of 30 to 50 colonies was averaged at critical temperatures and whenever there was any doubt as to the comparative growth rate on the two lots of media or between two temperatures. To obtain growth of *Rhizoctonia solani* for 6 days at temperatures favoring rapid growth it was necessary to use 20-cm Petri dishes instead of the usual 10-cm ones. It was also necessary to draw figure 2, representing the daily growth rate of *Rhizoctonia*, on a different scale from that used for all other figures.

TOMATO INOCULATIONS

Mature-green tomatoes, selected for freedom from decay and blemishes, were washed in water, after which they were sterilized by washing for 3 minutes in a formaldehyde solution bath (1 part commercial formaldehyde to 250 parts of water). The tomatoes were then rinsed twice in sterile water and stacked in a sterile inoculation chamber till dry. The fruits were inoculated by inserting bits of mycelium, or spores and mycelium, from pure cultures of the organisms into the wound made by puncturing the tomato with a sterile probe. The wound thus resulting was 2 mm in diameter and about 3 mm deep.

Immediately after inoculation the tomatoes were placed in new cellophane bags that were large enough to close tightly by folding the edge of the mouth over several times and yet leave sufficient air space about the enclosed tomato. The cellophane permitted observations to be made for several days without removing the tomato. Only when the lesions became very large was it necessary to open the bags to measure the curved surfaces. A duplicate experiment was run at some temperatures in which the tomatoes were placed in moist chambers instead of in cellophane bags, but no difference in results was noted. The juice of the tomato welled up into the wound sufficiently to moisten the inoculum and provide the necessary moisture for initial growth of the fungus. For the tomatoes used the average pH of ripe fruit juice was 6.01, and for green fruits it was pH 4.7.

In nearly all experiments the tomatoes in the cellophane bags were divided into two lots, the first half (lot 1) being placed at once in the temperature-control chambers, the remainder (lot 2) being held for 24 hours at room temperature before being exposed to the various temperatures ranging from 35° to 85° F. at 5° intervals. In most

cases there was little difference between these two lots at temperatures above 50°. All experimental lots remained for a period of a week to 10 days in the temperature chambers. Sufficient tomatoes were cultured after removal from the boxes to check the purity of the lesions and the reliability of the data obtained. In only one case was any contaminating organism other than bacteria present. It was easy to detect the presence of bacteria, and it is interesting to note that they sometimes retarded the rate of development of lesions and sometimes hastened it, but in many instances the presence of the bacteria did not alter the growth rate of the fungus through the tissues. Controls, similarly wounded, were held at all temperatures and remained sterile except for a few cases of bacterial infection. These infections were doubtless due to handling the fruits during measurement.

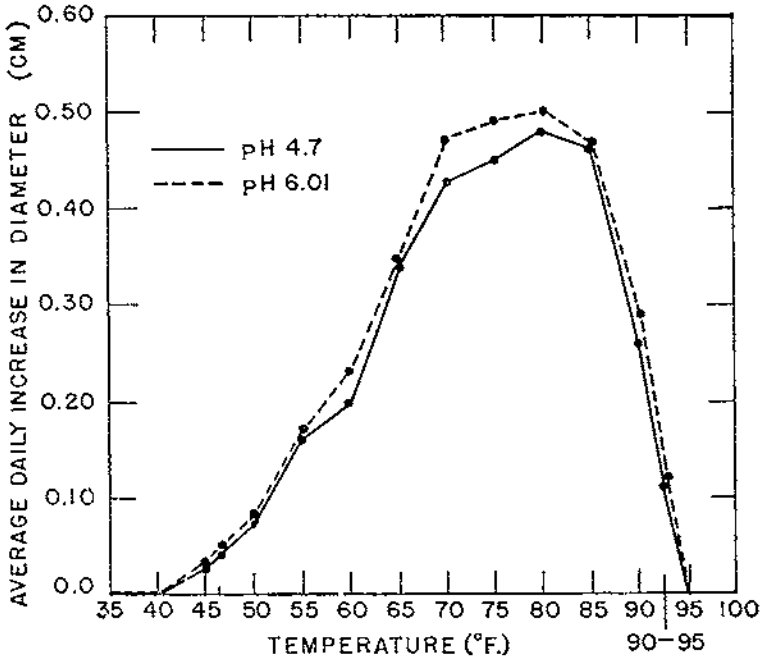

FIGURE 1.—Average daily increases in diameter of *Melanconium* colonies grown on potato dextrose agar of pH 4.7 and of pH 6.01 at temperatures ranging from 35° to 95° F.

Note was made of the ripening of the green fruits. Duplicate experimental inoculations were made on sterilized ripe tomatoes which were exposed to those temperatures most likely to occur during the ripening of commercial fruits. These tomatoes were placed in cellophane bags at the respective temperatures on the day of inoculation, and in some instances concomitant inoculations were made on green and turning fruits for comparison of the growth rate of the lesions developed on fruits of the various stages of maturity.

MELANCONIUM SP.

AGAR PLATE CULTURES

As will be noted from the curves shown in figure 1, *Melanconium* sp. grew best between 70° and 85° F., with an optimum temperature of

80° for the agar plate cultures. No growth was made after the first day at 40° or lower, although the fungus remained alive during a week's exposure to a temperature range of 33° to 38°. The minimum temperature for 1 week's continuous growth was 45°. The maximum temperature for continuous growth was found to be 90° to 95°. No growth took place at 93° to 97°, but the mycelium resumed growth when brought out into room temperature after 6 days in the temperature-control chamber. The growth on agar of pH 6.01 was slightly greater than that on agar of pH 4.7, but at no temperature was this difference in growth rate very marked, although at optimum temperatures the colonies could easily be distinguished by size at the end of a week. The greater development on the less acid agar plates is in keeping with the faster growth rate on ripe, less acid tomatoes than on green ones.

TOMATO INOCULATIONS

So far as is known, no studies have been made on the temperature relations of melanconium rot of tomatoes other than the work of Tisdale (*19*),[3] who reports that under favorable conditions (72° to 80° F.) the spores germinate within a few hours and are capable of infecting both green and ripe fruits. In the present tests it was found that at temperatures of 40° and lower (minimum 32°) no decay took place on mature green tomatoes put into the refrigerators immediately after inoculation, although the fungus remained alive, for lesions began to develop upon removal from the refrigerating room and in 4 days averaged 1.19 cm in diameter. In the case of the fruits held a day at room temperature before exposure to a temperature of 35° to 41° slight development became evident on the tenth to the eleventh day. Lesions that averaged 0.53 cm in diameter on the tenth day had increased to only 0.65 cm by the twelfth day, but growth was hastened by the removal of the fruit to room temperature. In all inoculated fruits held at temperatures higher than 40° there was 100 percent infection.

At 45° F. there was very slight evidence of decay on the eighth day (table 1) in fruits given a day's start at room temperature (lot 2), and a day later in those put into immediate storage (lot 1). Both lots averaged 0.56 cm in diameter on the tenth day.

At 50° F. on the inoculated fruits held at room temperature for the first day, the visible initiation of lesion development was 3 days ahead (fifth to seventh day) of those placed at 50° immediately following inoculation. On the tenth day the average diameter of the latter was 0.59 cm and of the former 0.78 cm. After a fortnight's development the lesions averaged 1.07 and 1.26 cm, respectively.

Although lot 2 showed initial lesion development on the fourth and fifth days (1 day ahead of lot 1), there was little difference between the two lots at 55° F., consequently all measurements were averaged together and the average diameter of the lesions on the tenth day was found to be 0.97 cm. Both lots of fruits exposed to a constant temperature of 60° ripened at about the same time and showed first signs of decay on the fourth to the fifth day, the average lesion diameter on the tenth day being 1.41 cm. At 65° lesions that appeared on the fourth day in both lots averaged 1.80 cm in diameter on the tenth day. The lesions on the riper fruits showed acervuli and spores, as did a few of those held at 60°.

[3] Italic numbers in parentheses refer to Literature Cited, p. 35.

TABLE 1.—*Decay produced by various organisms when inoculated into green tomatoes held at various temperatures for 5 to 10 days*

RHIZOCTONIA SOLANI

Storage period (days)	Average diameter of lesions in specified lots of green tomatoes held at indicated temperature													
	32°–41° F.		45° F.		50° F.		55° F.		60° F. (lots 1 and 2)	65° F. (lots 1 and 2)	70° F. (lots 1 and 2)	75° F. (lots 1 and 2)	80° F. (lots 1 and 2)	85° F. (lots 1 and 2)
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2						
	Cm	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*
5	0.0	0.0	0.0	0.0	0.0	----------	0.47	0.38	0.63	0.52	1.90	2.71	3.27	3.04
6	.0	.0	.0	.0	.0	----------	.50	.45	.82	.98	3.00	3.86	4.51	3.75
7	.0	.0	.0	.0	.0	0.43	[1].50	.53	1.29	1.65	4.14	5.34	5.65	4.54
8	.0	.0	.0	.0	.0	.46	.52	.64	1.76	2.36	5.25	6.13	6.60	5.42
9	.0	.0	.0	.0	.0	.51	.53	.88	2.20	2.96	6.09	7.15	7.63	[2]
10	.0	.0	.0	.0	.0	.57	[1].62	1.12	2.53	3.74	7.20	7.98	8.76	----------
COLLETOTRICHUM PHOMOIDES														
5	0.0	0.0	0.0	----------	----------	----------	----------	0.56	0.81	0.57	0.96	1.02	1.11	0.69
6	.0	.0	.0	----------	----------	0.60	----------	.66	.99	.88	1.26	1.72	1.46	1.11
7	.0	.0	.0	----------	----------	.67	----------	.72	1.12	1.39	1.70	2.19	2.01	1.63
8	.0	.0	.0	0.62	0.60	.74	0.57	.86	1.30	1.61	2.25	2.38	2.55	2.21
9	.0	.0	.0	.67	.73	.89	.65	1.00	1.59	2.37	2.80	3.15	3.17	2.88
10	.0	.0	.0	.72	.86	1.03	.78	1.15	1.90	2.92	3.45	3.68	3.79	3.46
PHOMA DESTRUCTIVA														
5	0.0	0.0	0.0	----------	----------	----------	[3] 0.51	----------	0.60	0.48	0.57	0.51	0.55	0.53
6	.0	.0	.0	----------	----------	----------	.58	----------	.71	.58	.63	.61	.60	.55
7	.0	.0	.0	----------	----------	0.51	.69	----------	.80	.67	.79	.80	.70	.60
8	.0	.0	.0	----------	----------	.53	.78	----------	.92	.78	1.00	1.07	.78	.64
9	.0	.0	.0	----------	0.49	.61	.83	----------	1.06	.93	1.27	1.27	.84	.64
10	.0	.0	.0	0.44	.54	.71	.88	----------	1.23	[4] 1.13 / [5] 1.28	[4] 1.57	[4] 1.58	.90	[6]
MELANCONIUM SP.														
5	0.0	0.0	----------	----------	----------	0.50	[3] 0.58	----------	0.71	0.87	1.12	1.18	1.25	1.05
6	.0	.0	----------	----------	----------	.54	.66	----------	.87	1.04	1.31	1.51	1.52	1.26
7	.0	.0	----------	----------	----------	.57	.77	----------	1.00	1.17	1.61	1.76	1.81	1.47
8	.0	.0	----------	0.53	0.45	.62	.85	----------	1.12	1.37	1.94	2.04	2.10	1.67
9	.0	.0	0.53	.55	.52	.70	.91	----------	1.27	1.59	2.19	2.37	2.41	1.83
10	.0	.53	.56	.56	.59	.78	.97	----------	1.41	1.80	2.43	2.70	2.85	1.98

[1] Some still look negative.
[2] Cultured.
[3] This and following values are averages of lots 1 and 2.
[4] Larger diameter inside; 0.89 cm deep.
[5] Inside measurement.
[6] Cultured; doubtful to slight growth.

At temperatures below 65° F. the initial growth rate was slightly faster on fruits in lot 2, but at all the higher temperatures employed it was the lesions on fruit of lot 1 that showed initially higher growth rate, since they were not exposed to 12 hours of night temperature ranging from 60° to 66°. There was, however, no marked difference in the diameters of the lesions after the first few days, consequently all measurements were averaged together.

At 70° F. and at 75° the lesions were visible on the third day and averaged 2.43 cm in diameter at 70° and 2.70 cm at 75° after 10 days, growth. At 80° the lesions appeared on the second to the third day, and their average diameter on the tenth day was 2.85 cm. At these three temperatures all lesions showed acervuli and spores, some as early as the seventh day after inoculation. Since the comparable average diameter at 85° was only 1.98 cm, it is clear that the optimum temperature for advance of the fungus within the tissues of the tomato corresponded with the optimum for growth on the potato-dextrose agar plates.

Melanconium grew faster on ripe tomatoes than on green ones, thus checking with field observations made in Texas by the junior writer. At any one temperature the lesions on those fruits that ripened first developed more rapidly. To check the rate of development on green and ripe fruits, some ripe tomatoes were inoculated for comparison. At 70° the lesions on ripe fruits averaged 2.88 cm in diameter on the tenth day. Contrasted with this is the 10-day average of 2.56 cm on fruits that had been inoculated while green and were not ripe until the sixth or seventh day thereafter. Similar results occurred at higher temperatures, although, since the green tomatoes ripened more quickly at higher temperatures, the difference in size between lesions on the fruits of the two maturities was not as marked at the conclusion of the experiment.

As the green tomatoes ripened, the rate of spread of *Melanconium* within the tissues increased, but after the lesions had attained considerable size the growth rate began to decrease, so that the average daily increase followed a curve that had its high point at approximately the time the fruit was just turning red. Therefore, in any one experiment the difference between the diameters of lesions on tomatoes inoculated while green and the ones on parallel fruits inoculated after ripening decreased as the green fruit ripened. This may be illustrated by the results from a lot of green and ripe inoculated fruits held at 75° F. On the ripe fruit the lesions averaging 1.34 cm in diameter on the fifth day had made an average daily growth of 0.27 cm, whereas those on the green fruit (now pink) had made an average daily growth of 0.23 cm and averaged only 1.15 cm in diameter. In the subsequent 24 hours the lesions on the ripe fruits grew 0.30 cm in diameter, while those on the green fruit (now red) grew 0.37 cm; consequently the respective diameters were now 1.64 cm and 1.52 cm, a difference between ripe and green fruit lesions of 0.12 cm, as compared with a 0.19 cm difference on the previous day. By the tenth day the daily increase of lesions on the ripe fruit had dropped to 0.26 cm and that of the ripened green fruits to 0.34 cm, making the average diameters practically identical. The growth rate of lesions on green tomatoes showed a subsequent drop, so that from the tenth to the fourteenth day the average daily increase in diameter was 0.22 cm. This type of curve for daily growth rate

over the 10-day period was characteristic of several of the fungi studied. Other workers have found a similar growth curve for other fungi.

DISCUSSION

The data obtained from agar plate studies of *Melanconium* sp. are in excellent agreement with the findings on tomato inoculations. The optimum for growth is high (80° F.).

So far the disease has been reported from few tomato-growing districts (*12*, *19*) and has been of little economic importance except in greenhouse tomatoes, although it has been found during several years in shipments from Texas, in some years more extensively than others.

In spite of the high optimum for growth, once the fungus is established it may continue to develop slowly within tomatoes even at low temperatures (35° to 41° F.). While low temperatures in transit may check the rate of advance of the fungus, development of the lesions proceeds rapidly as soon as the tomato is put into the ripening room.

The decay advances more rapidly in ripe than in green fruits, consequently larger lesions may be expected on tomatoes that are ripe at the end of the transit period. Since acervuli and spores develop sooner on ripe than on green fruits, the presence of these fruiting bodies is not an accurate diagnosis of the age of the lesion, though mature acervuli and spores are usually not present even on ripe tomatoes in less than 7 days after infection takes place.

RHIZOCTONIA SOLANI

AGAR PLATE CULTURES

As shown in figure 2, the growth rate of the tomato strain of *Rhizoctonia solani* Kühn did not vary greatly on the two media used except at temperatures ranging between 70° and 85° F., within which range the organism grew appreciably faster on the media having the hydrogen-ion concentration of the ripe tomatoes, namely, pH 6.01. At all other temperatures the growth rate was the same or even slightly better on the media having the acidity of green fruits, pH 4.7. Samuel and Garrett (*17*), working with a strain of *Rhizoctonia* isolated from potato, found that the optimum hydrogen-ion concentration for growth on potato-dextrose agar was pH 6.5 to 7.0. The optimum growth of the tomato strain on both pH 4.7 and 6.01 agars occurred at 80°, and this optimum coincides with that obtained for tomato decay. Wellman (*20*) reports practically the same optimum (25° to 27° C. or 77° to 80.6° F.) for the strain of *Rhizoctonia* causing bottom rot of cabbage, although Samuel and Garrett (*17*) found the optimum for wheat and potato strains to be slightly lower (23° to 26° C. or 73.4° to 78.8° F.). Monteith and Dahl (*6*), working with 9 strains of *Rhizoctonia solani* from grass, pea, and potato, found that in general these had their optimum temperature between 25° and 30° C. (77° to 86° F.).

The minimum temperature at which very scant growth of *Rhizoctonia solani* (tomato strain) occurred was found to be 45° to 49° F., which again agrees with the minimum (9° C. or 48.2° F.) cited by Wellman and with that (less than 10° C. or 50° F.) given by Monteith and Dahl, but is higher than that (4° C. or 39.2° F.) given by Samuel

and Garrett for their strains. It was found that the tomato organism could be killed by a week of exposure to temperatures around 35° and was sometimes killed within a week at 38°. Some product given off during the degeneration and death of the fungus at these temperatures cleared the agar for an average of 6 to 10 mm around the

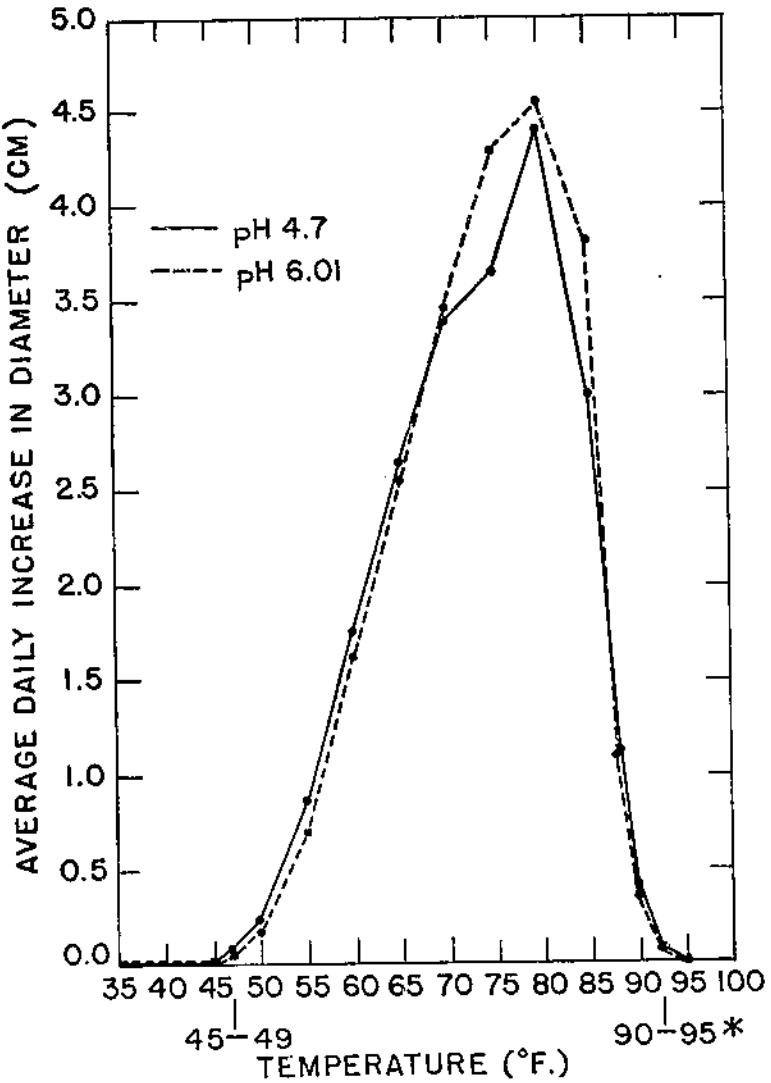

FIGURE 2.—Average daily increases in diameter of colonies of *Rhizoctonia solani* (tomato strain) grown on potato-dextrose agar of pH 4.7 and of pH 6.01 at temperatures ranging from 35° to 95° F. Scale is one-fifth that of all the other figures. (*Indicates that growth ceased before end of the week.)

colonies. At 40° no growth took place after the first day, but the mycelium was not killed by a week's exposure to this temperature. The first normal-looking mycelium occurred at 50°, where the average daily growth rate was around 0.20 cm.

The maximum temperature at which growth occurred was 90° to 95° F. with an average daily growth rate of less than 0.10 cm. When the temperature was raised to 92° to 95° the fungus ceased growing before the end of a week but was not killed. No growth occurred at 95° and the mycelium was dead by the end of a week, the same type of cleared area developing around the colony as occurred at temperatures low enough to kill the fungus. Both Wellman and Samuel and Garrett give 32° C. as about the maximum for the *Rhizoctonia* strains used in their investigations, while Monteith and Dahl give 35° C. (95° F.) as the maximum for their strains.

TOMATO INOCULATIONS

Although the fungus remained alive in many of the inoculated tomatoes after 2 weeks exposure to temperatures ranging from 32° to 41° F., all inoculations with *Rhizoctonia* were negative at temperatures lower than 50°, as shown in table 1. At this temperature the fruits placed in the chamber at once were still negative at the end of 15 days, but those that were held in the laboratory for 1 day after inoculation showed 63-percent infection. However, the lesions, which began to appear on the seventh day, developed very slowly and averaged only 0.57 cm in diameter after 10 days. The fungus was still alive in the 37 percent showing no decay and produced typical lesions when the fruits were removed to room temperature.

At 55° F. 100-percent infection occurred, though the lesions in the fruits in lot 1 were very small, averaging only 0.62 cm in diameter at the end of 10 days, while those in lot 2 averaged 1.12 cm. These were the first fruits to show measurable rhizoctonia lesions after 6 days, the lesions of lot 1 appearing on the fifth to sixth days, those of lot 2 24 hours earlier. From 60° upward there was little difference between lots 1 and 2, and cited diameters are an average of all lesions. Well-defined decay occurred in 5 to 6 days on both lots at 60°, the lesions averaging 0.82 cm on the sixth day and 2.53 cm on the tenth. The fruits held at 65° developed lesions averaging 3.74 cm in diameter by the tenth day.

At all higher temperatures most of the fruits were cultured earlier than the tenth day, inasmuch as the lesions grew so large on the ripened fruit that many cracked open or involved too much of the fruit to measure accurately. However, the 10-day diameters show a general increase to the maximum figure at the optimum of 80° F., where lesions over 8 cm in diameter developed in 10 days. From 70° upward the beginning of decay became evident on the second or third day. Under transit conditions with commercial stock Ramsey and Bailey (*11*) found that mature green tomatoes inoculated at 75° to 80° began to show decay on the second day and that lesions averaging over 2.0 cm in diameter were produced in 5 days. Under the conditions of the present experiments the rate of growth increased so rapidly at temperatures above 65° that a difference of only 2° made a noticeable difference in growth rate between any two lots. The decay spread rapidly at 85°, although there was a drop from the optimum rate of development most noticeable on green fruits.

Rhizoctonia, like *Melanconium*, grew more rapidly on ripe fruits, and the same relationships for comparative daily growth rate on green, turning, and ripe tomatoes held true. The optimum for development of rhizoctonia rot (soil rot) on both green and ripe

fruits lay at 80° F., where the decay became evident on the second day. Table 2 shows the comparative daily diameters of lesions developing on green, turning, and ripe tomatoes held at temperatures of 70° and above.

TABLE 2.—*Percentage of infection by Rhizoctonia solani and comparative diameters of lesions on tomatoes of different degrees of maturity*

Temperature (° F.)	Maturity of tomatoes	Diameter on—					Inoculated fruit showing decay
		Third day	Fourth day	Fifth day	Sixth day	Seventh day	
		Cm	*Cm*	*Cm*	*Cm*	*Cm*	*Percent*
70	Green	0.70	1.08	1.90	3.00	4.14	98
	Turning	1.06	1.70	2.57	3.48	4.25	89
	Ripe	1.11	1.88	2.86	3.88	4.42	100
75	Green	.92	1.50	2.71	3.86	5.34	82
	Turning	1.14	1.93	2.83	3.75	(1)	100
	Ripe	1.90	2.73	3.62	4.27	(1)	100
80	Green	1.21	2.11	3.27	4.51	5.65	97
	Ripe	1.77	3.16	4.06	5.14	5.80	100
85	Green	1.11	1.65	3.04	3.75	4.54	96
	Ripe	2.64	3.86	4.42	5.10	5.72	100

[1] Cultured.

DISCUSSION

The rapidity with which rhizoctonia lesions develop on both green and ripe tomatoes under conditions of transit and storage, together with the ability of the organism to spread from fruit to fruit through the paper wraps under humid conditions, makes this one of the most serious transit and storage diseases of tomatoes. Fruits showing very small spots at the time of packing may develop large lesions before reaching their destination. From the standpoint of spreading the disease in transit, the lesions occurring on ripe fruits are more important, since these develop faster and the tomatoes soon crack open, the juices wetting the wraps and providing excellent conditions for the spread of the fungus to other fruits in the basket or lug.

Temperatures of 50° F. and lower will prevent new infection. In early stages of infection the fungus may be checked and in some cases killed by holding the fruit at temperatures lower than 50°. However, since in many instances the organism is not killed, the decay, temporarily checked, will resume development when the fruits are removed to higher temperatures.

FUSARIUM SEMITECTUM

AGAR PLATE CULTURES

In the case of *Fusarium semitectum* Berk. and Rav. the difference between growth on agar of pH 4.7 and that of pH 6.01 was quite striking at temperatures between 60° and 95° F., so that within a few days there was a noticeable difference in the size of the respective colonies (fig. 3). This was especially true at the optimum temperature for growth, 75°, where the 10-cm Petri dishes containing agar of pH 6.01 were more than full of fungus growth after 8 days, whereas the colonies on the pH 4.7 agar averaged 8 cm in diameter. This difference in growth rate at the two acidities is in keeping with

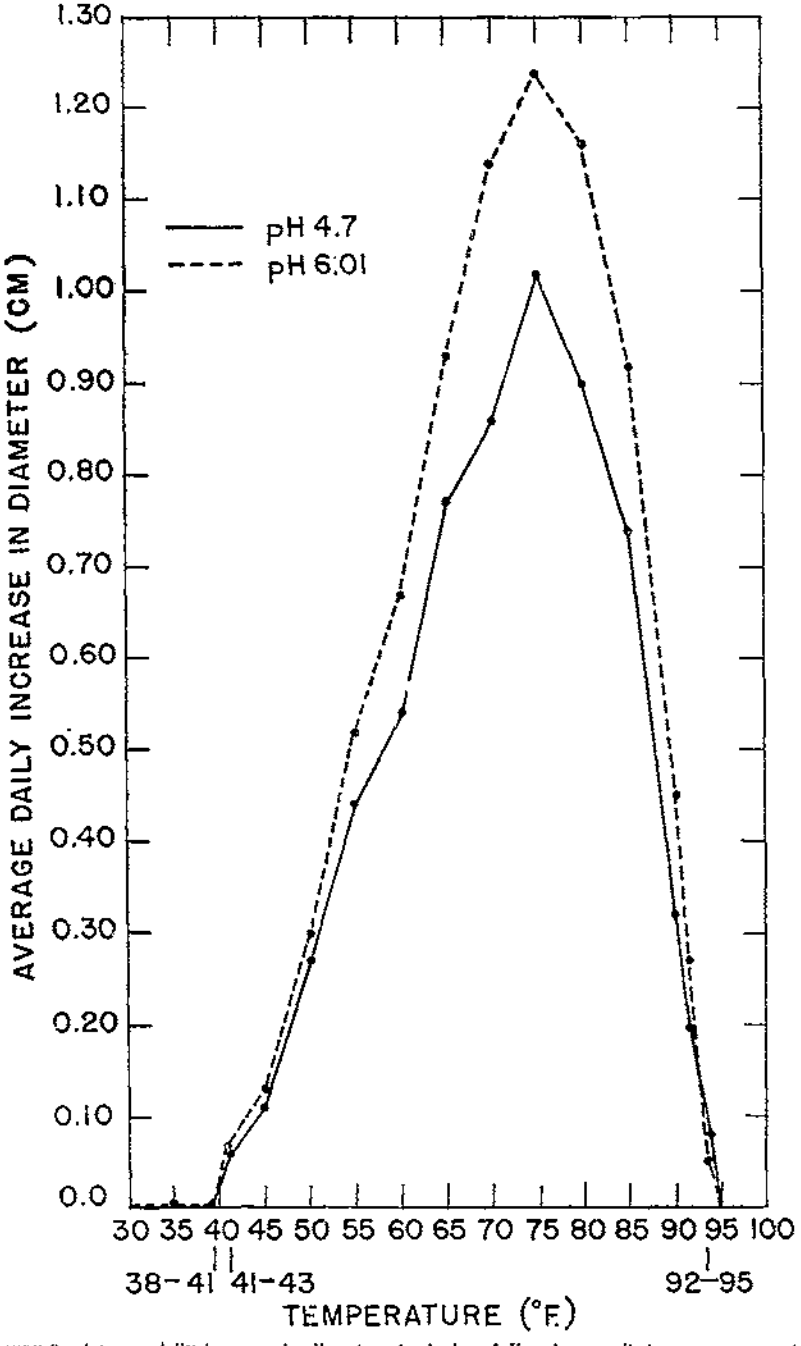

FIGURE 3.—Average daily increases in diameter of colonies of *Fusarium semitectum* grown on potato dextrose agar of pH 4.7 and of pH 6.01 at temperatures ranging from 30° to 95° F.

the development of lesions on green and ripe fruits. (See Discussion, below.)

A rapid drop in growth rate occurred at 85° F. The maximum temperature for 1 week's continuous growth was 92° to 95°, where the daily growth was less than 0.1 cm increase in diameter. No growth occurred after the first day at 95°, although the fungus was not killed during 4 successive days at this temperature.

The minimum for continuous growth of 1 week occurred at 41° to 43° F., the daily increases in diameter being about the same as at the maximum. At 40° the fungus grew for 2 days and then ceased growth, but at temperatures below this all growth ceased after the first day, although cultures exposed for 3 days at 30° to 34° were still alive.

TOMATO INOCULATIONS

Tomatoes inoculated with *Fusarium semitectum* showed no decay at temperatures lower than 45° F., and even at this temperature mature-green fruits developed decay only if they were allowed to stand for 24 hours at room temperature before being placed in the refrigerator. Fruits so treated developed lesions that averaged but slightly less than 1 cm in diameter 10 days after inoculation. Both those fruits placed in the cold chambers immediately after inoculation and those held at room temperature for 24 hours before inoculation developed lesions about 1 cm in diameter at 50° in the same period. The average diameter of all lesions after 10 days at 55° was 1.36 cm; at 60° about 1.5 cm; at 65°, 2.0 cm; and at 70°, 2.8 cm. At 70°, 58 percent of the inoculations were successful in producing decay. The optimum temperature for decay was reached at 75°, when 69 percent of the inoculated tomatoes developed lesions which grew to an average diameter of 3.5 cm and a depth of 2.5 cm in 10 days. At 80° the lesions developing on 46 percent of the inoculated fruits had an average diameter of 3.25 cm and an average depth of 1.8 cm in 10 days, whereas at 85° in the same length of time 43 percent of the inoculated tomatoes developed lesions whose average diameter was only 1.9 cm and average depth 1.3 cm.

Not only was the percentage of decay greater on ripe fruits (89 percent at 70° F., 92 percent at 75°, and 100 percent at 80°), but the *Fusarium* grew much faster on ripe and turning fruits than on green ones. The initial growth on ripe fruits was more rapid than on those that were turning, although the growth rate of lesions on the latter equaled that on the ripe tomatoes after several days of development. The relationship between the daily growth-rate curves at any one temperature for green and ripe fruits was similar to that described in the case of *Melanconium*. The average diameter of 10-day-old lesions on fruits inoculated after reaching ripeness was 2.60, 3.94, 4.21, and 4.23 cm, respectively, for temperatures of 65°, 70°, 75°, and 80°. It will be noted that the diameter of those at 80° was equal to the diameter of those at 75°, although this was not true of the lesions on tomatoes inoculated while green.

DISCUSSION

Although several species and strains of *Fusarium* are able to cause tomato decay in transit and storage when access is given through wounds or the lesions formed by other fungi (*12*), *Fusarium* spp. are generally not important as decay producers, except on ripe-

turning tomatoes. In this connection the close correlation between the comparative growth rates on agar of the two acidities and on inoculated tomatoes of the two maturities is interesting (table 3). It would seem that the lesser relative acidity is at least one factor in the greater susceptibility of ripe tomatoes to decay by *Fusarium semitectum*.

Although growth of *Fusarium semitectum* can be held in check by a storage temperature of 40° F., it is not advisable to hold tomatoes at this temperature for more than 5 to 8 days because of the danger of chilling injury (*21*), and it would be impossible to prevent its later development in the ripening room if infection had occurred. The speed with which this fungus develops in the tissues of tomatoes at ripening room temperatures causes total loss of infected fruits within a few days.

TABLE 3.—*Comparison of growth rate of Fusarium semitectum on inoculated green and ripe tomatoes and on agars of comparable acidities*

Temperature (° F.)	Average daily rate of growth in diameter on agar			Average 10-day diameter of lesions on inoculated tomatoes		
	pH 4.7	pH 6.01	pH 4.7[1] pH 6.01	Green when inoculated	Ripe when inoculated	Green[2] ripe
	Cm	*Cm*	*Percent*	*Cm*	*Cm*	*Percent*
65	0.77	0.93	83	2.60	2.60	77
70	.86	1.14	75	2.80	3.04	72
75	1.02	1.24	82	3.50	4.21	83
80	.90	1.16	78	3.25	4.23	77

[1] Rate of growth on agar of pH 4.7 expressed as percentage of rate of growth on agar of pH 6.01.
[2] Diameter of lesions on green tomatoes, expressed as percentage of diameter of lesions on ripe tomatoes.

COLLETOTRICHUM PHOMOIDES

AGAR PLATE CULTURES

A rapid development of *Colletotrichum phomoides* (Sacc.) Chester was obtained at favorable temperatures, 65° to 85° F. on both pH 4.7 and pH 6.01 potato-dextrose agar, with slightly faster growth always on the less acid medium. The optimum temperature for growth on media of both acidities was 80°, as shown in figure 4, and this agrees with the results of tomato inoculations. The development at 85° was somewhat less, but at temperatures above 85° the growth rate rapidly diminished.

This organism had a higher maximum temperature (95° F.) for continuous growth than any of the other fungi studied except *Alternaria solani*. In an incubator whose temperature fluctuated around 95° the organism continued to make slight growth for the week of the experimental observations, although the daily increase in diameter was less than 0.1 cm. The growth was not normal, however, the colony seeming almost to contract toward the center with a piling up of the mycelial growth in the center and a final pulling away of the entire colony from the agar. No acervuli were produced, but after a day or two at room temperature normal growth was resumed. When the average temperature was raised about 1° no growth occurred, but the fungus survived 4 days at this temperature and was able to resume growth when brought out of the incubator.

Colonies exposed for 20 days at 30° to 34° F. were not killed, but no growth was made after the first day in the refrigerators. The minimum temperature for growth was 35°, at which temperature a

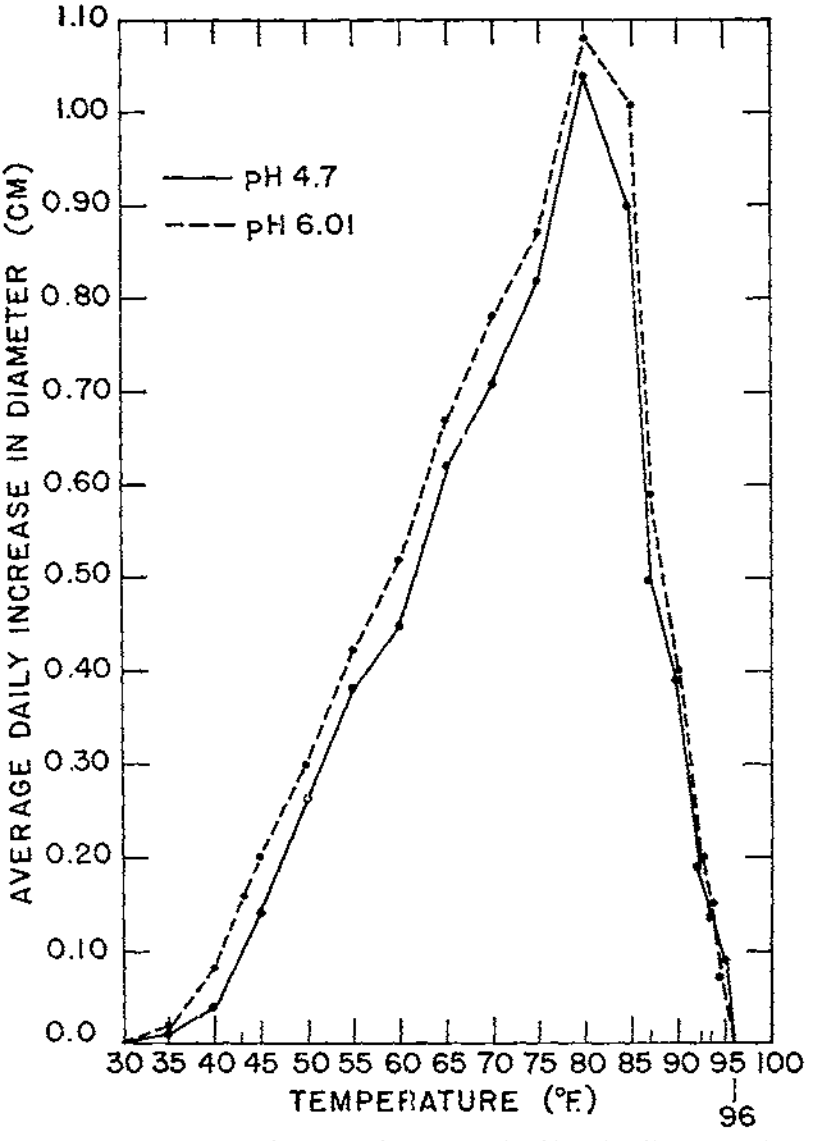

FIGURE 4.—Average daily increases in diameter of colonies of *Colletotrichum phomoides* on potato-dextrose agar of pH 4.7 and of pH 6.01 at temperatures ranging from 30° to 96° F.

barely perceptible growth (about 0.01 cm daily increase) occurred during the week of the tests.

TOMATO INOCULATIONS

Colletotrichum phomoides produced larger lesions more rapidly than any other fungus studied with the exception of *Rhizoctonia*

(tables 1 and 2), and, like *Rhizoctonia*, it was able to grow well at high temperatures. Although it grew more rapidly on ripe fruits, it was able to initiate growth on green fruits and developed rapidly as the fruit began to color. No growth took place at temperatures lower than 45° F., at which temperature infection developed only on the lot 2 tomatoes, where first symptoms became visible on the eighth day and the lesions averaged 0.7 cm in diameter on the tenth day. At 50° and at 55° lesions developed on the lot 1 fruits on the eighth day and averaged about 0.8 cm on the tenth day, whereas the fruits of lot 2 showed initial development on the fifth and sixth days and averaged 1 and 1.15 cm, respectively, on the tenth day.

From 60° F. with an average (both lots) 10-day lesion of 1.90 cm, the diameters increased with each rise of 5° in temperature until the optimum 80° was reached, when the average diameter on the tenth day was 3.79 cm. Certain fluctuations occurred (table 1) due to differential ripening of fruits, but the general curve was upward to 80° with a decrease in lesion size at 85°. The fungus often developed as rapidly through the tissues during the first days at 85° as at 80°, but later there was usually a drop in growth rate. The growth on ripe fruits was more rapid but showed the same upward trend to 80° and decrease at 85°, as is shown by table 4. Acervuli commonly were present on 10-day lesions at temperatures above 60°.

TABLE 4.—*Anthracnose (Colletotrichum phomoides) lesions on ripe tomato fruits held at various temperatures for 5 to 10 days*

Storage period (days)	Average diameter of lesions at—					Storage period (days)	Average diameter of lesions at—				
	65° F.	70° F.	75° F.	80° F.	85° F.		65° F.	70° F.	75° F.	80° F.	85° F.
	Cm	*Cm*	*Cm*	*Cm*	*Cm*		*Cm*	*Cm*	*Cm*	*Cm*	*Cm*
5	1.35	1.49	1.52	2.04	1.95	8	2.68	2.87	3.68	3.91	3.38
6	1.82	1.97	2.41	2.74	2.71	9	3.19	3.28	4.19	4.59	4.06
7	2.23	2.41	2.99	3.32	2.89	10	3.43	3.64	4.81	5.08	4.54

DISCUSSION

Colonies of *Colletotrichum phomoides* grew faster on the less acid agar. This fact agrees with the results of fruit inoculations and with the general opinion held by plant pathologists that anthracnose is a "ripe rot" of tomatoes. As pointed out above, most rapid development of decay took place in the ripe fruits. Since the curves for growth made on media of the two acidities corresponding to green and ripe fruits, pH 4.7 and pH 6.01, respectively, are so close together (fig. 4), and not far apart as in the case of the *Fusarium* sp. colonies, it appears that possibly other chemical or physical changes in the fruit during ripening are more important than changes in acidity in influencing the development of decay. Certain it is that ripening speeds up the growth rate, for often on green fruit lesions that have shown no growth for several days, then slow growth as the tomato yellowed, have suddenly doubled in size overnight when the fruit reddened and have continued to develop rapidly from then on.

Since the temperature range of the fungus is so great, one cannot hope to check satisfactorily its progress in tomatoes already infected with it.

PHOMA DESTRUCTIVA

AGAR PLATE CULTURES

Phoma destructiva Plowr. grows well on potato-dextrose agar, but, as indicated in figure 5, it develops much more slowly on media having the acidity of green tomatoes (pH 4.7) than on that more comparable to ripe fruit (pH 6.01). This difference in growth rate is especially noticeable at the temperatures most favoring growth.

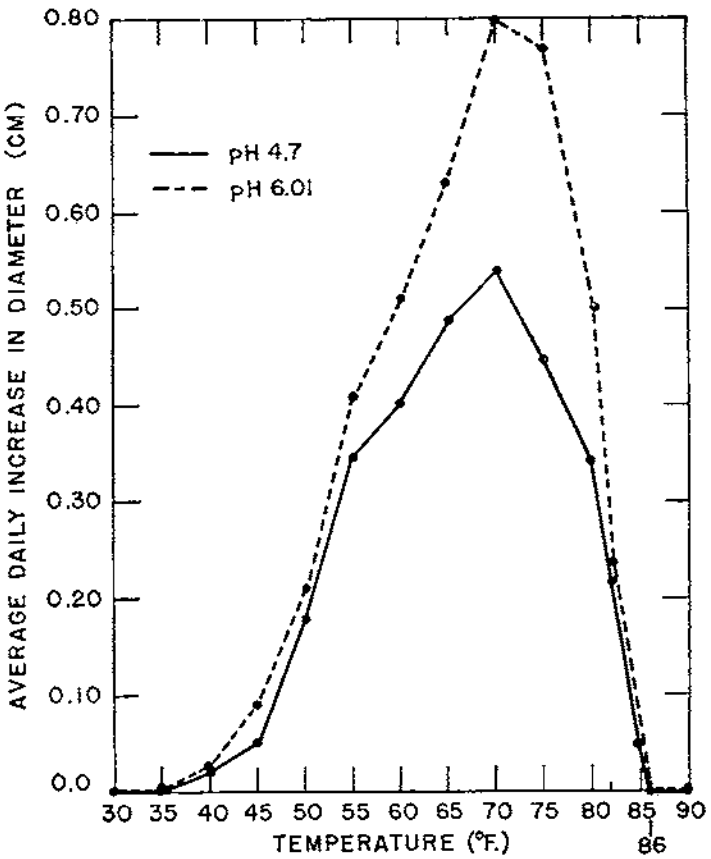

FIGURE 5.—Average daily increases in diameter of colonies of *Phoma destructiva* on potato-dextrose agar of pH 4.7 and of pH 6.01 at temperatures ranging from 30° to 90° F.

The optimum for growth, under the conditions of these experiments, occurred at 70° F.

It had been previously found that strains of *Phoma* carried for some time in artificial culture and subcultured at infrequent intervals sometimes lost their ability to produce pycnidia and produced only a uniformly light-gray mycelial growth quite unlike that of the parent strain. During the course of the present cultural studies it was learned that strains recently isolated from tomato lesions and producing numerous pycnidia in the culture tubes had a tendency to produce saltants by sectoring. Saltants seemed to occur more frequently

on the agar of pH 6.01 than on the more acid agar. Such sterile saltants were not peculiar to *Phoma* but occurred also in the *Colletotrichum* colonies and in *Pleospora*, only in the case of these two organisms the saltant grew more rapidly than the parent strain.

As a rule the saltants that did not produce pycnidia grew at a slightly slower rate on both kinds of agar, but when duplicate series of agar-plate colonies were run it was found that the optimum fell at 70° F. for both parent strain and saltant, with 75° but slightly less favorable for most rapid growth. On the agar plates usually the difference in daily growth rate between the sterile strains and the fertile parent strain was less than 0.05 cm.

In both cases the minimum temperature for continuous growth of 1 week was 40° F.; the daily growth rate was 0.02 cm on the more acid medium and but slightly over this on the less acid medium. At 35° no growth occurred but the fungus was not killed. In fact, it even withstood a temperature of 32° F. for 20 days.

Neither the newly isolated strains nor the old sterile strains were able to grow for a week at temperatures higher than 85° F., in many instances the slight growth (less than 0.1 cm per day on each of the agars) having ceased by the end of the week even at this temperature. At 86° no growth occurred after the first day, and though the fungus was not killed in 7 days at 90°, some of the colonies did not resume growth until the third day after removal to room temperature.

All of the critical temperatures obtained in these tests were lower than those cited by Jamieson (*4*), i. e., minimum 6° C. (42.8° F.), optimum 28° C. (82.4° F.), maximum 32° to 33° C. (89.6° to 91.4° F.); however, her determinations were made by estimating the abundance of mycelial growth in flasks of agar at the end of 10 days' growth at the respective temperatures.

In so many instances the daily growth rate of *Phoma destructiva* colonies, especially on the more acid agar, seemed to increase with the size of the colony that a check was made on the pH of the agar at or near the conclusion of a 7-day growth period. It was found that the fungus gradually changed the pH of the medium in advance of its growth so that the agar just outside of a colony on agar of pH 4.7 was often about pH 5.33 and that outside of a colony on agar of pH 6.01 was pH 6.7 or higher after 7 days' growth. Concomitantly, with the lessening of acidity of the medium there was an increase in growth rate. These facts agree well with the data obtained by *Phoma* inoculations on green and ripe tomato fruits.

The other fungi studied, with the exception of *Rhizoctonia*, all showed this same tendency to change the pH of the medium in an alkaline direction. *Rhizoctonia* alone had no appreciable effect upon the pH of the agar in advance of the mycelium, and hydrogen-ion determinations showed that the mycelium of all the other fungi studied were much more alkaline than that of *Rhizoctonia*. *Colletotrichum phomoides* had more effect upon the hydrogen-ion content than any other fungus studied, sometimes bringing the pH of the 4.7 agar to pH 6.7 in advance of the mycelium and changing the pH 6.01 medium to pH 7.6 at the edge of the colony. This characteristic of the fungus doubtless explains why it is able to grow with such ease on the more acid medium although it is essentially a "ripe rot".

TOMATO INOCULATIONS

Phoma destructiva grows very slowly on green fruits; consequently, there was little difference in growth rate at the different temperatures until after the tomatoes had begun to ripen. In storage experiments at 66° to 76° F. with naturally infected stock, Nightingale and Ramsey (7) found that an average gain in diameter of only 0.33 cm took place in a week on green tomatoes, whereas on ripe fruit the gain was 0.71 cm. Jamieson (4) reports lesions only 1 to 1.5 cm in diameter developing in 2 weeks in the greenhouse on green fruit, whereas on ripe fruits the lesions were 2 to 3.5 cm in diameter.

In the present experiments no growth took place during 12 days at temperatures below 45° F., but the fungus was not killed, for later, after 2 days at near-optimum temperatures, early stages of decay were visible. In a few fruits of those held for 1 day at room temperature before exposure to temperatures of 45° there was slight evidence of decay on the tenth day, but in none of the fruits in lot 1 did lesions appear while in storage at this temperature. At 50° initial decay appeared on the ninth day in lot 1 fruits and on the seventh day in lot 2 fruits. By the tenth day the former lesions averaged 0.54 cm and the latter 0.71 cm in diameter. At 55° the lesions appeared on both lots on the fifth to sixth days, and the average of all lesions was 0.88 cm on the tenth day, when the fruits were green to pink in color. At all remaining temperatures there was no difference between fruits in lot 1 and lot 2.

As is shown in table 1, difference in growth rate at the various temperatures began to appear about the eighth day in those temperatures that permit ripening of the fruits to take place. Even after that, owing to differential ripening of individual fruits, there were daily fluctuations in the curve, which should have its peak at the optimum of 70° F. Often, too, the lesions developed more extensively within the locule than on the surface of the fruits. Such inner development could be observed only when the fruits were cut open; consequently, it is noted after the tenth day and helps to explain such irregularities as occur in the curve at 60° and 65°.

Since green fruits ripened more rapidly at 75° F. than did those at 70°, the initiation of decay took place earlier at 75° in most cases; consequently, the average diameter given for 75° is as high as that at 70°. However, when fruits were sufficiently mature to ripen quickly at 70° the fastest growth rate occurred here. In most experiments with green fruit the optimum occurred at this temperature, but in those cases in which the fruits ripened much faster at 75° the fastest growth rate occurred at 75°, the difference in growth rate decreasing as the fruits in the 70° temperature ripened. It will be noted from the curve of daily growth rate on agar plates (fig. 5) that the optimum for growth occurred at 70°, but that the rate at 75° was only slightly less. When ripe fruits were inoculated the optimum was always 70°. For a comparison of growth rate on green and ripe fruits at temperatures of 65° to 75° compare tables 1 and 5.

TABLE 5.—*Phoma lesions on ripe tomato fruits held at various temperatures for 5 to 10 days*

Storage period (days)	Average diameter of lesions at—			Storage period (days)	Average diameter of lesions at—		
	65° F.	70° F.	75° F.		65° F.	70° F.	75° F.
	Cm	*Cm*	*Cm*		*Cm*	*Cm*	*Cm*
5	0.53	1.06	1.06	8	1.14	2.04	1.78
6	.74	1.38	1.35	9	1.41	2.30	2.10
7	.93	1.70	1.59	10	1.70	2.65	2.30

Though fruits ripened quickly at 80° F. there was a decided drop in the growth rate of the fungus in the tissues, the lesions averaging only 0.90 cm in diameter in 10 days, while 33 percent of the fruits showed no decay. At 85° also, 33 percent of the inoculations failed; on the remaining 67 percent there was slight development for a few days and the growth ceased. When the lesions, averaging 0.64 cm in diameter, were cultured on the ninth day they were found to be doubtful to very slightly positive, although the fungus was still alive.

Old strains kept for several years under artificial culture and non-pycnidia-forming strains obtained by saltation from pure cultures of recently isolated pycnidia-forming strains advanced more slowly within the tomato than did recently isolated strains. Although the lesions produced by the saltants appeared identical with typical forms in the earlier stages of decay, they failed to develop pycnidia on the surface in the advanced stages.

DISCUSSION

Since *Phoma destructiva* grows so slowly in green tomatoes and the initial infections about the stem scar are so easily overlooked at packing time, it is not surprising that the disease caused by it is primarily a transit and market disease of southern-grown tomatoes. Yet, as with the other tomato-rotting organisms, its ability to withstand low temperatures and to resume activity at ripening temperatures make it impossible to control the disease once infection has taken place. Storage at 45° F. of already ripe fruits having small lesions might, however, preserve them from more rapid decay until they could be marketed.

The fact that the fungus grows a third again as fast at optimum temperatures on the less acid medium and that the growth rate increases as the acidity of the agar decreases, is in keeping with the very slow advance of decay on green fruits and the more rapid rate of decay as the tomato ripens. *Phoma* is most destructive on ripe tomatoes, and the indications are that the change of acidity of tomato fruits during the ripening process is one of the important facts favoring rapid development of the rot in both turning and ripe fruits during transit and storage.

CLADOSPORIUM FULVUM

AGAR PLATE CULTURES

The strains of *Cladosporium fulvum* Cke. isolated from tomatoes received on the market tended to sporulate profusely on the media employed in these tests and to produce practically no mycelial growth

at any temperatures save 60° to 70° F. Within this range a uniform mycelial growth took place, producing a circular, well-developed colony that bore a velvety dark-green layer of normal spores. It will be noted from the growth curve in figure 6 that the daily growth rate within this temperature range was much greater than at temperatures above and below, where the colonies were very small, irregular in outline, and greenish brown in color and the spores varied more in size and shape.

The optimum for growth occurred at 70° F., at which temperature the daily growth rate on the more acid medium was 0.41 cm and on the less acid 0.43 cm. It is interesting to note that at temperature

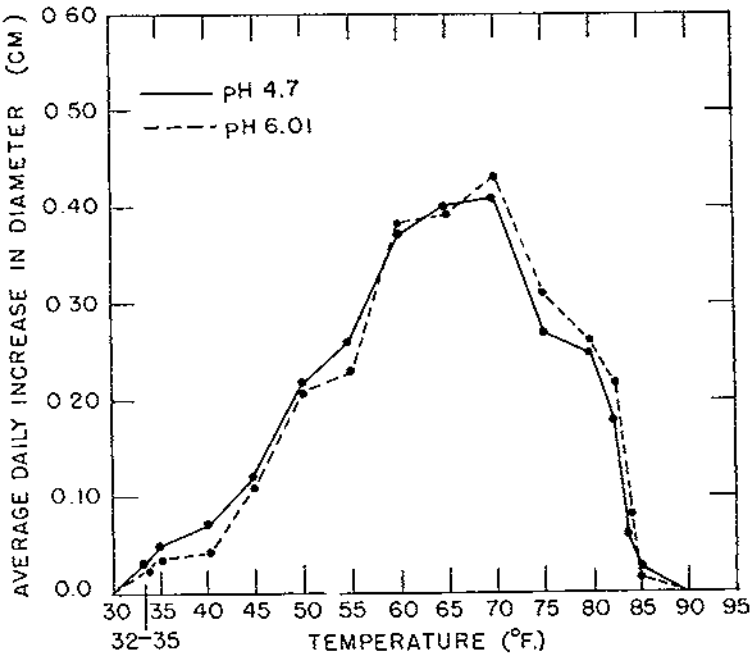

FIGURE 6. Average daily increases in diameter of colonies of *Cladosporium fulvum* on potato-dextrose agar of pH 4.7 and of pH 6.01 at temperatures ranging from 30° to 95° F.

below 60° the fungus grew slightly more rapidly on the medium of pH 4.7, whereas at 60° and above, the medium of pH 6.01 favored more rapid growth. These differences were very slight, however, and in the inoculation experiments here conducted there was no evidence that the organism grew more rapidly on the more acid green tomatoes than on ripe ones at temperatures lower than 60°.

The minimum for growth was 32° to 35° F., the average daily growth rate being 0.03 cm. The fungus was not killed, however, by 3 days' exposure at 30° to 32°. Gardner (*2*), in working with a stem-end infection of greenhouse tomatoes, reported no growth on potato-dextrose agar at 2° C. (35.6° F.), but his other figures agree well with those for the strain here considered. He found that the optimum temperature for growth coincided with that reported by Makemson (*5*) as 20° to 24° C. (68° to 75.2° F.). Gardner states that no growth occurred at 35° C. (95° F.). In the present writers' tests the maxi-

mum for continuous growth of 1 week was 85° F., the average daily growth being only 0.02 to 0.03 cm. At 1° above this the fungus failed to grow after the first 2 days, and at still higher temperatures no growth took place after the first day, during which the cultures had not yet reached the temperature of the incubator. Although no growth took place at temperatures around 87° to 93° the culture was only rarely killed by a week's exposure to such temperatures. The mycelium was killed, as evidenced by microscopic examination and failure of the colonies to resume growth until 4 or 5 days after removal from the incubator. New growth seemed to result from the germination of some of the more heat-resistant spores. Small (*18*) found the thermal death point of the spores of *Cladosporium* to be 115°.

TOMATO INOCULATIONS

Under the conditions of these experiments *Cladosporium fulvum* produced very little decay. There was no decay in 10 days in any fruits held from the day of inoculation (lot 1) at temperatures lower than 45° F., and those of lot 2, which were held for 1 day at room temperature first, showed only doubtful evidence of lesion development on the tenth day. However, the organism was still alive within the fruit even after 10 days at 32° to 41°.

At 50° F. 25 percent of the inoculated green fruit showed small lesions at the conclusion of the tests, while at 55° and at 60° the percentage of infection was doubled but the lesions still measured about 0.5 cm on the tenth day. Even at 65° and 70°, where there was 66 percent and 54 percent infection, respectively, the external lesions on green fruits did not average over 0.5 cm in diameter in 10 days, although in some cases the fungus spread out in the locule so that the area covered with mycelium was greater than 1 cm in diameter and the average internal diameter of all lesions was about 0.7 cm. Those at 70° were very slightly if any larger than those at 65°.

Sometimes the fungus formed merely a thin mycelial mat between locule wall and pulp, although usually it sporulated freely within the moist cavity; but frequently it attacked the seeds, darkening them or forming spores on their surfaces. No soft decay of the pericarp tissues occurred unless bacteria found entrance, but the presence of the mycelium in the carpel wall was evidenced by the brown to blackish network visible on its inner face. Gardner (*2*) makes note of a similar symptom in the subepidermal tissues of some infected fruits in the greenhouse. A greater amount of internal infection occurred in green fruits held at 65° and 70° F. than at 75°. At 75° there was scarcely any surface development, the average lesion being only 0.3 cm in diameter after 10 days, and but 33 percent of the inoculations resulted in the production of small lesions or in fungus discoloration within the seed cavity.

The extent of the surface lesions on the inoculated ripe tomatoes was not much greater than that on inoculated green ones, averaging but slightly over 0.5 cm in diameter even at the optimum temperature for growth. However, the diameter of the inner infected areas averaged 0.71 cm in 10 days at 65° F., 1.08 cm at 70°, and 0.91 cm at 75°. There was no evidence of lesion development on either green or ripe fruits at 80° or at 85°. Several attempts to reisolate the fungus at the end of the test exposures of 10 days in these incubators by replanting the dried inoculum on agar plates failed. Evidently the

fungus died more quickly in the humid atmosphere inside the tomatoes than it did on agar plates held at the same temperatures.

The greatest development of *Cladosporium* within the tissues occurred in those fruits that were held at optimum temperature (65° to 70° F.) and exposed to more humid conditions than those used in the rest of the temperature experiments. In one test in which the tomatoes were kept in a saturated atmosphere in moist chambers some lesions spread out in the interior of the carpel wall or throughout the adjacent seeds to a diameter of 1.5 to 2 cm. There was no case such as that produced in natural stem-end infection of greenhouse fruits or that reported by Reinmuth (*14*) as occurring in the field. Neither Makemson (*5*) nor Gardner (*2*) were able to produce this type of decay by artificial inoculation.

DISCUSSION

It is evident from these experiments that *Cladosporium fulvum* is not a very virulent pathogen under ordinary conditions of transit and storage. Nevertheless, it occurs frequently on tomatoes from California and Mexico (*12, p. 14*), usually occurring as small lesions in shoulder bruises or following insect or other injury. Sometimes a dozen or more lesions occur on one fruit. In some years it has occurred frequently on the Chicago market as a secondary organism following mailhead, especially on Mexican fruits, and often when these two weak pathogens occurred together the resultant lesion reached 1 cm or more in diameter, whereas either pathogen by itself would have made only a small lesion. Under proper conditions of field infection pure *Cladosporium* lesions may reach a diameter of 1.3 cm by the time the fruit is ripe on the market (*12, p. 15*), and in any case of infection it offers an opening to more rapidly developing decay-producing organisms.

ALTERNARIA SOLANI

AGAR PLATE CULTURES

Alternaria solani (Ell. and Martin) Jones and Grout produced an abundant growth of grayish-brown to dark-brown mycelium throughout a wide range of temperatures and on potato-dextrose agar showed the characteristic yellow pigment diffusing through the agar with the development of a brick-red to wine-red color on older colonies, especially at higher temperatures. On the more acid medium the yellow color was more predominant than on the less acid medium. The development of the wine-red color as the colonies increased in size was doubtless tied up with the change in acidity of the medium toward the neutral side, described on page 18.

This fungus was not killed by 3 days at freezing temperatures (30° to 32° F.), but no continuous growth was produced below 35°, where the average daily increase in diameter for a week's growth was about 0.05 cm. Although the better growth was consistently made on the less acid agar at all temperatures, the difference in the acidity of the pH 4.7 and pH 6.01 media did not greatly influence the growth rate of the fungus except at 70° to 85°, where the colonies growing on pH 6.01 were decidedly favored (fig. 7).

The optimum temperature for development on both pH 4.7 and pH 6.01 media was found to be 80° F. At this temperature colonies on agar of both acidities grew so rapidly that they practically filled

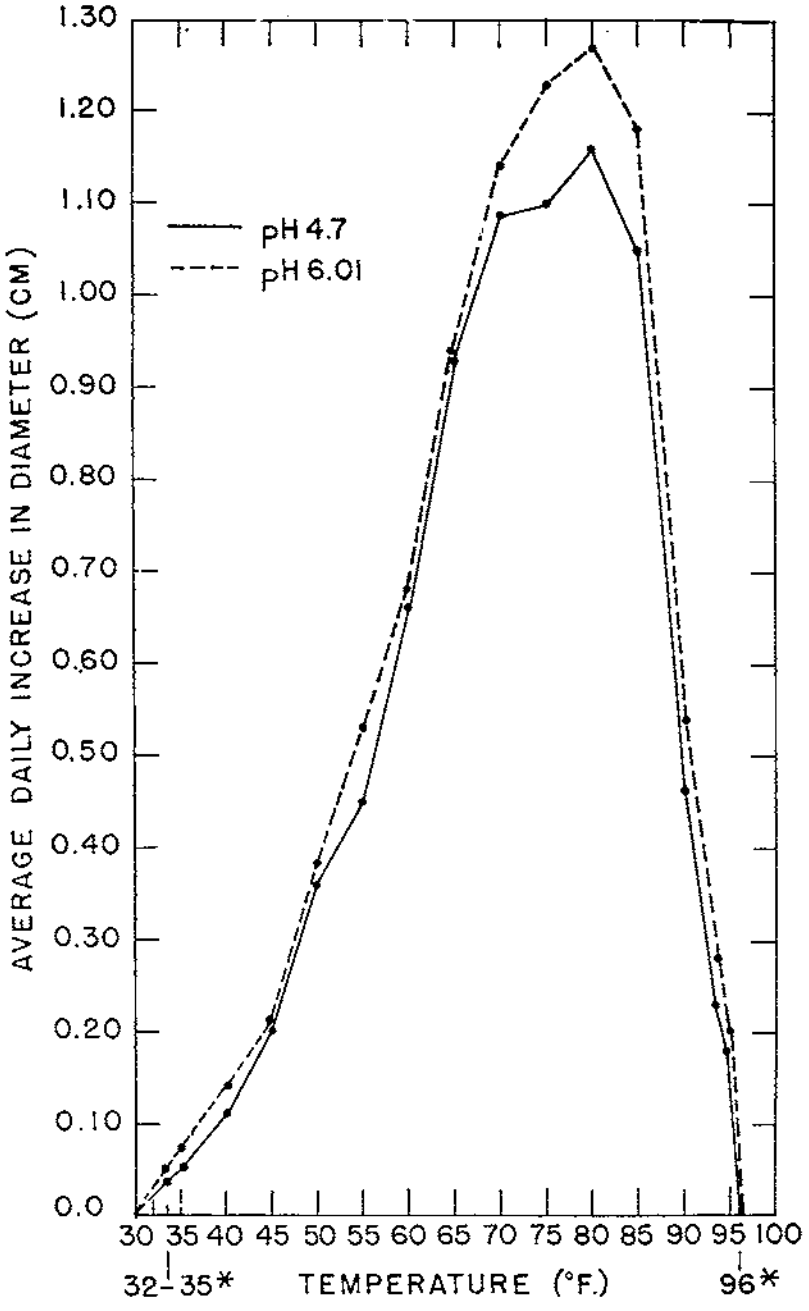

FIGURE 7.—Average daily increases in diameter of colonies of *Alternaria solani* on potato-dextrose agar of pH 4.7 and pH 6.01 at temperatures ranging from 30° to 98° F. (* Indicates that growth ceased after about 3 days.)

the Petri dishes during the week in the incubator, those with the less acid medium often being full a day before those with the more acid agar.

At 75° and 85° F. the growth was slightly less, but there was a decided decrease in growth rate at 90°. At a mean temperature of 95° the fungus grew steadily with a daily increase of slightly under 0.2 cm, but when the temperature was raised to fluctuate about 96° the fungus sometimes grew steadily for a week and sometimes did not grow after the first day. The maximum lay somewhere near 95° to 96°, and it is believed that the temperature at which the inoculum had been growing previously had something to do with its ability to grow slowly or not at all in an incubator fluctuating between 93° and 99°. Monteith and Dahl (*6*) found this to be true of *Rhizoctonia*. Rands (*13*) gives a wide range for the maximum temperature for his potato strain of *Alternaria solani*, which he found to have a maximum between 37° and 45° C. (98.6° to 113° F.). The other cardinal temperatures for the tomato organisms correspond very well with those he cites for his potato strain, i. e., minimum 1° to 2° C. (33° to 36° F.) and optimum 26° to 28° C. (79° to 82° F.).

TOMATO INOCULATIONS

The optimum temperature for development of alternaria rot on green tomatoes was 80° F., at which temperature the first symptoms of decay were visible on the second day and the average surface diameter of lesions on the tenth day was about 0.9 cm, with the inside diameter of the lesions averaging about 1 cm. From 50° through 65° the average diameter of the lesions developed in 10 days was slightly over 0.5 cm, but the decay penetrated to a depth of 0.8 cm. No decay occurred at temperatures lower than 45°. At this temperature there was no visible lesion development until the tenth day, at which time the average diameter was less than 0.5 cm but the lesion penetrated to a depth of 0.8 cm.

At 75° F. the surface diameter of the lesions was only 0.7 cm, but many of them spread out under the locule wall so that the internal diameter was slightly over 1 cm. The lesions at 70° developed more uniformly through the locule wall and the locule and averaged 0.85 cm surface diameter with no additional spread within. At 85°, the average surface diameter was 0.65 cm with a few lesions spreading out slightly wider within the locule. No inoculations were made at temperatures higher than 85°.

With the exception of the minimum temperature at which alternaria rot develops in tomatoes these data furnish a striking contrast to those obtained by Gratz and Bonde (*3*) for the development of alternaria rot in potatoes. In storage experiments they found the minimum temperature for development was about 5° to 7° C. (41° to 44.6° F.), the optimum 13° to 16° C. (55.4° to 60.8° F.), and the maximum about 25° C. (77° F.).

On ripe tomatoes the decay developed very much more rapidly than on green ones. At the optimum temperature (80° F.) the lesions were about 1.5 cm in diameter in a week and 2.35 cm after 10 days, penetrating to a depth of 1.4 cm. They ranged from 1.5 to 4.0 cm in diameter on the tenth day. Lesions 1.0 to 2.0 cm in diameter developed within a week at ripening room temperatures (65° to 73°). Rands (*13*), who inoculated tomatoes in the greenhouse with his

potato strain, found that after 15 days there was only slight invasion about the points of inoculation on the green fruits, whereas with the ripe fruits almost complete rotting occurred.

DISCUSSION

Alternaria solani, which grows much better on ripe fruits, displays quite a marked difference in growth rate on agars adjusted to the acidities of green and of ripe tomatoes, whereas *A. tomato* (see later discussion) shows practically no difference in development on the two media and is almost entirely a parasite of green tomatoes. *A. solani* grows so rapidly at temperatures of 70° to 85° F. that stock held in the ripening room very often shows great loss due to this organism (*12*). So quickly does it progress through the tissues once it has gained entrance through some wound or lesion caused by another organism that infected fruit has little or no market value. This organism is often found associated with *A. tomato*, which is able to penetrate the skin of green fruits and to make small nailhead lesions that offer access to the decay-producing *A. solani*.

ALTERNARIA TOMATO

AGAR PLATE CULTURES

As in the case of some of the other fungi, *Alternaria tomato* (Cke.) Brinkman [4] was able to withstand several days of exposure to temperatures as low as 30° to 32° F. and remain alive, but the minimum for a week's continuous growth on potato-dextrose agar plates was 35°, where the average daily growth for a week was but slightly over 0.05 cm on both media. An abundant growth of grayish-brown mycelium, not so dark as that of *A. solani*, was produced on both pH 4.7 and pH 6.01 agar through a wide range of temperatures, and there was only very slight difference in growth rate on these two media (fig. 8).

The fungus showed a very rapid rate of development, particularly at the higher temperatures, the most rapid growth being made at 85° F. This optimum is 5° higher than that of *Alternaria solani*, which is generally considered one of the high-temperature organisms.

From an average daily increase in diameter of 1.16 cm on pH 4.7 agar and 1.20 cm on pH 6.01 agar at 85° F., the growth rates diminished to 0.53 and 0.58 cm, respectively, at 90°. The maximum temperature of a week's continuous growth of about 0.1 cm daily on agar of both series was 92° to 95°, although in some instances slight growth occurred for 4 or 5 days at 95°. In an incubator whose temperature ranged from 93° to 99°, no growth occurred except for one colony on pH 4.7 agar which grew very feebly for 5 days and then ceased growth. All colonies remained alive, however, with the exception of one which for no detectable reason (as compared with the other colonies) was killed by 7 days' exposure to this temperature. The maximum for this organism quite evidently is lower than that for *Alternaria solani*, which is able to resist the higher temperatures for a longer time. Of course, the choosing of 7 days' growth as the standard for measuring maximum growth is purely arbitrary, but it was found that whenever the reaction to a given maximum temperature of all the colonies in the several duplicate experiments was consistent, growth usually continued over a week's time.

[4] For discussion of name see Brinkman (*1*).

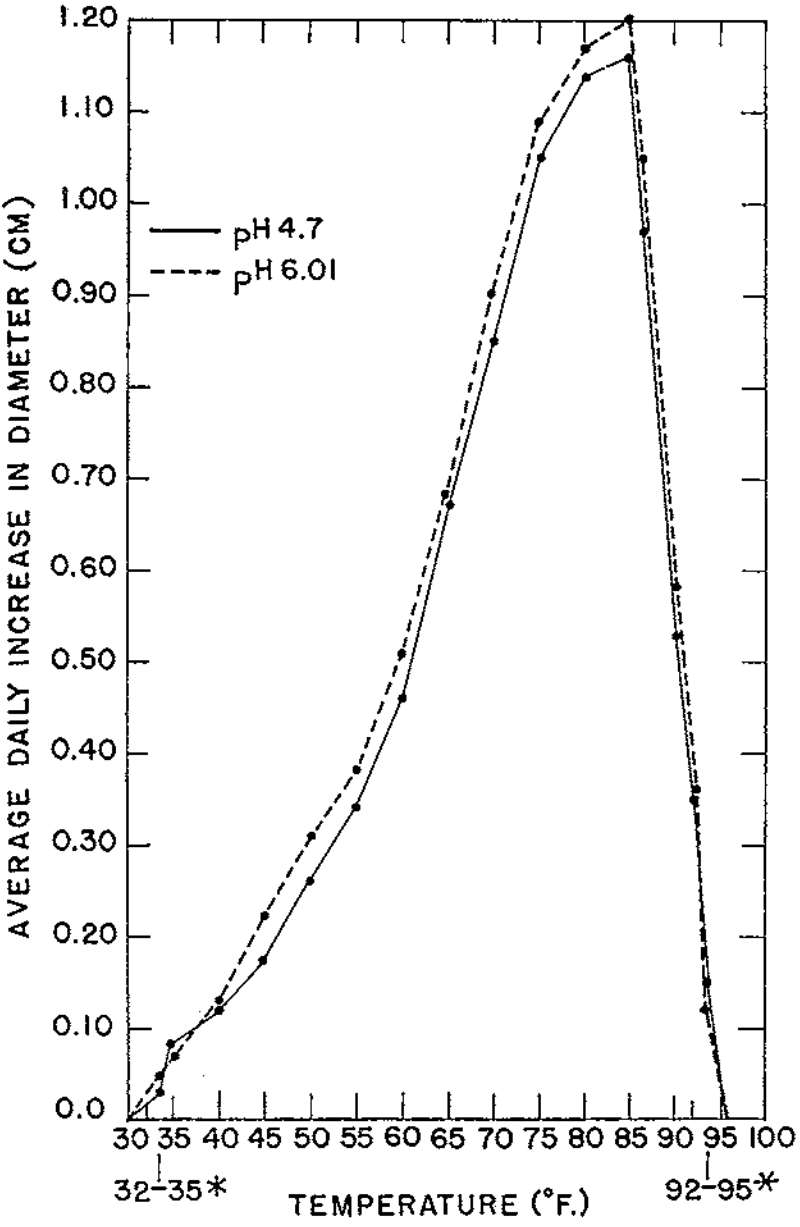

FIGURE 8.—Average daily increases in diameter of colonies of *Alternaria tomato* on potato-dextrose agar of pH 4.7 and pH 6.01 at temperatures ranging from 30° to 96° F. (* Indicates that growth ceased after about 3 to 5 days.)

TOMATO INOCULATIONS

Tomato fruits inoculated with *Alternaria tomato* showed no evidence of decay in 10 days at temperatures lower than 50° F. except in those fruits held for 1 day at room temperature before exposing to low temperatures. In these lots there was very slight evidence of decay on the eighth day both at 32° to 41° and at 45°, the average diameter of the lesions on the tenth day being 0.50 cm in the former and 0.52 cm in the latter. At 50°, 55°, and 60° lesions showed slight signs of infection on the fifth day, and the average diameter on the tenth day was slightly over 0.6 cm in each case. At 65°, 70°, and 75° the average diameter of the lesions on the tenth day was about 0.7 cm. At 80° the average external diameter of the lesions was no larger than at the three preceding temperatures, but an occasional lesion spread quite rapidly inside the locules, averaging about 2.5 cm in diameter inside by the conclusion of the experiment. At 85° there was a greater number of large lesions, the decay spreading more rapidly both inside and outside. On the tenth day the average surface diameter was 1.20 cm and the internal diameter 1.40 cm, with an average depth of 0.75 cm. Rarely a lesion reached 3.0 cm in internal diameter and 1.2 cm in depth. No inoculations were made at higher temperatures.

Ripe tomatoes decayed more rapidly and more uniformly than green ones. In a week, lesions 1.5 cm in diameter occurred at ripening room temperature (75°) and 2.5 cm in diameter at the optimum temperature (85°). By the end of the tenth day in the ripening room the lesions were over 3 cm, whereas at the optimum they averaged about 4 cm.

DISCUSSION

In spite of the fact that *Alternaria solani* has a greater resistance to very high temperatures than *A. tomato*, the optimum of the latter for growth on agar plates is higher, and under the conditions of these experiments it was able to produce larger lesions on both green and ripe fruits.

Although when extensive decay is produced by *A. tomato* such decay develops faster on ripe than on green fruits, nevertheless *A. tomato* is best known as a parasite of green tomatoes, on which it is able to produce small lesions, usually not more than 0.5 cm in diameter, without wounds being necessary, if infection occurs while the green tomatoes are still young (*16*). In the light of this characteristic adaptability for infecting green fruits it is not surprising that the fungus was able to grow almost equally well on the pH 4.7 and the pH 6.01 media.

Infections of tomatoes by *Alternaria tomato* are usually confined to the nailhead type of lesion, which seldom reaches a diameter of over 6 cm and enlarges very little in transit and storage (*10*); therefore the small size of most of the lesions in the present experimental inoculations is not surprising. However, the organism is occasionally found in the fields, causing a much larger spot, which Rosenbaum (*15*) designates as a "spreading Macrosporium spot." Strains of *A. tomato* isolated from such spots he found were capable of producing typical nailhead spots when young green fruits were inoculated, without injury, with cultures of the organism.

The writers have made repeated isolations from large lesions centered about nailhead spots and have found that both *Alternaria tomato* and *A. solani* were capable of producing such lesions. Although the causal organism was more often the latter species following the nailhead as a secondary organism, the occurrence of *A. tomato* in this capacity was quite frequent and the resultant lesions were often as large as those found in the present inoculation tests at the higher temperatures. It would seem that if conditions permit the entrance of the nailhead organism into the inner locule, it is capable under favorable conditions of developing a more extensive decay. In the present tests this was due to the fact that inoculations were made into wounds; in the field it could occur in those old nailhead lesions which sometimes show a break or crack.

PLEOSPORA LYCOPERSICI

AGAR PLATE CULTURES

Pleospora lycopersici E. and E. March grew very well on media of both hydrogen-ion concentrations, although at all temperatures it grew slightly better on the less acid medium (pH 6.01). The difference between the more acid medium (pH 4.7) and the less acid was more marked at the temperatures near optimum for growth (fig. 9). This is in keeping with the relative pathogenicity on green and ripe fruits, since *Pleospora* grew better on the ripe fruits whose fresh expressed juice averaged about pH 6.01.

The strains of *Pleospora lycopersici* causing decay on tomato fruits were found by Ramsey (*9*) to have a marked tendency to develop saltants, especially when they had been recently single-spored. Some of these saltants cease to produce perithecia and the type of mycelial growth of the *Pleospora* mother strain, but develop instead a heavier mycelium producing only *Macrosporium*-type spores. When such a saltant was grown at temperatures of 70° to 80° F. it grew at a faster rate than did the unsaltating parent strain, as shown in figure 9. Below and above these temperatures there was no difference in growth rate between the *Macrosporium* and *Pleospora* type subcultures.

This difference is especially interesting because the *Macrosporium* phase of the fungus developed optimum growth at 75° F. whereas the *Pleospora* phase had its optimum for growth at 70° (*9*). Consequently, the differences in colony size were especially great at the temperatures between 70° and 80°, at which the *Macrosporium* was still growing vigorously while the growth rate of the *Pleospora* stage was dropping off. This ability of the *Macrosporium* stage to do better at higher temperatures than the *Pleospora* stage results in a difference in pathogenicity on fruits held at higher temperatures.

The minimum for both stages of the fungus on potato-dextrose agar was 32° to 34° F. where the daily growth was 0.06 cm on pH 4.7 medium and 0.07 cm on pH 6.01 medium. The organism was not killed by 10-days' exposure to this temperature, nor by a week at 93° to 97°, although no growth was made after the first day at the latter temperature. At 90° the growth ceased after the third day, and even at 87°, where the average daily growth for a week was 0.07 cm on the pH 4.7 agar and 0.09 cm on the less acid medium (pH 6.01), the growth ceased after the sixth day.

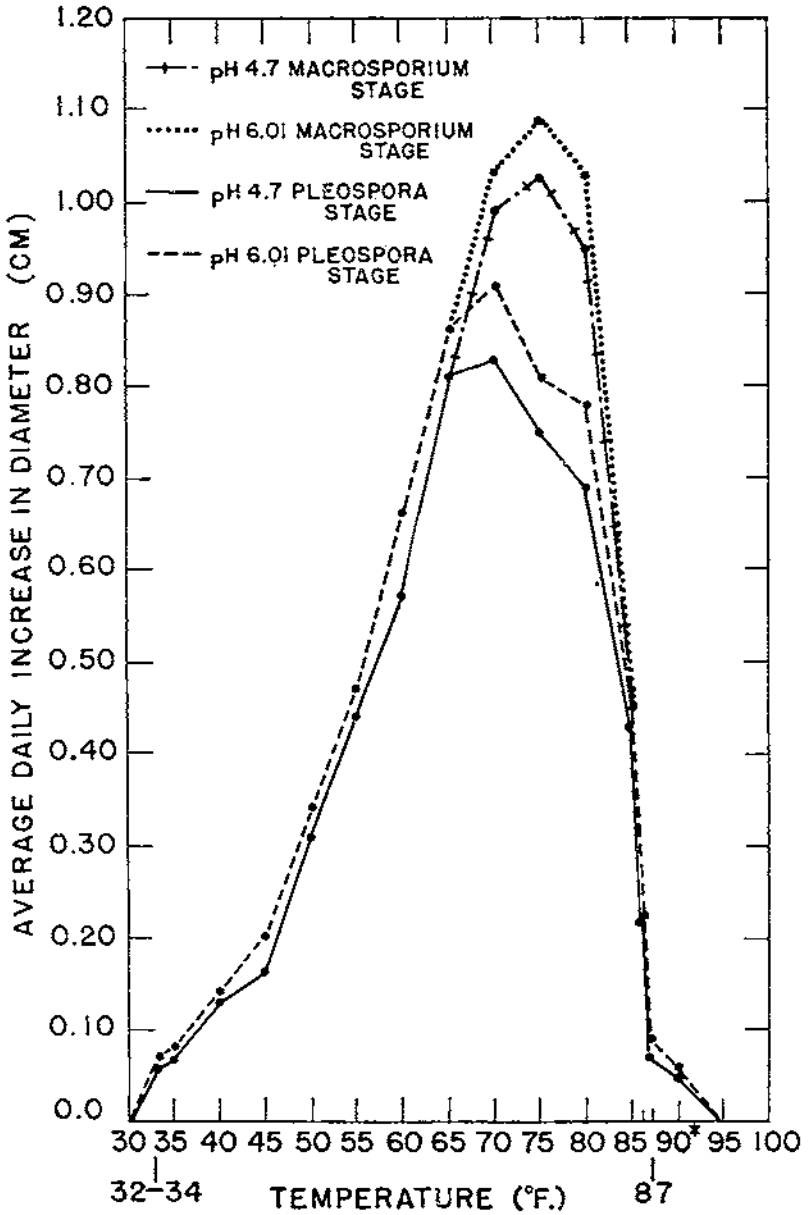

FIGURE 9.—Average daily increases in diameter of colonies of the *Pleospora* and *Macrosporium* stages of *Pleospora lycopersici* on potato-dextrose agar of pH 4.7 and pH 6.01 at temperatures ranging from 30° to 95° F. (* indicates that growth ceased after 3 days.)

TOMATO INOCULATIONS

All of the fruits in lot 2 held at temperatures of 50° F. and lower developed lesions averaging about 0.60 cm in 10 days. The lot 1 fruits placed at 32° to 41° were still negative on the tenth day but developed lesions when removed to room temperature. Some of the lot 1 tomatoes at 45° showed slight decay on the tenth day; others were negative; but all of those held at 50° showed early signs of decay, the average diameter of the lesions at this time being 0.45 cm. In fruits of both lots held at 55° the lesions appeared in 7 to 8 days and averaged 0.68 cm at the end of 10 days, whereas on the 60° fruits the lesions showed up on the fifth day and averaged 0.90 cm on the tenth day.

Although the optimum growth of the *Pleospora* stage of this organism on artificial medium was found to take place at 70° F. the faster development on green fruits took place at 65° where the lesions averaged 0.80 cm in 10 days on the surface of the fruit, but spread out in the locule to an average diameter of 1.2 cm and a depth of 0.80 cm. The lesions developing in the same length of time at 70° averaged 0.60 cm in diameter on the exterior of the fruit, but 0.85 cm internal diameter with a depth of 0.60 cm. At 75° the lesions did not spread out far through the internal tissues but were more uniform in size, averaging 0.60 cm in diameter with a depth of 0.77 cm.

At 80° F. the lesions were only 0.56 cm in average diameter at the end of the experiment, though rarely a lesion developed to 1 cm or more in diameter; this might have been due to the development of the *Macrosporium* stage, which grew faster at 80° than the *Pleospora* stage. At 85° the inoculations were negative to doubtful.

The percentage of pleospora decay was slightly greater, and the decay also developed faster on ripe fruits than on green fruits at comparable temperatures. Lesions on ripe tomatoes developed slightly faster at 70° than at 65° F. although lesions at both temperatures averaged in exterior diameter about 1.5 cm after 10 days, and in both cases there was 65 percent decay. There was a noticeable drop in the percentage of decay (54 percent) and in the diameter of the lesions in fruits held at 75°, the average diameter being 0.85 cm. At 80° the comparable diameter was 0.65 cm and only 33 percent infection occurred following inoculation. However, when inoculations were made on ripe tomatoes with a culture that showed only the *Macrosporium* stage there was 54 percent decay at 80° and the lesions averaged about 1 cm in diameter. At the end of 2 weeks these lesions had reached an average diameter of 2 cm and were still free from contaminations.

The *Macrosporium* stage grew faster than the *Pleospora* stage at 75° F. also, but at 70° the *Pleospora* stage developed slightly larger lesions in the same period of time, while at 65° and below there was no difference in growth rate within the fruit. This is quite in keeping with the growth rate of the two stages on agar plates, for the *Pleospora* stage had its optimum at 70°, whereas the *Macrosporium* stage grew most rapidly at 75°. On plates held at 80° also, the *Macrosporium* stage grew much faster than the *Pleospora* stage, but at temperatures above and below these mentioned there was no difference in growth rate.

DISCUSSION

That the change in acidity during ripening is one factor in the more rapid development of decay on ripe fruits is indicated by the fact that the fungus consistently grew better on the less acid medium.

Pleospora is able to develop slowly in both green and ripe tomatoes at low temperatures, but most rapid development of decay takes place at those temperatures usually found in tomato-ripening rooms. Since both the *Macrosporium sarcinaeforme* Cav. stage (*8*) and the *Pleospora* stage are able to cause decay, temperatures favoring either phase of fungus growth will favor decay. The optimum temperature for lesion development is made more extensive in range, since the *Macrosporium* stage is able to develop at temperatures higher than those favoring fastest spread of the *Pleospora* stage in the tomato tissues. The fungus is essentially a high-temperature organism, and so far the disease has been limited to California and Mexican stock.

SUMMARY

In an attempt to determine influences of temperature and maturity of fruits upon development of decay after tomatoes leave the packing house, studies of growth rate were made with pure cultures of nine organisms commonly found producing decay of tomato fruits during transit and storage.

The daily diametric growth of colonies of the organisms was determined on two series of Petri-dish cultures grown on potato-dextrose agar of pH 4.7 and of pH 6.01, respectively. The first corresponded in acidity to the average hydrogen-ion concentration of the juice of green fruits of the variety employed in the inoculation tests; the agar in the second was adjusted to the average acidity of the ripe-tomato juice. The plates were held in incubators ranging from 30° to 95° F. at intervals of 5°.

The rate of development was studied on green and on ripe tomatoes inoculated with pure cultures of the organisms and held at temperatures ranging from 32° to 85° F. at 5° intervals.

Cardinal temperatures given were determined by comparing average daily increase in diameter of colonies grown for 1 week on Petri dishes containing potato-dextrose agar of pH 4.7 and pH 6.01. Unless exceptions are stated, the cardinal temperatures for lesion development on tomatoes agree with those given for growth on agar. No inoculated tomatoes were held above 85° F. If the maximum lies above this temperature, determinations were made from growth on agar plates only.

It was found that *Fusarium semitectum* Berk. and Rav., *Phoma destructiva* Plowr., *Alternaria solani* (Ell. and Martin) Jones and Grout, and *Pleospora lycopersici* E. and E. March, as well as the conidial stage (*Macrosporium sarcinaeforme* Cav.) of the latter, all grew much better on the agar of pH 6.01 than on that of pH 4.7. This is in keeping with the fact that lesions induced by these organisms grew much faster on ripe fruits than on green ones. It would indicate that change in acidity of the tomato during the ripening process is an important factor in determining their ability to produce greater decay on ripe fruits. In the case of *P. destructiva* there was a marked increase in rate of decay as green fruits began to turn red.

While *Rhizoctonia solani* Kühn and *Melanconium* sp. grew somewhat better on the agar of pH 6.01 at those temperatures at which best growth took place (65° to 85° F.), there was no marked difference in growth rate on the two sets of media. Both of these fungi are able to produce large lesions on green tomatoes, although both grow somewhat better on ripe fruits.

The strain of *Melanconium* sp. causing ring rot of tomatoes develops most rapid growth on agar and on tomatoes of all stages of maturity at 80° F. The maximum temperature for growth on agar plates was 90° to 95°, no growth occurring at 93° to 97°, although the colonies resumed growth when removed to room temperature. The fungus was not killed by a week at 33° to 38°, nor did it grow on agar plates held at 37° to 41° for 10 days; but in fruits inoculated and held for a day in the room before exposure to a temperature of 35° to 41°, slight evidence of lesion development was noted on the tenth to eleventh days. The minimum for a week's continuous growth on the agar plates was slightly higher than this, 45°. Optimum development on both tomatoes and agar took place at 80°.

Rhizoctonia solani developed optimum growth at 80° F., lesions 8 cm in diameter occurring on tomatoes in 10 days at this temperature. The maximum temperature for a week's growth on agar plates was 90° to 95°, although feeble growth (chiefly beneath the surface of the agar) occurred at 92° to 95°. The mycelium was killed by a week at 95° and at 35°. It was sometimes dead after a week at 38°, and the minimum temperature for scant growth on agar plates was found to be 45° to 49°. Although the *Rhizoctonia* remained alive in many of the inoculated tomatoes after 2-weeks' exposures to temperatures ranging from 32° to 41°, all inoculations were negative if the tomatoes were held at temperatures lower than 50°. Lesions only 0.57 cm in diameter developed in 10 days at this temperature on inoculated green fruits held for 1 day at room temperature before being placed in the refrigerator.

A strain of *Fusarium semitectum* isolated from decaying tomato fruit and used for inoculation tests developed optimum growth rate on both fruit and agar plates at 75° F. The maximum temperature for growth on agar plates was 92° to 95°. The fungus was not killed by a week at 95°. The minimum for a week's continuous growth on agar occurred at 41° to 43°, although the minimum for development of lesions on tomatoes was 45°. Cultures grown on agar plates at 30° to 34° for 3 days were still alive, although those held at 40° ceased growth in 2 days.

Colletotrichum phomoides (Sacc.) Chester is usually considered a ripe rot, and in inoculation tests here reported it grew much faster on ripe than on green fruits; in green fruits lesion development was speeded up as the tomatoes ripened. In agar-plate cultures, however, the growth rate on the medium whose acidity corresponded to that of ripe-tomato juice was not much greater than on the more acid medium. It appears that possibly other chemical or physical changes occurring in the tomato during ripening are more important than changes in acidity in influencing the development of decay.

Colletotrichum phomoides developed fastest at 80° F. both on agar and on tomatoes, but the minimum temperature, 35°, for growth on agar plates was lower than that for lesion development, 45°.

Colonies of the fungus on agar plates exposed for 20 days at 30° to 34° were not killed even though no growth occurred, nor were those held for 4 days at 34° to 37° dead, although growth ceased at this temperature. The maximum temperature for continuous growth on agar plates was 95°, very small abnormal colonies developing in a week.

Phoma destructiva had a much lower optimum, 70° F., than the anthracnose organism (*Colletotrichum phomoides*) and did not grow at such extremes of temperature. The minimum temperature for growth on agar was 40°, although the agar cultures withstood a 20-days' exposure to 32° and the organism remained alive in tomatoes held for 12 days at 32° to 41°. The minimum temperature for decay of fruits was 45°, the symptoms being very slight to doubtful on the tenth day. The maximum temperature for development of agar plates was 85°, at which temperature tomato inoculations were doubtfully successful. The agar cultures held at 86° did not grow, and those held for a week at 90° did not resume growth until the third day after removal to room temperatures.

Newly isolated pycnidia-bearing strains of *Phoma* grew slightly faster and produced decay more quickly than did old strains held for several years in artificial culture or nonpycnidia-forming strains obtained by saltation.

There was no consistent difference in the reaction of *Cladosporium fulvum* Cke. on agar of the two acidities used, the colonies usually growing faster, however, on the less acid medium at temperatures above 60° F. The decay produced by *Cladosporium* was not extensive on either green or ripe fruit, but at optimum temperatures the organism spread out a little more extensively within the locule of ripe fruits than in green ones.

Cladosporium fulvum had an optimum temperature for growth of 70° F. and a maximum on agar plates of 85°. At 86° no growth occurred after the second day, and no growth took place in an incubator whose daily fluctuation was 87° to 93°. Only rarely was the colony killed by the latter temperature, although microscopic examination showed the mycelium to be dead and development of the colony was not resumed until after 4 or 5 days at room temperature. Evidently some of the spores resisted the high temperature. The minimum for growth on agar plates was 32° to 35°, but the colony remained alive for 3 days at 30° to 32°. Fruits held at 45° showed only doubtful evidence of decay, though the organism remained alive for 10 days in fruits stored at 32° to 41°. Small lesions occurred at 50°. The optimum for lesion development was 70°, but no decay occurred on either green or ripe fruits at 80° and 85°.

The optimum of *Alternaria solani* was 80° F. and its minimum 35°. Its maximum temperature for continuous growth, 95°, was slightly higher than in the case of the nailhead organism. Sometimes the fungus continued to grow steadily for a week at 96°, but at other times growth ceased after the first day. The colonies on agar did not grow but remained alive at 30° to 32°. No tomato decay occurred at temperatures lower than 45°.

Alternaria tomato (Cke.) Brinkman, in contrast with *A. solani*, is more often a parasite of green tomatoes. Correlated with this is the fact that growth was only very slightly favored by the less

acid agar, and very rapid development of the colonies occurred on agar of pH 4.7.

Alternaria tomato had a higher optimum temperature for growth than any of the other eight organisms, i. e., 85° F. The maximum for continuous growth of 1 week was 92° to 95°, although in some instances a very slight mycelial development continued for 4 to 5 days at 95°. No growth took place at 93° to 99°. The minimum for a week's growth was 35°, although the fungus was not killed by a week at 30° to 32°. There was very slight evidence of decay on the eighth day in green fruits of lot 2 stored at 32° to 41°.

The minimum temperature for development of both stages of *Pleospora* both on agar and on tomatoes was 32° to 34° F. However, the perfect stage (*P. lycopersici*) had a lower optimum for growth on agar, i. e., 70°, whereas the best temperature for growth of the conidial stage (*Macrosporium sarcinaeforme*) was 75°. Both stages grew equally well at all temperatures below 70° and above 80°, their maximum for growth occurring at 87°. Growth ceased after the third day at 90°, but, although no growth occurred at 93° to 97°, the fungus remained alive for a week. On ripe fruits the optimum for lesion development of the *Pleospora* stage occurred at 70° (as on agar plates), but on green fruits its best development occurred at 65°. Strains of the *Macrosporium* stage grew faster within the tomatoes at 75° and at 80° than did the perfect stage.

In the case of several of the fungi studied, as the green fruits ripened the rate of spread of the organism within the tissues increased, but after the lesions had attained considerable size the growth rate began to decrease, so that the average daily increase followed a curve that had its high point at approximately the time the fruit was just turning red. Therefore, in any one experiment the difference between the diameters of lesions on fruits inoculated while green and the ones on parallel fruits inoculated after ripening decreased as the green fruit ripened.

LITERATURE CITED

(1) BRINKMAN, A.
1931. DE ROODNEUZEN-ZIEKTE VAN PHASEOLUS VULGARIS L. VEROORZAAKT DOOR PLEOSPORA HERBARUM (PERS.) RBH. 86 pp. [Thesis.] Amsterdam.

(2) GARDNER, M. W.
1925. CLADOSPORIUM LEAF MOLD OF TOMATO: FRUIT INVASION AND SEED TRANSMISSION. Jour. Agr. Research 31: 519–540, illus.

(3) GRATZ, L. O., and BONDE, R.
1927. INFECTION OF POTATO TUBERS BY ALTERNARIA SOLANI IN RELATION TO STORAGE CONDITIONS. Fla. Agr. Expt. Sta. Bull. 187, pp. 165–182, illus.

(4) JAMIESON, C. O.
1915. PHOMA DESTRUCTIVA, THE CAUSE OF A FRUIT ROT OF THE TOMATO. Jour. Agr. Research 4: 1–20, illus.

(5) MAKEMSON, W. K.
1919. THE LEAF MOLD OF TOMATOES, CAUSED BY CLADOSPORIUM FULVUM CKE. Mich. Acad. Sci. Ann. Rept. (1918) 20: 311–348, illus.

(6) MONTEITH, J., JR., and DAHL, A. S.
1928. A COMPARISON OF SOME STRAINS OF RHIZOCTONIA SOLANI IN CULTURE. Jour. Agr. Research 36: 897–903, illus.

(7) NIGHTINGALE, A. A., and RAMSEY, G. B.
1935. DEVELOPMENT OF PHOMA ROT OF TOMATOES IN TRANSIT AND STORAGE. U. S. Dept. Agr. Circ. 371, 8 pp.

(8) RAMSEY, G. B.
1934. PLEOSPORA LYCOPERSICI E. AND E. MARCH., A TOMATO PATHOGEN IN THE UNITED STATES. Science (n. s.) 79: 294.
(9) ———
1935. PLEOSPORA ROT OF TOMATOES. Jour. Agr. Research 51: 35–43, illus.
(10) ——— and BAILEY, A. A.
1929. DEVELOPMENT OF NAILHEAD SPOT OF TOMATOES DURING TRANSIT AND MARKETING. Jour. Agr. Research 38: 131–146, illus.
(11) ——— and BAILEY, A. A.
1929. THE DEVELOPMENT OF SOIL ROT OF TOMATOES DURING TRANSIT AND MARKETING. Phytopathology 19: [383]–390, illus.
(12) ——— and LINK, G. K. K.
1932. MARKET DISEASES OF FRUITS AND VEGETABLES: TOMATOES, PEPPERS, EGGPLANTS. U. S. Dept. Agr., Misc. Pub. 121, 44 pp., illus.
(13) RANDS, R. D.
1917. EARLY BLIGHT OF POTATO AND RELATED PLANTS. Wis. Agr. Expt. Sta. Research Bull. 42, 48 pp., illus.
(14) REINMUTH, E.
1934. TOMATENFRUCHTFÄULE DURCH CLADOSPORIUM FULVUM CKE. Ztschr. Pflanzenkrank. 44: 558–560, illus.
(15) ROSENBAUM, J.
1920. STUDIES WITH MACROSPORIUM FROM TOMATOES. Phytopathology 10: [9]–21, illus.
(16) ——— and SANDO, C. E.
1920. CORRELATION BETWEEN SIZE OF THE FRUIT AND THE RESISTANCE OF THE TOMATO SKIN TO PUNCTURE AND ITS RELATION TO INFECTION WITH MACROSPORIUM TOMATO COOKE. Amer. Jour. Bot. 7: 78–82.
(17) SAMUEL, G., and GARRETT, S. D.
1932. RHIZOCTONIA SOLANI ON CEREALS IN SOUTH AUSTRALIA. Phytopathology 22: 827–836, illus.
(18) SMALL, T.
1931. TOMATO LEAF MOLD. Cheshunt, Herts, Exp. and Research Sta. Ann. Rept. (1930) 16: 40–56.
(19) TISDALE, W. H.
1916. A MELANCONIUM PARASITIC ON THE TOMATO. Phytopathology 6: [390]–394, illus.
(20) WELLMAN, F. L.
1932. RHIZOCTONIA BOTTOM ROT AND HEAD ROT OF CABBAGE. Jour. Agr. Research 45: 461–469, illus.
(21) WRIGHT, R. C., PENTZER, W. T., WHITEMAN, T. M., and ROSE, D. H.
1931. EFFECT OF VARIOUS TEMPERATURES ON THE STORAGE AND RIPENING OF TOMATOES. U. S. Dept. Agr. Tech. Bull. 268, 35 pp., illus.

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