WATERY-ROT OF TOMATO FRUITS¹

A PHYSIOLOGICAL FORM OF OOSPORORA LACTIS; EFFECT ON THE HOST; PENETRATION OF THE CELL WALLS BY ENZYMIC ACTION

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INTRODUCTION

A new rot of tomato fruits closely resembling the rot caused by *Bacillus carotovorus* has frequently been found in shipments of southern grown tomatoes and sent to the Office of Cotton, Truck, and Forage Crop Disease Investigations by the Bureau of Markets' inspectors since the spring of 1921. It is also prevalent in the vicinity of Arlington, Va., and Washington, D. C.

This rot is characterized by the extremely watery appearance and condition of the affected tissues, by the absence of any other discoloration, and by the occasional oozing of water from the surface—features by which it can readily be distinguished from other fungous softrots. The rotted areas usually develop in the form of sectors extending from the stem scar toward the blossom end. In very humid air they are partly covered with a white velvety to granular fungous growth, but under average atmospheric conditions this is absent. The illustration shown in Plate 4, A, is fairly typical of this rot except for the low point of origin and the presence of a surface growth.

That this rot has frequently been mistaken for the rot caused by *Bacillus carotovorus* is quite likely, as the two are similar in macroscopic appearance. However, it differs from the bacterial rot in rapidity of development; in fact, fruits kept in the laboratory 10 days after this rot had made considerable progress were not completely softened, while those infected by *B. carotovorus* usually collapsed in about 3 days.

As an examination of the affected fruits obtained from the Bureau of Markets always disclosed the presence of an Oospora, experiments were made to determine the relation of this fungus to the disease.

INVESTIGATION

MATERIAL AND METHODS

The Oospora used in the inoculations was grown chiefly on carrot agar, as it grew better on it than on most other kinds of media. It grew well also on turgid raw carrots kept in a moist atmosphere, producing a distinct rot (Pl. 4, C), but this material was used only for morphological comparisons.

In the inoculation work, green, ripe, and partly ripe tomato fruits free from blemishes were submerged for 30 minutes in a 1:1000 aqueous solution of bichlorid of mercury, washed in distilled water, and inoculated with a pure culture of the Oospora obtained from the rotted fruits.

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This treatment caused some discoloration of the fruits but no softening. It had no visible effect on susceptibility to infection by this fungus, for fruits treated with weak solutions of dichlorid of mercury or formaldehyde as well as fruits not treated were quite as readily infected.

Controls were liberally used in all the experiments. Both the controls and the inoculated fruits were usually kept in closed glass chambers to prevent contamination from the air, but inoculated fruits kept in open dishes were quite as readily infected.

The decomposition of the cell constituents was observed on roots of carrots and on green tomato fruits. Sections of carrot roots, chiefly from the heart, and of green tomato fruits, 250 and 500 \( \mu \) thick, respectively, and free-hand sections of varying thickness were used as fresh material. Pieces of green tomato fruits from spots 2 days old were treated with Flemming's medium killing and fixing solution, embedded in paraffin, and sectioned and stained for the study of fixed material.

The cultures were made as described by Brown (3) on thin layers of media (carrot decoction, and beef bouillon + 2½ per cent glucose) in small flasks or Petri dishes, inoculated with an optimal quantity of spores, and kept at a temperature of 22° to 26° C.

The action of the enzymes on the host cells was determined by means of the live organism, the ground-dried organism, the extract from the ground-dried organism, the filtrate from cultures, and the alcoholic precipitate from the filtrate.

The organism was separated from the culture medium by passing the liquid through a double layer of Whatman's No. 50 filter paper. A few small cells passed through the filter but showed no signs of germination during the experiment.

The enzymic material was precipitated by adding 4 volumes of 95 per cent alcohol to each volume of the filtrate. The liquid was then filtered and the precipitate washed in 95 per cent alcohol, dried in a warm air current, dissolved in a very small quantity of distilled water, and used immediately.

The ground-dried organism was used in aqueous suspension. The extract of the same material was prepared by soaking the powdered fungus in water for 24 hours.

The decomposition experiments were run for 24 hours at temperatures ranging from 24° to 45° C. Growth of microorganisms was prevented by adding chloroform or toluene to the liquid in the proportion of 10 to 25 per cent, but duplicate experiments in which no antiseptic was used were also made. Chloroform and toluene inhibited growth without apparently hindering the action of the enzym.

RESULTS OF INOCULATION

The results obtained with both pricked and unpricked fruits are summarized in Table I.

Of the 277 pricked fruits inoculated, 208, or 75 per cent, became infected. The ripe fruits seemed to be somewhat more susceptible than the green fruits, but both were easily infected through punctures. The infections of unpricked fruits took place only through the stem scar. This was frequently observed in preliminary experiments not recorded. Moreover, it appears to be the cause of the position of the rotted areas of this type on most shipped fruits.

Reference is made by number (italic) to "Literature cited," p. 905.
### Watery-Rot of Tomato Fruits

**Table I.—Results of inoculating tomato fruits with the Oospora associated with watery-rot**

<table>
<thead>
<tr>
<th></th>
<th>Green</th>
<th>Ripening</th>
<th>Ripe</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pricked</td>
<td>160</td>
<td>90</td>
<td>27</td>
<td>277</td>
</tr>
<tr>
<td>Unpricked</td>
<td>16</td>
<td>7</td>
<td>5</td>
<td>28</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Per cent.</th>
<th>Number</th>
<th>Per cent.</th>
<th>Number</th>
<th>Per cent.</th>
<th>Number</th>
<th>Per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pricked</td>
<td>118</td>
<td>74</td>
<td>64</td>
<td>71</td>
<td>26</td>
<td>62</td>
<td>96</td>
<td>0</td>
</tr>
<tr>
<td>Unpricked</td>
<td>208</td>
<td>75</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Infected through the stem scar.*

Although these infections were obtained with an apparently pure culture of Oospora, the possibility of contamination by *Bacillus carotovorus* was not overlooked. The cultures were repeatedly plated and examined microscopically for the presence of bacteria but no evidence of bacterial contamination was found in either the plates or microscopic mounts. Moreover, the fungus pricked into halved potato tubers caused only a slight superficial growth, even when allowed to stand for a considerable period of time, while *B. carotovorus* produced a very decided rot (PL 4, B). The infections of the inoculated tomato fruits were therefore unquestionably caused by the fungus.

The stems and leaves of 15 vigorous tomato seedlings about 4 inches tall were thoroughly sprayed with the Oospora spores and kept in a moist chamber 60 to 72 hours but no infections developed. Ten similar tomato seedlings were thoroughly pricked in stems and leaves and smeared with the spores of Oospora, but the fungus was unable to invade the tissues. It therefore seems to have little if any parasitic action on tomato plants.

**Morphology of the Fungus**

The Oospora causing watery rot of tomato fruits consists of a hyaline, septate mycelium with granular contents and numerous short branches arising near the septa at an angle of about 45° from the main filament (Pl. 3, A) and other longer branches of a two-or-three-forked type (Pl. 3, B–D).

The hyphae vary from 2.5 to 7.5 μ in diameter. The younger branches are narrower than the main filament but the taper is very gradual.

Reproduction is accomplished by the breaking up of the hyphae into their cells which serve as spores. The branches shown in Plate 3, A, divide into numerous short cells which round at the ends (Pl. 3, E, F, H) and separate. The rounding appears to begin in the apical cells (Pl. 3, G), but occurs in all cells of a branch almost simultaneously. At a certain stage of their development these cells appear to be chains of spores arising from the main part of the mycelium (Pl. 3, E, F), but by the time they lose their coherence, or shortly afterward, the main filament itself breaks up by cell division and separation into numerous cells of different lengths, which round at the ends, and when short are often indistinguishable from those formed from the lateral branches. They are hyaline and granular and are capable of germinating immediately.

The transverse diameter of these sporelike cells (oidia), which for convenience will be referred to hereafter as spores, varies from 2.5 to 7.5 μ; the length from 3.2 to 40 μ and in some cases even to more than 60 μ.
Germination of the spores may start from the end (Pl. 3, I, L), from the side (Pl. 3, J, K), or from a corner (Pl. 3, I, M). Development from a corner of the cell is quite similar in point of origin to the development of the branches shown in Plate 3, A.

COMPARISON WITH OOSPORA LACTIS

The Oospora obtained from rotted tomato fruits shipped from the Gulf States was compared morphologically with the Oospora lactis that commonly grows on the surface of tissues in the cracks of ripe tomatoes; with two cultures of O. lactis received from Dr. Charles Thom, one isolated by him from pickle scum, the other sent to him from Germany; and with an Oospora isolated from green tomato fruits affected by watery-rot at the Government Experimental Farm, Arlington, Va. The two Oosporas causing watery rot and the one from Germany did not always break up into their cells so readily and completely as those from the cracks of ripe tomatoes and from pickle scum, but this habit varied with the age of the culture and with the kind of culture medium used. Aside from this difference, which was not constant, and slight differences in quantity of mycelial growth, there were no peculiarities, except in parasitism, by which one form could be distinguished from the others. The Oospora received from the Gulf States and the one obtained from green tomato fruits at the Government Experimental Farm reproduced the watery-rot in a large percentage of the fruits inoculated, but the others appeared to have no such parasitic action on tomato fruits. It would seem, therefore, that the Oospora causing watery-rot of tomato fruits, both in the Gulf States and at the Government Experimental Farm, is a physiological form of O. lactis. Consequently we have given it the trinomial O. lactis parasitica, form phys., to distinguish it from the parent species. As it is indistinguishable morphologically from O. lactis, it needs no further description.

It would be interesting to know how this form compares with the 9 varieties of Oospora lactis that Schnell (8) grew on sliced potato tubers, but as he made no inoculations on tomato fruits an accurate comparison of their parasitism is impossible. Six of the 9 varieties grown on sliced potatoes, 4 of which grew also on sliced cucumbers and 2 on plums, produced a discoloration of the potato tissues which would distinguish them from the form isolated from green tomatoes. Two of the others produced slimy colonies on sliced potatoes—a character not obtained with the watery-rot fungus. The remaining strain, viz, Oid. 1. 557, made a feeble growth on potato tubers, agreeing in this respect with the one isolated from tomato fruits affected by watery-rot, but this has no significance with reference to its parasitism on tomato fruits.

TEMPERATURE RELATIONS

The effect of temperature on growth and infection by Oospora lactis parasitica is shown in Table II.

The minimum temperature obtained for germination of the spores, for growth of the mycelium, and for infection of pricked tomato fruits was approximately 2° C., the optimum temperature 30°, and the maximum 38.5°, except for infection of fruits, which was 37.5°. As there was a difference of 1° to 2° between the temperatures of adjoining chambers and some fluctuation within each chamber, these temperatures are only approximately correct.
TABLE II.—Relation of temperature to growth and infection by Oospora lactis parasitica

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Growth on culture media. a</th>
<th>Infection of pricked tomato fruits.</th>
<th>Germination of spores.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum</td>
<td>38.0°C</td>
<td>37.5°C</td>
<td>38.5°C</td>
</tr>
<tr>
<td>Optimum</td>
<td>30.0°C</td>
<td>30.0°C</td>
<td>30.0°C</td>
</tr>
<tr>
<td>Minimum b</td>
<td>2.0°C</td>
<td>2.0°C</td>
<td>2.0°C</td>
</tr>
</tbody>
</table>

a Carrot agar and glucose agar.
b The experiments on the minimum temperature were run about a month.

EFFECT ON THE HOST

ACTION ON THE CUTICLE

It was shown in the inoculation experiments described above that this fungus is unable to infect uninjured tomato fruits except through the stem scar or other similar areas not covered by the cuticle. This is also illustrated in Plate 1, A. The fungus lay in masses on the surface of the fruit but was unable to penetrate it. However, when once inside the fruit it invaded the epidermal cells quite readily.

ACTION ON THE PROTOPLASM

Invasion of the host cells is soon followed by a gradual consumption of their protoplasmic contents. Some of the steps in this process are illustrated in Plate 1, in which B and C show an early stage, D a medium early stage, and E–J late stages. This action of the fungus on the protoplasm of the invaded cells is quite evident soon after the penetration of the wall, as it causes dissolution of the protoplasm in advance of the growing tip (Pl. 3, R). These transparent areas, or digestion vacuoles immediately surrounding the filaments, are shown more fully in Plate 1, B–D, and Plate 3, U. It would seem from these figures that the proteolytic enzym secreted by the fungus acts chiefly in the region of the growing tip. There was apparently no preference for the nucleus, as it often remained intact after most of the other cell contents had disappeared.

Quite different results were obtained with the ground dried organism, the filtrate, and the alcoholic precipitate from the filtrate. Repeated experiments with these substances produced no visible effects on the protoplasm. The failure to obtain a proteolytic action with any of these substances may be due to a rapid deterioration of the enzym, to inhibitors, or to lack of suitable technic.

ACTION ON THE MIDDLE LAMELLA

The growing fungus, as shown in Plate 1, dissolves the middle lamella slowly, causing the loss of cell coherence. This loss of coherence and the breaking up of the fungus filaments into their individual cells produce the extremely watery consistency which distinguishes this rot from other softrots in which the fungus filaments remain intact and hold the host cells together. A similar action was produced on the middle lamella by the ground dried organism, the extract from the ground dried organism, the filtrate, and the alcoholic precipitate from the filtrate of cultures 2 to 3 days old, but this activity diminished as the age of the culture
increased. In fact, the alcoholic precipitate from cultures 6 days old and the filtrate from cultures 21 days old produced no visible effect on the middle lamella. This deterioration or inhibition of the action of the pectinase from age is quite different from the rapid action obtained with pectinase from cultures of *Bacillus carotovorus* 21 days old.

**ACTION ON THE CELLULOSE**

The passage of this fungus through the cell walls is shown by the drawings in Plate 3, N-T, and by the intracellular filaments in the photomicrographs of Plate 1. The drawings in Plate 3, N-T, were made from partly destroyed cells of a disintegrating area of the fruit. Only the tips of growing hyphae are shown passing through the somewhat wavy and more or less separated walls. The penetration of the cell walls of normal cells by young germ tubes was also observed by means of the microscope. The more important details of this process will be described later.

No visible effect was made on the walls by the filtrate, by the alcoholic precipitate from the filtrate, nor by the mycelium in the presence of sufficient chloroform or toluene to inhibit its growth. Moreover, bits of filter paper placed in fresh cultures of the fungus and allowed to remain there for 10 days to 2 weeks did not disintegrate. The fungus filaments passed between the fibers, causing the paper to tear apart somewhat more readily after the breaking up of the hyphae than similar bits of paper kept in distilled water, but microscopic examination of the fibers failed to reveal any corroding effects. There was apparently no chemical action on the filter paper.

It might seem from the foregoing facts that pressure rather than enzymic action enables the fungus to penetrate the cell walls as described for *Pythium debaryanum* by Hawkins and Harvey (7), but further observations do not substantiate this means of penetration for *Oospora lactis*.

Before a fungous filament can penetrate a cell wall by means of pressure, it must attach itself to the wall, or, if in a cell, to the protoplasm in order to prevent pushing itself away from the wall as it elongates. Spores of this fungus germinated either in water or in culture solution do not attach themselves to the slide or the cover slip. Moreover, when germinated in cells of tomato fruit tissue they do not adhere to the wall or the protoplasm. When the tip of such a sporeling comes into contact with the wall, its more or less continuous growth in length usually pushes it aside, which causes it to slide along the wall. Not infrequently the position of the whole filament is thus changed, as well as the position of other sporelings lying in contact with it. In fact, such a filament may even shift its position in such a way as to remove its tip some little distance from the wall. Such short filaments go through the walls more easily at the corners of the cell because there is less chance to slide along the wall. When a filament has passed through a wall it pierces other walls more rapidly because the anchorage thus obtained holds the growing tip against a single point better than does the free spore end of a germ tube that has no anchorage.

The phenomena accompanying the penetration of a cell wall by a germ tube of this fungus throw some light on the means by which it is accomplished. By placing spores of the fungus on the top of thin sections of tomato fruit tissue mounted and covered on a glass slide and furnished with a constant supply of water, the growth activities of the
spores that settle in the cells as well as the penetration of the wall and the effects resulting from it are easily observed by means of the microscope. Some short germ tubes lying near a wall and approaching it perpendicularly go directly through it without the use of any support or anchorage to increase their pressure. The opening made in the first half of the wall is a hole, not a basin or general depression such as would be produced by pressures, although the second half of the wall, i.e., the wall of the cell undergoing invasion, is sometimes pushed back. Whenever an invading filament completely fills the hole in the first half of the wall it attains a certain amount of anchorage which no doubt enables it to make some use of its growth pressure. This causes the second half of the wall to bend back before the filament has passed through it (Pl. 3, P, T), but it is not essential to the penetration. Moreover, this bending is usually absent, because the hole made in the first half of the wall, as shown by focusing sharply with the microscope, is usually a little larger than the filament (Pl. 3, V). It is also destitute of radiating cracks or fragments such as would be likely to accompany the bursting of the wall by pressure. By pressing the cover slip with a needle so as to produce vertical and lateral movements the angle between a germ tube and the wall through which it has passed may be varied more than 90°. This is caused by the pressure of the liquid against the filament, which changes its position in the wall without bending it at the edge of the hole. The two ends of such a filament usually move in opposite directions, especially when the part extending through the wall is three or four times as long as the part in the original cell. If the filament were rigidly fixed in the wall, the angle between it and the wall would not change unless the filament were bent at the edge of the hole. Moreover, an occasional filament can be made to slide in the hole. These phenomena are possible only when the hole is larger than the filament. In view of these facts, it would seem that this fungus invades the cells by means of a cellulose-dissolving enzym (cellulase) secreted by the growing tips while in contact with the wall.

**COMPARISON WITH BACILLUS CAROTOVORUS**

As this Oospora and *Bacillus carotovorus* cause similar rots of tomato fruits, a comparison of their effects on the host is interesting. Oospora invades the cells and destroys the protoplasm before it causes much separation of the walls (Pl. 1, B–F). Although it is also found in the intercellular spaces, it apparently makes little use of them except as passages. *Bacillus carotovorus*, on the other hand, remains in the intercellular spaces until it destroys the middle lamellae of the adjoining cell walls (Pl. 2, A–D) and enters the cells usually after it has destroyed their coherence. That an earlier entrance is sometimes effected, however, is evident from Plate 2, E. In later stages (Pl. 2, F–H) it not infrequently fills the cells.

**ENZYM VERSUS PRESSURE**

If we assume that the penetration of the cuticle by all fungous parasites is by "sheer mechanical pressure," as concluded for *Botrytis cinerea* by Brown (4, 5) and by Blackman and Welsford (1) and for *Sclerotinia*

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8 An excellent history of the work done on the softrots of vegetables (also tomato) caused by the *Bacillus carotovorus* group of organisms is published in "An Introduction to Bacterial Diseases of Plants," by Erwin P. Smith (9).
Libertiana by Boyle (2), we may be greatly misled. This would apply also to the conclusions of Hawkins and Harvey (7) regarding the penetration of cellulose walls. Fungi produce a variety of enzymes by means of which they decompose substances and obtain food. Moreover, it is quite likely that they produce many more enzymes than have been isolated. Failure to isolate an enzyme therefore does not disprove its existence, especially as the physiological factors involved in the production of enzymes and the activators and inhibitors controlling their activities are little understood. Moreover, the observation by Hasselbring (6) that the cavities made in the waxy covering of Berberis Thunbergii berries by the anthracnose fungi are much larger than the germ tubes and the conclusions by Ward (10, 11) from an exhaustive study of the brownrust of bromes that there is absolutely no relation between thickness of walls, number and size of stomates, hairs, and other mechanical structures and immunity to rust, present a striking contrast to the pressure theory of parasitic invasion. That growth pressure often accompanies enzymic action is obvious. It may also speed up the process of penetration and in certain cases serve as the chief, if not the sole, means of invasion, but it plays only a minor and nonessential part in the invasion of the tomato fruit cells by Oospora lactis and no doubt acts only in a secondary capacity in most cases. This is fortunate, for if the development of disease-resistant fruits depended upon thickness of walls, quality and resistance would often be diametrically opposed.

Since Oospora passes through the cellulose walls of two adjoining fruit cells without attaching itself to the wall or protoplasm and not infrequently makes an opening larger than the filament without causing a depression in the wall of the occupied cell, nor lateral cracks, or ruptures, in the wall of the invaded cell, its means of penetration can not be ascribed to pressure. The only other known means by which a fungus can make an opening of this description is by the use of an enzyme, such as cellulase, which has been isolated from fungi.

There are features in the penetration of cell walls by some fungi, especially species of the genus Pythium, which cast considerable doubt on the penetration of cell walls by pressure. In his study of Pythium gracile, Ward observed that an oospore which had germinated at some little distance from a cress seedling, produced as it grew several bends in its germ tube and passed around a small algal cell at right angles before reaching a cress cell. On coming into contact with this cress cell its "apex became closely pressed against the cuticle, apparently lifting the whole hypha slightly in the process," evidently a result of pressure, but thereafter made no further movement nor change in its position as it produced a small hole in the cuticle and cellulose wall, passed through, and enlarged to normal size within the cell. As this fungus filament consisted of a single cell, the pressure within it was equally distributed. If the pressure had been sufficient to penetrate the host cell wall, which was many times as thick as the fungus wall, it would have straightened the fungus filament. As a matter of fact, however, it did not straighten a single bend nor change its position in the least, although such filaments bend easily, even by the motion of delicate water currents. Moreover, if pressure had caused the penetration of the wall, the fungus would have made a hole as large as its filament instead of a small hole, for the pressure on every unit area of its wall surface was equal. A more probable cause of this type of penetration is that cellulase is formed solely at a single point on the tip of the filament.
In view of the foregoing evidence, it would seem that the pressure theory of cell wall penetration by fungi is not so well supported as the enzym theory.

**DISTRIBUTION OF THE FUNGUS**

As this physiological form of _Oospora lactis_ has been isolated frequently from tomato fruits shipped from the Gulf States, it is probably common in at least several of the Southern States. It is difficult from the reports of the Bureau of Markets' inspectors to estimate how often it really occurs in shipped tomatoes, as they report all such rots as "soft rot." It was found to be common at the Government Experimental Farm, Arlington, Va., and in gardens in the vicinity of Washington, D. C., where no doubt it has carried on its parasitic activities for some time, but has been overlooked because of the similarity of the rots produced by it and _Bacillus carotovorus_ and the not infrequent association of the two organisms in the same fruits.

**POSSIBLE MEANS OF CONTROL**

As this fungus infects tomato fruits quite readily between 9° and 32° C. and can infect them at temperatures ranging from 2° to 37.5°, the practicability of controlling it by means of low temperatures seems doubtful.

Some experiments were made to determine the effects of antiseptics on the control of this organism. The fungus was immersed for 30 minutes in an aqueous solution of the antiseptic and transferred to sterile carrot decoction, in which, if it were still viable, it grew readily. The results are summarized in Table III.

**Table III.—Effect of antiseptics on the viability of _Oospora lactis parasitica_**

<table>
<thead>
<tr>
<th>Antiseptic</th>
<th>Concentration of solution</th>
<th>Subsequent growth in carrot decoction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antiseptic.</td>
<td>Water.</td>
</tr>
<tr>
<td>Chlorid of lime</td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>Potash alum</td>
<td></td>
<td>(a) (a)</td>
</tr>
<tr>
<td>Potassium permanganate</td>
<td></td>
<td>400</td>
</tr>
<tr>
<td>Copper sulphate</td>
<td></td>
<td>(a) (a)</td>
</tr>
<tr>
<td>Mercuric chlorid</td>
<td></td>
<td>5,000</td>
</tr>
<tr>
<td>Formaldehyde, 37 per cent.</td>
<td>I</td>
<td>300</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Saturated.

Immersing the fungus for 30 minutes in an aqueous solution of chlorid of lime 1:40, potassium permanganate 1:400, formaldehyde (37 per cent) 1:300, or mercuric chlorid 1:5000, prevented its subsequent growth, but a similar treatment with a saturated solution of potash alum or copper sulphate was ineffective. The resistance of this fungus to copper sulphate—a fungicide of wide use—is surprising. Although the experiments with this treatment were repeated several times, only negative action was obtained.

Some experiments were also made on the use of the antiseptics as washes. Tomato fruits varying in maturity from green to ripe were lightly pricked in several places and submerged for 5 minutes in an aqueous suspension of _Oospora_ spores, then drained, washed in an antiseptic solution for 30 minutes, and placed in moist chambers. From 20
to 40 pricked fruits, 8 unpricked fruits, and a number of controls equal to the number of treated fruits were used in each treatment. The results are summarized in Table IV.

**TABLE IV.—Effect of washing green to ripe tomato fruits for 30 minutes in an antiseptic to control watery-rot**

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Strength of solution: parts antiseptic to water</th>
<th>Pricked fruit infected 6 days after treatment</th>
<th>Unpricked fruit infected 12 days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potash alum</td>
<td>1:50</td>
<td>31</td>
<td>25</td>
</tr>
<tr>
<td>Do</td>
<td>1:40</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Chlorid of lime</td>
<td>1:50</td>
<td>14</td>
<td>12 1/2</td>
</tr>
<tr>
<td>Do</td>
<td>1:40</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Formaldehyde (37 per cent)</td>
<td>1:300</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Do</td>
<td>1:240</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>57</td>
<td>37 1/2</td>
</tr>
</tbody>
</table>

The percentage of infected fruits is higher in the pricked than in the unpricked series. Moreover, it is higher than in shipped fruits. As shipped fruits usually become infected through the stem scar instead of through the epidermis, they are probably comparatively free from punctures and therefore compare more nearly with the unpricked series. Formaldehyde and chlorid of lime (calcium hypochlorite) caused considerable reduction in the percentage of infected fruits. It is quite likely that sodium hypochlorite, which is a cheap convenient solution, would give more effective results than chlorid of lime. As the latter is partly insoluble it should, if used on a commercial scale, be dissolved in a separate tank so the clear liquid can be drawn off for use, or dissolved in a washing tank having a removable perforated sheet a few inches above the bottom to prevent the solid particles from adhering to the fruits.

The lower effectiveness of these antiseptics in the presence of fruits as compared with their effect on free spores (Table III) is probably due to chemical reaction of the germicides with substances on the surface of the fruits, which reduces their strength.

The economical use of a fungicide for washing tomato fruits should not be measured by its effect on a single rot, for if it controls one, it will give at least partial control of several others that cause heavy loss during shipment. The selection of a solution for this purpose should therefore be based on its control of this whole group of rots as well as on its cheapness and ease of handling.

**SUMMARY**

(1) A new rot of tomato fruits, closely resembling the rot caused by *Bacillus carotovorum*, has been common since the spring of 1921 in tomatoes shipped from the Gulf States. It is also prevalent in the vicinity of Arlington, Va., and Washington, D. C.

(2) The rot is characterized by dark-colored water-soaked areas which start in the stem scar and spread slowly toward the blossom end of the fruit without the production of a pronounced odor.

(3) The causal organism is a physiological form of *Oospora lactis*, which, though incapable of penetrating the epidermis, usually enters the fruits through the stem scar, but may enter through any place not cov-
ered by the cuticle, such as wounds, cracks, and punctures. It shows a little preference for ripe fruits, but infects green fruits quite readily. It invades the cells, destroys their protoplasmic contents, and causes loss of cell coherence through a slow dissolution of the middle lamellae of the cell walls.

(4) The minimum temperature for the germination of its spores, growth of its mycelium, and its infection of tomato fruits is approximately 2°C, the optimum 30°C, and the maximum between 37.5°C and 38.5°C.

Immersing the fungus spores (cells) in an aqueous solution of an antiseptic for 30 minutes and transferring them to sterile carrot decoction had the following effects on their viability: Chlorid of lime 1:40, potassium permanganate 1:400, formaldehyde (37 per cent) 1:300, and mercuric chlorid 1:5,000, no growth; potash alum or copper sulphate in saturated solution, considerable growth. The percentage of tomato fruits infected after inoculation with this fungus was considerably reduced by washing them for 30 minutes in an aqueous solution of chlorid of lime 1:40 or formaldehyde (37 per cent) 1:240. It would seem from these results that an effective wash could be developed for the control of a large number of these rots during shipment.

LITERATURE CITED


(9) SMITH, Erwin F. 1920. AN INTRODUCTION TO BACTERIAL DISEASES OF PLANTS. XXX, 688 p., 453 fig. [pl.] Philadelphia, London. Literature at end of most of the chapters.


Sections of a 2-day-old spot of a green tomato fruit affected by watery-rot (*Oospora lactis parasitica*).

A.—Masses of the fungus lying on the surface unable to penetrate the cuticle.
B–G.—Consumption of the protoplasm by the fungus; B–C, early stages; D, medium early stage; E–G, late stages.
H.—Cells beginning to lose their coherence after destruction of their protoplasm.
I.—A more complete stage of cell separation than shown in H.
J.—Very late stage. Protoplasmic contents completely destroyed; cells free; walls thin, irregular, and inconspicuous.

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Watery Rot of Tomato Fruits

PLATE I - A

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Watery-Rot of Tomato Fruits

PLATE 2

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Sections of a tomato fruit infected by *Bacillus carotovorus*.
A.—Early stage of invasion; bacteria confined to the intercellular spaces.
B–D.—Medium early stages. B–C.—Intercellular spaces enlarging through dissolution of the middle lamellae of the adjoining cell walls; bacteria beginning to invade the cells.
D.—Cells losing their turgidity and coherence; middle lamellae dissolved.
E.—Bacterial invasion of a firmly attached cell.
F–H.—Late stages. Cells occupied by the bacteria.
PLATE 3

*Oospora lactis parasitica*

A–D.—Types of branches. A.—Short branches which arise near the septa at an angle of about $45^\circ$ with the filament and break up into more or less rounded, irregularly shaped cells which function as spores. B–D.—Long branches which bear lateral branches of type A, but also break up into numerous cells capable of immediate germination.

E–F.—Segmented branches of type A; also early stages in the segmentation of the central filament.

G.—Order of segmentation in branch of type A.

H.—Highly magnified detached cells of lateral branches and central filament.

I–M.—Germinating detached cells.

N–T.—Penetration of irregular, wavy, somewhat separated walls of partly destroyed cells by tips of growing hyphae.

U.—Cells of tomato fruit tissue containing hyphae surrounded by digestion vacuoles.

V.—Penetration of the cell wall of a tomato fruit cell by a germinating spore. (a) Germinating spore. (b) Cell wall. (c) Hole made in the wall by the germ tube. Somewhat diagrammatic.
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PLATE 4

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A.—Watery-rot of tomato fruits produced by *Oospora lactis parasitica*. The fruits were inoculated in needle punctures below the stem scar and kept in a moist atmosphere.

B.—Halved potato tubers.  (a) Inoculated with *Oospora lactis parasitica*; (b) inoculated with *Bacillus carotovorus*.

C.—Halved carrot roots.  (a) Pricked with a sterile needle; (b) inoculated with *Oospora lactis parasitica* isolated from watery-rot lesions of tomato fruit.