A CYTOLOGICAL STUDY OF THE TOMATO LEAF SPOT AND ITS CAUSAL ORGANISM, SEPTORIA LYCOPERSICI (SPEG.) SACC.

by

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One of the serious diseases of tomato (Lycopersicon esculentum Mill. var. commune Bailey) is caused by Septoria lycopersici (Speg.) Sacc. This disease is commonly called late blight, blight, and leaf spot. The latter name is the most widely used, since it eliminates confusion with blights caused by other organisms. Levin (1916) reviews the historical background of the disease. It has been known since 1882 when it was first reported by Spegazzini in Argentina. Later it was found to occur in Italy, Australia, France, Germany, Austria-Hungary, and England. In the United States it was first observed and reported by Halstead in New Jersey in 1896. It has since been found in the various states where tomatoes are grown. It was first reported in Maryland in 1899 by Bessey.

The symptoms and the methods of control are contained in numerous experiment station and Farmer's bulletins. The symptoms of the disease may be considered briefly here. The first sign of the disease is the appearance of water soaked areas, visible from the under side of the leaf, especially if held against the light. As the spot enlarges it becomes more or less circular, with a definite margin. Later the affected tissue becomes dry, shrunken, and brownish grey. The spots are variable in size from 2mm. to 2 cm. in diameter, depending on the number of lesions in a given area and on their proximity to the midrib or veins. Several spots may coalesce forming an irregular lesion. The size of the spot is not related to

its age or degree of maturity. Within five days pycnidia begin to appear and are evident as black dots, from which yellowish white masses of spores exude when the pycnidium is mature.

Spots on the stem and the calyx are similar to those on the leaves, although more elongated and not so well defined. Levin (1916) includes a description of the spots on the fruits, but adds that these do not occur unless the skin is injured, thus allowing the entrance of the organism.

Other members of the genus Septoria cause leaf spots on numerous cultivated plants. Among these are Septoria ribis Desm. on gooseberry and currants, S. fragariae Desm. on strawberry, S. pruni Ellis on plum, S. apii Chester on celery, S. lactucae Pass. on lettuce, and S. tritici Desm. on wheat. All of these form spots similar to those on the tomato caused by S. Lycopersici (Speg.) Sacc. and reproduce by the formation of pycnidia.

REVIEW OF LITERATURE

The chief concern of the literature relating to the leaf spot caused by Septoria lycopersici has been a description of the symptoms and the methods of control. These aspects of the disease have been covered in a number of experiment station and Farmer's bulletins. The literature relating to the cytological study of the fungus, S. lycopersici, and its relation to the host tissue, with which this paper is concerned, is limited. This necessitates an extensive reference to similar studies on other fungi. To facilitate the discussion

of the literature it may be considered under four heads: (1) the method of penetration of the organism into the host tissue, (2) the penetration of artificial membranes by fungus hyphae, (3) host relationships, and (4) the development of the pycnidium.

Method of Penetration

Numerous and varied fungus species have been employed in the study of the method of penetration of the fungus into the host tissue. These will be discussed according to their systematic arrangement, as the Archimycetes, Phycomycetes, Ascomycetes, Basidiomycetes, and the Fungi Imperfecti.

De Bary (1887) found that the spores of Synchytrium sp. germinate forming a germ tube which directly penetrates the cell wall. Cook and Nicholson (1933) found that the penetration hypha arising from the zoospores of Woronina polycystis Cornu. parasitic upon Saprolegnia and Achyla, enters the cell wall in a similar way. The contents of the spore flow through forming a plasmodium within the cell.

Swarm spores of Cystopus sp., Phytophthora omnivora, Peronospora nivea, and several unidentified species of Peronospora were shown by de Bary (1887) to cause infection by penetrating the epidermal cells. The penetration tube arises from the spore without the formation of a germ tube. Busgen (1893) observed that the germinating swarm spores of Peronospora parasitica form appressoria on the surface of the host cells. Appressoria were also formed in water mounts on glass slides from which Busgen concluded that appressoria are formed as the result of contact between the germ tube and an impenetrable object. Hawkins and Harvey (1919), studying the

infection of potato tubers by Pythium debaryanum Hesse, found that the organism enters by means of a penetration hypha. It appears to enter by mechanical means since a dent is formed in the wall of the peridermal cell by the pressure of the infection hypha.

Concerning the reaction of the germ tubes and hyphae of Peziza tuberosum and P. sclerotiorum to the stimulus of contact, Brefeld (1881) found that both form a much branched, short, brown group of hyphae, which he calls an Haftorgan. De Bary (1887) using the same two species Sclerotinia (Peziza) tuberosa and S. (Peziza) sclerotiorum and in addition S. ciborioides and S. fuckeliana found similar organs of attachment formed on contact with impenetrable surfaces, but poorly formed, or not at all, when growing on the surface of plant parts. With Protomyces macrosporus he found that tubes are sent directly from the ascospore into the host tissue at the line of union between the epidermal cells. The species of the Erysiphaceae, which he studied, on germination form appressoria at the tips of the germ tubes. These are closely appressed to the surface of the plant tissue, the infection hypha arising from the under side and entering the host cell. De Bary (1887) mentioned that the penetration of Polystigma rubrum and Claviceps sp. is directly through a firm membrane, but he does not add the method of entrance. Busgen (1893) studied the germination of the conidia of Erysiphe communis and found that appressoria are formed and that from these the penetration tubes arise. Smith (1900), investigating the formation and entrance of

haustoria of the Erysophaceae also observed the formation of an appressorium and penetration hypha with E. communis. The hose cell forms a thick band of cellulose about the infection hypha, which the hypha penetrates, dissolving it by enzymatic action. Uncinula salicis forms a lobed appressorium from which several infection hyphae may arise, resulting in the formation of several haustoria. Phyllactinia sp. on Xanthoxylum americanum, forms appressoria not only on the surface of the epidermal cells but also intercellularly within the host tissue. Boyle (1921) found the formation of appressoria and infection hyphae to be the method of entrance for Sclerotinia libertiana. Since there is a marked denting of the epidermal cell he believes that a mechanical force is exerted by the penetration hypha. Penetration by means of a single hypha was shown by Conant (1927) with Thielavia basicola Zopf on tobacco which he believes to be accomplished by mechanical means. Pearson (1931) found infection of corn seedlings with Gibberella saubinetii (Mont.) Sacc. to occur in a similar way. The germ tube constricts as it passes through the cell wall, later swelling to normal size. At the coleorhiza infection occurs through the plugs, which are formed between the epidermal cells, probably as the result of the deposition of materials in the spaces left during the period of rapid growth. The germ tube enters here without resistance. There is evidently an enzymatic action since the plugs react differently to stains after penetration.

De Bary (1887) found that the germ tubes formed from uredospores and aeciospores of the Uredineae enter the host

tissue through the stomata, whereas those of the sporidia cause infection by penetration through the epidermal cells. Leptopuccinia dianthi he found to be an exception since the germ tubes arising from the sporidia entered the stomata. Similar results have been obtained with uredospore, aeciospore, and sporidial infection caused by Uromyces fabae, U. poa, and U. polygoni by Busgen (1893); with uredospores and aeciospores of Puccinia dispersa Erikss. on the bromes by Ward (1902,1905). Ward showed that an appressorium is formed over the orifice of the stoma from which an infection hypha arises which later forms a substomatal vesicle. From this the actual penetration hypha arises. Similar observations have been made by Marryat (1907) on the infection of wheat by uredospores of P. glumarum; by Stakeman (1914) on the infection of wheat by uredospores of P. graminis tritici and P. graminis avenae; by Waterhouse (1921) with the uredospore infection of wheat by P. graminis Pers. and the sporidial infection of Berberis vulgaris with the same fungus. In the latter appressoria may or may not be formed. If they are formed, a style-like tube, the infection hypha, arises from them, penetrating the epidermal cell, apparently by mechanical means. When within the epidermal cell a vesicle is formed from which branches arise. Allen (1926, 1932) has shown that uredospore infection by P. triticina Erikss. on wheat and the sporidial infection of P. coronata Cda. on Rahmnus cathartica L. follows the same procedure as has been described by Ward, Marryat, Stakeman, and Waterhouse.

De Bary (1887) showed that infection by Tuburcina trientalis

occurred through the epidermis at the line of union between the cells. That germ tube from the conidia of Ustilago avenae (Pers.) Jens. penetrate the epidermal cells of oats, through small openings in the walls, was demonstrated by Kolk (1930). Evans (1933) found no evidence of the formation of appressoria on the germ tubes of Urocystis cepulae Frost from which penetration tubes arise entering the onion tissue. He believes that penetration is accomplished by a combination of enzymatic and mechanical action.

Busgen (1893) and Voges (1910) found that germ tubes of Fusicladium pirinum and F. sp., respectively, formed dark brown appressoria when in contact with an impenetrable surface, adhering closely to the surface by means of a gelatinous sheath. Germ tubes from conidia and ascospores of Ascochyta sp. infect the tomato tissue by means of germ tubes entering through the epidermal cells, without the formation of appressoria, according to Klebahn (1921). Rands (1917), concerned with the infection of potato leaves with Alternaria solani (E. and M.) J. and G. found that infection might be either stomatal or cuticular. Young (1926) showed that Alternatia sp. formed appressoria from which infection pegs arise, on wheat, oats, sorghum, cabbage, radish, and on several other plants, whereas penetration was stomatal on the leaves of pea and tomato. When penetration was cuticular, the host cells formed callosities. Similar results were obtained with Diplodia zeae, Cephalosporium acremonium, Colletotrichum nigrum, Acrothecium sp. and Helminthosporum graminum. That appressoria, and infection hyphae, are formed by

Stagnospora curtisii (Berk.) Sacc. on narcissus was shown by Creager (1933).

Poorly developed clusters of short hyphae were shown by de Bary (1887) to be formed when the germ tubes of Botrytis cinerea were in contact with the leaf tissue. Nordhausen (1888), Ward (1888), Busgen (1893), and Pfaff (1925) noted the formation of appressoria with B. sp. and B. cinerea, penetrating the cell wall by means of infection hyphae. Busgen believes entrance is effected by a combination of mechanical and toxic action with which Smith (1902) agrees, whereas Brown (1915) believes it is an enzymatic action since an extract on which B. cinerea has been grown causes a disintegration of lettuce tissue. Blackman and Welsford (1916) did not observe an enzymatic action with the infection of leaves of Vicia with B. cinerea, but observed a denting beneath the appressorium and penetration tube from which they concluded that it was mechanical rather than enzymatic. Godfrey (1923) found that infection by the conidia of Sclerotinia ricini Godf. occurred as that described by Blackman and Welsford.

Appressoria and infection hyphae were first observed on Gloeosporium (Colletotrichum) lindemuthianum Sacc. et Magnus by Frank (1883), formed as the result of the stimulus of contact. Hasselbring (1915) observed the same method of infection with G. fructigenum on berries of Berberis thumbergii, the penetration hypha arising from a definite pore in the appressorium and entering the leaf by dissolving the cuticle and perforating the cell wall. Dey (1919) found penetration by infection hyphae from appressoria of Colletotrichum lindemuthianum to be

to be mechanical since a dent was formed in the cell wall below the hypha and since the appressorium was often lifted from the surface of the leaf. Walker (1921) agrees with this in the infection of onion by C. circinans. Dey (1933) concerned with the factors influencing the formation of appressoria was able to show that they are due to the stimulus of contact in the case of C. gloeosporioides Penz.

That Septoria graminum Desm. infecting wheat forms appressoria and infection hyphae was shown by Mangin (1899). He concludes that the fungus secretes some material, probably an enzyme, since there is a change in the reaction of the cell wall to stains around the point of infection. He adds that penetration is definitely not stomatal. Cochran (1932), comparing the leaf spots of celery caused by the two species of Septoria, S. apii (Br. and Cav.) Chester and S. apii. graveolentis Dorogin, also found that penetration was directly through the epidermal cells. Levin (1916) believes that penetration of the tomato tissue by S. lycopersici Speg. is stomatal. Killian (1920) shows that S. lycopersici penetrates the membrane directly, forming a hypha within the epidermal cell which he calls an haustorium. He does not find that entrance is gained by the formation of an appressorium and penetration tube, but by the growth of the germ tube through the cuticle, cell wall, and into the cytoplasm.

Penetration of Artificial Membrane

In order to understand the method of penetration and the factors influencing it more clearly, artificial membranes with

various nutrient materials have been employed by several workers. Both Botrytis cinerea and Penicillium glaucum were used by Miyoshi (1895) to show the method of penetration of such artificial membranes as cellulose, collodion, gold leaf, parchment paper, and onion skin. With these he showed that the hyphae penetrated the membrane if there was a nutrient solution below, but the germ tubes grew over the surface if the nutrient medium was lacking. From this Miyoshi concluded that a chemical stimulus is present which influences the formation of appressoria and infection hyphae, although penetration may be effected by the germ tube without the formation of an appressorium. He believes that there is also a mechanical effect arising from the fungus hypha itself, since a dent is often formed in the membrane at the point of penetration. Fulton (1906) conducted various tests using a number of fungi and several artificial membranes. He was unable to show any chemotropic influence, but believes that cytotropism and hydrotropism do affect the direction of growth of the fungus hyphae.

Brown (1922a,1922b) believes that germination is stimulated by the presence of materials in the infection drop, which diffuse out from the plant tissue. If this nutrient material is present and suitable, attack of the host plant occurs. The presence of such materials was shown by the increase in conductivity of the water on the tissue surface.

Brown and Harvey (1927) agree with Fulton that a chemotropic effect is not responsible as an individual influence in bringing about penetration. With neither artificial

membranes, as paraffin and formalized gelatin, nor natural membranes, as onion skin, were they able to show any chemotropic influence in the penetration of the germ tubes of Botrytis cinerea. They believe penetration is mechanical since infection hyphae of B. cinerea can penetrate a 40 per cent gelatine membrane and not a 50 per cent gelatine membrane. Brown and Harvey believe that penetration is the result of a contact stimulus, although there is in addition a specific relation between the host and the fungus which exists and so disqualifies this theory to a certain extent.

Johnson (1932) attempted to show the specificity of attack by sowing spores of Colletotrichum circinans (Berk.) Voglino on the surface of various plants, including onion, pea, buckwheat, bean, cotton, and tomato. He found that under certain conditions, not always the usual, C. circinans appeared to be parasitic on a number of species of plants. He states that specificity against attacking fungi seems to be due to several limiting factors as failure of spores to germinate, non-formation of appressoria, non-penetration, or the rapid death of the infection hypha after entering the tissue.

Relation of Host and Pathogen.

After penetration has occurred and the fungus hyphae are within the host tissue they may proceed in two ways, either intercellularly or intracellularly. Edson (1915) finds that the hyphae of Phoma betae, (Oud.) Fr. infecting beet tissue, grow intracellularly consuming the cytoplasm and causing the disintegration of the host nuclei. The fungus dissolves the

middle lamella and causes the disintegration of the cellulose walls. That the mycelium of Alternaria solani within the potato tissue is intercellular was shown by Rands (1917) as is the mycelium of Sclerotinia libertiana according to Boyle (1921). He observed that the death of the cells extends beyond the area of hyphal invasion, showing that it must be due either to an enzyme secreted by the hyphae or due to the products of disorganization of the host cells. This effect is observed as a swelling of the plastids and their disorganization finally forming a densely granular mass. The nucleus moves to the side of the cell nearest the infected area and finally disorganizes. Around the infection hypha there is formed a halo which suggests a change in the structure of the cell wall and cytoplasm. Pearson (1931) made similar observations in corn seedlings infected with Gibberella saubinetii (Mont.) Sacc., finding plasmolyzed cells beyond the invading hyphae, which showed a greater affinity for stains than the healthy cells. Allen (1926), studying the infection of wheat by Puccinia triticina, observed little if any effect of the fungus on the host cells. The hyphae are intercellular sending haustoria into the cells. The only noticeable effect was the migration of the nucleus of the host cell toward the haustorium, sometimes accompanied by a swelling of the nucleus. The hyphae of Ustilago avenae (Pers.) Jens. infecting oats are shown by Kolk (1931) to be intracellular after entering the host tissue in the tissues of the coleoptile, mesocotyl, coleoptile node, and in the internode between this and the first leaf. In the growing

point the mycelium is intercellular.

Cunningham (1928a,1928b) was concerned chiefly with the formation of a phellogen resulting in the formation of a phellem, which he calls a cicatrice, thus preventing the further spread of the fungus. Such a layer was found around infected areas of citrus leaves caused by Sphaceloma fawcettii Jenkins, those of Prunus domestica L. caused by Cocomyces prunophorae Higgins, those of beet caused by Cercospora beticola Sacc., as well as several others. Those in which no such layer was formed were more numerous, including lesions caused by Septoria petroselini Desm., S. podophyllina Pk., S. conspicua Ell. and Mart., S. cirsii Niessl., S. verbasicola B. and C., S. acerina Pk., and S. osmorhizae Pk.

Klebahn (1907), describing the lesions on Pyrus communis caused by Septoria piricola Desm. says that the invading hyphae are intercellular, present as thin brown threads in the intercellular spaces. The mesophyll cells are filled with dark brown masses, especially at the edge of the leaf spot. Cochran (1932) found that the hyphae of S. apii (Br. and Cav.) Chester and of S, apii graveolentis Dorogin were intercellular being present in knots and fasicles, in the intercellular spaces. He found the hyphae present beyond the collapsed areas but not beyond the dead cells. He believes that the death of the host cells precedes the growing hyphae. Levin (1916) states that the infection of tomato leaves with S. lycopersici Speg. may occur from either side of the leaf. Lesions occurring from the lower side are usually broad whereas those from the upper surface are narrow. He states

that when entrance has occurred through the stomata the fungus forms haustoria which penetrate the host cells, although not deeply. The mycelium is not confined to the collapsed area. He adds that within 5-13 days after infection there is a definite shrinkage and blackening of the tissues. Killian (1920) shows that the secondary hyphae arising from the tube entering the epidermal cell continue to grow intercellularly. He finds, as does Levin, that the spread of the fungus depends on the tissue through which the hyphae are growing. He also agrees with Levin that haustoria are formed by the intercellular hyphae. He describes the effect of the fungus on the host cells as a coagulation and disintegration of the protoplasm and adds that the nucleus shrinks forming an unrecognizable clump. He finds that the injured cells show a greater affinity for haematoxylin.

Development of the Pycnidium.

Pycnidia may be initiated in two ways, either meristogenously or symphyogenously, according to de Bary (1887). Those of Pleospora sp. described by him are typical of neither group since they arise from the intercalary portion of an hyphae as the result of successive divisions and by the branching of adjoining hyphae. Kempton (1919) summarizes the early work on the formation of the pycnidium. After an investigation of their formation by a number of fungi he found that many were intermediate between the two groups as defined by de Bary. In Septoria polygonorum (Desm.) Shear and Dodge he found that pycnidia sometimes developed meristogenously,

sometimes symphyogenously, and at other times they seemed to be a combination of the two. Pycnidia of S. helianthi E. and K. and S. scrophulariae Pk. were found to form meristogenously.

In the study of the pycnidia of Phyllostictina carpogena Shear, Sclerotiopsis concava Desm., and Schizoparme straminea Shear Dodge (1923) was concerned with the development of the central and ostiolar cavities. He outlines three stages in the growth of a pycnidium: (1) rapid cell division whereby a certain amount of fundamental and undifferentiated tissue is formed, (2) formation of the central cavity, organization of the wall, determination of the sporogenous tissue, in many the production of an ostiole, (3) spore production.

The formation of the pycnidium of Septoria lycopersici Speg. is described by Levin (1916). It is formed by the interweaving and anastomosing of adjacent hyphae. Its development follows the steps outlined by Dodge.

STATEMENT OF THE PROBLEM

The purpose of this work is to study in detail, using cytological methods, the method by which the germ tubes of Septoria lycopersici enter the leaf tissue of the tomato, the effect of the fungus hyphae on the host cells, the progressive formation of leaf spots, and finally the development of the pycnidium. A brief consideration of these aspects of the problem have been given by Levin (1916) and by Killian (1920). Levin believes that infection occurs by the growth of the germ tube through the stomata, Killian that it is effected by direct entrance into an epidermal

cell. Both Levin and Killian have shown that after penetration into the leaf tissue the hyphae grow intercellularly sending haustoria into the host cells. They describe the appearance of the leaf spots, which depends on the point of infection, and discuss the extent of the hyphae in the leaf tissue. Levin describes briefly the stages in the growth of the pycnidium.

MATERIALS AND METHODS

The culture of Septoria lycopersici, for use in inoculations, was isolated from diseased plants obtained from a nearby garden. The organism obtained was definitely identified as S, lycopersici. Both potato dextrose and malt extract agar were found to be suitable for growing the organism. Two to four week old cultures were used in inoculations.

The plants to be used for inoculations were grown in the greenhouse. Both entire plants and single leaflets were used in inoculations. The plants kept in the greenhouse were inoculated by spraying with a suspension of spores in tap water. The plants were kept shaded by moist papers for two days and were sprayed twice a day for two days with tap water. Infection was successfully obtained in this way but the number of spots on each leaf was not large. A larger number of leaf spots on one leaf was obtained when separate leaflets in moist dishes were inoculated. Healthy leaflets were washed thoroughly in tap water and then laid on moist filter paper on copper wire frames in the moist dishes, the bottoms of which were kept covered with water. The filter

paper was kept moist by the use of paper wicks. In this way there was very little, if any, wilting of the leaflets. The leaves were sprayed with a spore suspension and the dishes kept on a north window sill in the laboratory.

Bonny Best plants were used in all the series of inoculations except one in which the variety Marvel was used. The age of the plant had no apparent effect on the ease with which it was infected with Septoria lycopersici, so plants of no definite age were used.

For the study of the development of the pycnidium cultures grown on potato dextrose and malt extract agar were used. Squares of the colonies and the underlying agar were used in fixations.

For fixation of material both Flemming's medium and formalin acetic alcohol were used. The latter was found to be most satisfactory since it eliminated the lengthy washing and bleaching necessary with Flemming's medium and gave a good fixation of the fungus and the leaf tissue. Fixations were made daily between nine and ten o'clock in the morning for from onr to twelve days after inoculations were made. In order to obtain the best fixation it was found necessary to eliminate the air from the leaves. The material was placed in the fixing solution and then placed in the suction flask and the air exhausted until bubbles ceased coming from the leaves. After fixation the material was carried through alcohol, xylol, paraffin, and mounted for staining in the usual manner. Sections were cut at four to six microns as this was found to give the best sections for study.

The sections were stained with Haidenhain's iron alum haematoxylin, alone and counter stained with erythrosin, and with Flemming's triple stain. The haematoxylin stain alone proved to be satisfactory, since the hyphae of the fungus stained somewhat darker than the leaf tissue, allowing accurate differentiation between the two.

Temporary mounts for the study of the germination of the spores and the growth of the germ tubes over the epidermis were made by stripping the epidermis from the leaves, immersing in 95 per cent alcohol, and mounting for examination in a glycerine alcohol medium.

RESULTS

Method of Penetration

The spores of Septoria lycopersici germinate within twenty-four to forty-eight hours, depending on the age of the spores and on the temperature. Spores from cultures over four weeks old gave poor germination. During the winter, when the moist dishes containing the inoculated leaves were kept on the north window sill, fewer spores germinated, and those more slowly, than when cultures were kept at warmer temperatures.

Germination and early germ tube growth were studied by stripping the epidermis from inoculated leaves and mounting in glycerine and alcohol. Before germination, the cells of the spore absorb water, becoming greatly swollen. From each cell germ tubes may arise, often two from each cell. There is no evidence that the germ tubes are attracted to the stomata or that in growing across an open stoma they may enter the

leaf tissue at that point. Figure 1 shows a germinating spore from which three germ tubes have arisen. Two of these are growing directly away from the open stoma while the third is growing away from it, though not as decidedly as the other two. Figure 2 shows a germ tube growing directly across the pore of the stoma without entering. Figure 3 shows an open stoma over which one germ tube passes twice, crossing at A, bending and crossing again at B, while at C the second germ tube is crossing it without entering. Of added interest is the case in which the germ tube circled half way around the stoma and then grew away from it, and another in which the germ tube grew entirely around the pore without entering. A number of germ tubes were observed to grow over several open stomata without entering the leaf tissue. Of a total of 209 stomata observed over which one to four germ tubes were growing, or had grown, none showed the entrance of the germ tube into the leaf through the stomatal opening.

The germ tubes may grow over the surface of the leaf for a greater or a lesser distance, crossing a number of epidermal cells before penetration occurs, seeming to depend on the amount of water present on the surface of the leaf. Eventually the tips of the germ tubes become attached to the surface of the lea . In temporary mounts germ tubes were often observed to swing freely behind the point of attachment in currents of the mounting medium, when the cover glass was gently pressed. In permanent mounts of transverse sections of leaves the germ tubes w ere growing across the epidermal cells, as may be seen in figure 4, which shows a germ tube at the end of which is an appressorium, the medium of attach-

ment to the leaf surface.

From permanent mounts appressoria and the penetration hyphae arising from them were observed. In figure 5 the surface view of an appressorium is seen as it lies on an epidermal cell. The appressorium appears as a much swollen tip of the germ tube. Two nuclei are evident and the cytoplasm appears to be vacuolate. Surrounding it there is a non-staining wall, which is visible as a shining halo in reduced light. This may possibly be gelatinous in nature which would aid in the adherence of the appressorium to the leaf surface. It seems that the appressoria may be formed almost immediately after the germ tube is formed or later on a more elongated germ tube as in figure 4. Figure 6 shows an appressorium formed immediately from the spore. It appears to form a dent in the wall of the cell on which it lies. The epidermal cell is drawn showing the downward slope of the cell wall at A, and at B the internal view of the cell. The contents of the appressorium are densely cytoplasmic and binucleate.

The position of the appressoria on the surface of the epidermal cells is variable. They may be present near the line of union of two epidermal cells as in figures 4 and 6, or at the top of the slope of the epidermal cells as in figures 7 and 8.

Penetration of the host cell is accomplished by the formation of an infection hypha arising from the lower side of the appressorium. Figure 7 shows an early stage in the formation of the penetration tube. It appears to be dense at the tip. The contents of the appressorium are densely

cytoplasmic and one nucleus is visible. Around the penetration hypha a glistening halo, the wall of the infection hypha, is visible, similar in appearance to the wall around the appressorium. In this drawing the infection hypha has passed through the cell wall and is touching the cytoplasm. The host cell has not changed in any visible way.

As penetration progresses the tube increases in length and width as is shown in figure 8. With reduced light a wall is evident around the entire penetration hypha. The appressorium now appears vacuolate, with one nucleus visible. The host cell does not appear to be changed.

Host Relationships

After the penetration hypha has entered the epidermal cell wall, it enlarges and branches within the invaded cell. A section showing the connection between the penetration hypha and the intracellular hypha was not seen, although several were observed which were broken in the center and bent toward the top of the epidermal cell. The contents of the invaded cell seem little changed. The cytoplasmic layer within the cell wall appears normal. The nucleus is shown in figure 10, lying above the intracellular hypha at the point at which the break has occurred, does not appear altogether normal since the reticulum stains a faint gray with haemotoxylin instead of the usual blue color.

From this intracellular hypha branches arise which penetrate the cell wall, increase in length, branch, and grow intercellularly from then on. Figure 9 shows an intracellular

hypha having three branches, one of which has penetrated the cell wall passing into the underlying intercellular space. The intracellular hypha is vacuolate, and multinucleate at the points at which branching is to take place. The branch hypha has apparently penetrated the wall by means of a small style-like hypha, which later swells to its original size. The young branch hypha is densely cytoplasmic. Figure 10 shows a similar intracellular hypha and its branches, though more advanced. The hypha evidently penetrated the cell wall at the side since the portion through the cell wall is not visible.

From this point the fungus continues to branch and grow through the surrounding leaf tissue, through the entire thickness of the mesophyll and extending for some distance in breadth. As the hyphae progress through the mesophyll the host cells, previously in contact with the hypha, begin to show the effect of their presence. The first sign of this effect is shown by the cytoplasm which becomes densely granular showing a great affinity for stain. Later both the plastids and the nucleus show a tendency toward darker staining with haemotoxylin. There is no sign that either the nucleus or the plastids swell. Both may be distinguished up until the time that the entire mass stains a deep black, when differentiation is impossible. Even with extreme destaining this is still the case. The cell wall remains unchanged so far as can be told by the reaction to stains. It is usually visible around the dark mass of protoplasm. The shape of the cells is irregular and they appear shrunken. Figure 15 shows an

intermediate stage in the death of the cell. In figures 11 to 14 and 18 to 20, cells showing the final stages of death and collapse are illustrated.

The breadth of the spread of the fungus depends chiefly on the side of the leaf at which infection occurred. If the fungus enters from the under side, the lesion is wide and shallow at first, since the hyphae spread rapidly through the intercellular spaces between the loosely packed spongy parenchyma cells. Figure 12 shows an early infection occurring from the lower epidermis, in which the hyphae is just growing through the spongy layer. The epidermal cells and first layer of parenchyma cells have collapsed. Figure 11 shows the entire thickness of the leaf in which the hyphae have reached the upper epidermis, although only the lower epidermal cells and the adjacent layer of spongy parenchyma cells show signs of injury. If infection occurs through the upper epidermis the breadth of the lesion is less, due to the smaller intercellular spaces and the tightly packed, elongated palisade parenchyma cells around which the hyphae must grow. Figure 13 shows such a lesion in which an epidermal cell, three underlying palisade cells, and three cells of the spongy parenchyma are affected. The fungus hypha is seen growing away from the collapsed cells.

In none of these lesions has the entire thickness of the leaf been involved in the collapse of the cells. Figure 14 shows a young lesion in which the entire thickness of the leaf is affected, although not all the cells in the invaded area have been completely killed. Such a lesion is comparatively small since only four palisade cells are yet affected.

Figure 20 shows a lesion of about twice the size of that illustrated in figure 14. It covers a breadth of about nine palisade cells. Collapsed cells fill the entire thickness, although there are a few remaining uninjured cells scattered among the collapsed cells.

Figure 16 is a diagram of a mature leaf spot to aid in locating the position of figures 17, 18, and 19. Figure 17 is an enlarged view of the area at A, figure 18 of B, and figure 19 of C. In figure 17 the section is from the center of the collapsed area of the leaf spot in which all the cells are completely killed. Their contents form a dense darkly staining mass. In the spaces between, fragments of the hyphae are evident except at the upper right corner which shows the much branched primordium of the pycnidium. In figure 18 is seen the portion of the leaf spot at the margin of the collapsed area in which the cells on the right have been killed and in those on the left the cytoplasm is beginning to appear more granular. The number of hypha is greater in this area than in the completely collapsed area. Figure 19 shows the farthest extent of the hyphae. The host cells here do not show any effect of the presence of the hyphae.

The hyphae within the leaf spot are not numerous in the collapsed area except at the time of the formation of a pycnidium, when several adjacent hyphae become branched in its initiation. The hyphae are most numerous at the margin of the collapsed area, gradually diminishing in number from that point, often extending for a dozen or more palisade cells beyond the margin of the leaf spot. The host tissue does not form a cicatrice in order to prevent the growth of the hyphae.

The hyphae are multicellular, uninucleate, or often multinucleate, especially when their progress is hindered by a host cell, when branching occurs as a result. Often the nuclei are present in pairs indicating a recent division. The vegetative hyphae are usually vacuolate, although sometimes densely cytoplasmic. The hyphae which are to assist in the formation of the pycnidium are coarser, septate, usually vacuolate, and uninucleate. These are usually distinguishable since they stain more darkly than the vegetative hyphae.

Formation of the Pycnidium

The formation of the pycnidium is initiated by the intercalary division and branching of several adjacent hyphae. Figure 21 shows as early a stage in the formation of the pycnidium as can be distinguished from the tangled mass of hyphae as seen in artificial cultures. Here four hyphae seem to be involved in the formation of the primordium, entering the hyphal knot at A, B, C, and D. This branching and interweaving of the hyphae continues, resulting in an increase in the amount of the undifferentiated ground tissue, appearing

as a loosely aggregated mass. This is illustrated in figure 22; and the photomicrograph, figure 24. Each is larger than the former, but is reproduced at a different magnification. In figure 24 coarse brown hyphae are seen scattered over the surface of the ground tissue, which will later form the peridium. When the primordium is about one third mature size the outer hyphae form a compact layer, a well defined peridium. The central cavity is filled with a close net work of more delicate hyphae. Figure 25 shows the peridium made up of coarse brown hyphae and the central network of finer, lighter stained hyphae. As growth continues the hyphae of the central cavity become more slender as if being stretched, with a resultant break in the net work. The stretching and early stages of disintegration are shown in figure 26. The disintegration of the central hyphae continues from the center, until only a few non-staining fragments are evident in the central cavity. This disorganization of the tissue often involves the base of the peridium as well as the central tissue in pycnidia produced in artificial media. Such is the case in the pycnidia shown in figures 27 and 28. Figure 27 shows a young pycnidium in the central cavity of which there are only fragments of the disintegrated hyphae surrounded by the developing sporangeous layer just within the peridium. This layer begins to form before the disintegration of the central hyphae is complete, being formed into a closely woven layer by the branching of the hyphae just inside of the peridium. The upper enlarged cells,the sporophores, form a surface layer

parallel to each other and perpendicular to the peridium. This layer of sporogenous tissue is continuous with the peridium lining the central cavity. In figure 23 is seen the formation of the first group of spores. These arise as buds from the sporophores, later appearing as elongated branches. One to three spores may be formed from the same sporophore at the same time. Often spores may be formed by the terminal cell and the one beneath at the same time. The formation of spores is shown in figure 23. At A is shown a sporophore from which two branches are arising, one still very small and the other nearly mature. At B a spore is being formed from the side of the cell beneath the cell A. At C is a sporophore from which are formed three spores. Other sporophores form only one spore as is seen at D. The spores reach maturity before they are pinched off the base and a new group formed. The production of spores continues until the central cavity is filled. An ostiole is formed by a break in the peridium due to the pressure of the spore mass from below. The spores exude as a yellowish mass from the ostiole. A mature pycnidium with exuding spores is shown in figure 29.

In the leaf tissue the pycnidia are formed in much the same way as in artificial culture, though never attaining as large a size. They arise by the branching and interweaving of adjacent hyphae as has been shown for their formation on agar cultures. An early stage of the formation of the primordium is illustrated in figure 17, a section from the collapsed area of a mature leaf spot. Later a peridium is formed, although not as well defined as in the artificially

produced pycnidia. The primordia are embedded in the tissue of the leaf, but expand pushing the surrounding cells away, thus emerging at the leaf surface. This is shown in figure 30. The spores are formed as previously described, filling the cavity until their pressure on the peridium causes it to break, allowing their escape. This is shown in figure 31, in which a part of the peridium and the remainder of the surrounding leaf tissue is being pushed aside by the spore mass. Figure 32 shows a mature pycnidium in which the ostiole is definitely formed and the spores are escaping.

DISCUSSION

The method of penetration of Septoria lycopersici by means of an infection hypha arising from an appressorium follows the more general form of infection by many of the conidial forms of the perfect fungi as well as the imperfect fungi. All of the members of the Fungi Imperfecti do not cause infection in this way, as was shown by Klebahn (1921) with Ascochyta sp. in which infection is effected by the germ tube alone. Others of this group may not always behave in the same way as has been shown by Rands (1917) and Young (1926) with Alternaria sp. which may enter the tissue through the epidermal cells or through the stomata. That infection with Septoria lycopersici occurs by means of appressoria and penetration hyphae entering through the epidermal cells agrees with the results of Mangin (1899) with S. graminum and of Cochran (1932) with S. apii and S. apii graveolentis. The formation of the appressoria and the infection hyphae, and the passage of the germ tubes over the stomata, without

entering the leaf tissue disagrees with the suggestion of Levin (1916) that penetration occurs through the stomata. Both Mangin and Cochran state definitely that penetration is not stomatal. Killian (1920) does not find that penetration is stomatal, but by means of the germ tube growing through the cell wall. He does not report the formation of appressoria.

Before penetration occurs appressoria are formed. They seem to arise through the stimulus of contact. This seems likely since the internal hyphae of the fungus form similar swellings at the ends when in contact with an impenetrable cell wall. There is ample opportunity for the germ tube to arrive at a point beyond which it can not grow due to the irregular surface of the tomato epidermis, or to the drying of the infection drop, which would tend to draw the tip of the germ tube nearer to the surface. Those observed were sometimes present at the line of union of two cells which would form a depression, and sometimes on the slope of the cell wall. If appressoria are formed as the result of contact with an impenetrable object it would be expected that they would be formed in hanging drop cultures when growing against the cover glass. Mounts were made using both water and tomato decoction. In both of these media extensive hyphal growth was obtained, but in neither were appressoria formed. Drying which would draw the hyphal tips closer to the cover glass did not bring about the formation of appressoria as Dey (1933) was able to show with Colletotrichum gloeosporioides. He demonstrated that in hanging drop cultures appressoria were

formed as the result of contact between the hyphal tip and the cover glass. When allowed to dry somewhat, thus drawing the mycelium closer to the glass, appressoria were formed on the conidiophores as well as on the vegetative hyphae. That appressoria are formed as the result of stimulus of contact has been suggested by a number of investigators prior to Dey, among whom are Brefeld (1881), de Bary (1887), Busgen (1893), and Hasselbring (1915).

Penetration occurs by means of an infection hypha arising from the lower side of the appressorium, as has been shown in the case of S. graminum by Mangin (1899) and with S. apii and S. apii graveolentis by Cochran (1932). Similarly the germ tubes formed from other conidial forms as Colletotrichum lindemuthianum, C. circinans, Fusicladium pirinum, Botrytis cinerea, and Stagnospora curtisii cause infection. The entrance of the infection hypha into the cell may occur either by mechanical means, enzymatic action, or a combination of the two. If a dent is present beneath the appressorium at the point of entrance of the penetration tube, a mechanical force is considered to be the method of entrance. This was the conclusion of Blackman and Welsford (1916) with the infection of Vicia by Botrytis cinerea, of Boyle (1921) with infection by Sclerotinia libertiana, and of Waterhouse (1921) with the sporidial infection due to Puccinia graminis. Dey (1919) finding the appressorium often raised above the leaf surface of the host, as well as the presence of a dent at the point of infection, also suggests the mechanical factor in penetration. Using artificial membranes for the study of penetration, several

investigators have concluded that penetration is the result of either a chemical or a contact stimulus occurring by mechanical means. Miyoshi (1895) concludes that due to a chemical attraction germ tubes of Penicillium glaucum and Botrytis cinerea may penetrate artificial membranes, passing through them by mechanical pressure. Brown and Harvey (1927) do not agree to the theory of a chemical attraction as a stimulus to penetration, but do agree that penetration is accomplished mechanically. They conclude that since infection hyphae of B. cinerea will pass through a 40 per cent formalized gelatine membrane and not a 50 per cent membrane, a mechanical force and not an enzymatic action must be responsible for their entrance. They consider that the stimulus of contact causes the formation of infection hyphae.

The presence of a clear area, or an area reacting to stains differently than the remainder of the cell wall, has led to the conclusion that there is a chemical influence, probably enzymatic in nature, which changes the structure of the cell wall, thus aiding the infection hypha in its passage through the cell wall. Smith (1900) suggests this as a method of entrance from his study of the formation of the haustoria in the Erysiphaceae. Brown (1915) showed the disintegration of lettuce tissue by means of an extract of the culture medium on which Botrytis cinerea had been grown. From this he concluded that there was an enzymatic action of the fungus which changed the tissue so that penetration could occur without resistance.

Other investigators as Dey (1919) and Evans (1933) consider

a combination of the two factors, mechanical force and enzymatic action, as the more likely method. Dey believes that a mechanical force is first necessary for the infection hypha of Colletotrichum lindemuthianum to penetrate the cuticle. He observed a clear area in the cell wall around the infection hypha which he considers to be due to the dissolution of the cell wall. Mangin (1899) shows that there is a difference in reaction to cellulose stains in the cell wall around the infection hypha of Septoria graminum, from which he concludes that a material, probably an enzyme, is secreted by the fungus which changes the cellulose wall, thus facilitating penetration.

With Septoria lycopersici dents were occasionally observed in the wall of the epidermal cell beneath the appressorium. Around the infection hypha a clear halo is evident in the wall of the host cell and extending into the cytoplasm with the growth of the infection hypha, which corresponds to the wall around the appressorium and the walls of the internal hyphae. Stains to show a difference in the reaction of the wall around the infection hypha were not used, but no difference was observed with erythrosin, haematoxylin, or with Flemming's triple stain. From these observations a conclusion as to the method by which the hypha enters can not be definitely reached. Although the formation of a dent below the appressorium is the usual basis for the conclusion that a mechanical force is exerted, it seems that there must be a force exerted on the penetration hypha due to the adhesion of the appressorium to the surface of the cell, even though a dent in the epidermal wall is not present.

After penetration has occurred the infection hypha increases in breadth as well as length. There is a clear space about the infection hypha which might be considered as an area in the cell wall and cytoplasm which has been changed due to the action of an enzyme from the hypha, but it corresponds to the wall around the appressorium and the hyphae within the leaf tissue. This would lead to the conclusion that it is the wall of the infection hypha and not a modified area of the cell wall and cytoplasm. A combination of the two factors seems to give a more adequate explanation of the method by which penetration is effected. It seems entirely possible that the cell wall may be changed around the point of infection by the secretion of a material, possibly enzymatic in nature, which would facilitate the entrance of the infection hypha. Yet there is probably a mechanical force exerted by the adherence of the appressorium to the cuticle which aids the penetration tube in piercing the cuticle and cell wall. The change in the nature of the wall would make penetration less difficult.

As the infection hypha increases in length the appressorium becomes vacuolate. This suggests that the contents of the appressorium flow into the penetration hypha until it is well established and can absorb the necessary materials for further development.

There is no visible effect on the host cell up to this point. There is no thickening of the cell wall as a possible attempt of the cell to prevent infection as reported by Smith (1900) in the formation of the haustoria of the Erysiphaceae,

in which the host cells, in some cases, formed additional cellulose layers on the walls, often filling the entire cell. The formation of callosities by the host cells was described by Young (1926) as the result of infection by a number of fungi as Alternaria, Colletotrichum, and Diplodia. The cytoplasm appears to be normal amd the nucleus has not been observed to move towards the point of infection as described by Pearson (1931) in the corn cells infected by Gibberella saubinetii.

The infection hypha and the first branches arising from it are intracellular. Usually two branches are formed, enlarging and extending for the breadth of the epidermal cell. From this intracellular hypha branches arise, penetrating the host cell wall and continuing to grow in the intercellular spaces. Killian (1920) also shows that the secondary hyphae, arising from the hypha which has entered the epidermal cell, grow intercellularly. After entrance into the intercellular spaces the hyphae may be present singly or in groups. When growing in the limited spaces in the palisade parenchyma they are often present in fasicles, whereas in the spongy layer where the intercellular spaces are larger and more numberous the hypha are usually single, at least not in closely associated groups. The path of the hypha in the tomato tissue agrees with the descriptions of Klebahn (1907) and of Cochran (1932) with other species of Septoria. Levin (1916) and Killian (1920) describe the formation of shallow haustoria from the intercellular hyphae

by S. lycopersici. None were observed in any of the sections examined. The hyphae spread rapidly through the tissue, especially the spongy layer, so that lesions formed from infections on the lower side of the leaf are broader than those formed from the upper side of the leaf. Here a narrow space between the palisade cells which must be traversed by the hyphae and the elongated nature of these cells prevents a rapid spread. These observations agree with those of Levin and Killian.

Death of the host cells does not occur ahead of the growth of the hyphae, or coincident with their passage through the tissue, but after the fungus is established. This is contrary to the opinion of Cochran (1932) who finds the hypha of S. apii and S. apii graveolentis beyond the area of the collapsed cells but not beyond the dead cells. He believes that the death of the cells is due to a secretion of a toxic material by the fungus, which kills the cells for some distance beyond the location of the hyphae. Boyle (1921) finds that a similar condition exists in areas infected by Sclerotinia libertiana. If the death of the tomato cells, due to the presence of the hyphae of Septoria lycopersici, is due to an enzymatic or toxic material secreted by the fungus, the effects are not immediate. Likewise the effect is delayed if the death of the cells is due to the diffusion of the products of decomposition of other host cells which have been killed. Death as the result of a secretion of some material by the fungus hyphae seems the more likely cause of the injury to the cells. There is no reaction of the

host cell to the presence of the invading hyphae such as Boyle (1921) reported with lettuce cells when invaded by Sclerotinia libertiana and by Pearson (1931) with corn cells in the presence of the hyphae of Gibberella saubinetii. In both cases the nucleus was observed to move within the host cell toward the infected area. Neither the plastids nor the nucleus were observed to swell. Neither was there any evidence of disorganization of either the nucleus or the plastids before the protoplasm became an undifferentiated black mass. Killian (1920), however, describes a shrinking of the nucleus of tomato cells in areas infected with Septoria lycopersici. Under natural conditions these masses of cells appear brown. Boyle (1921) describes the swelling and the disorganization of the nucleus of the lettuce cell as the result of the presence of Sclerotinia libertiana. The greater affinity for stains shown by dead tomato cells has been described by Killian (1920). Pearson (1931) has also described it for corn cells infected with Gibberella saubinetii. In the case of the tomato cells the cytoplasm, with the nucleus and plastids following, is the first to show this effect, staining more darkly with haematoxylin than normally and appearing to be granular. The density of the stain increases until it is impossible to distinguish between the nucleus, plastids and the cytoplasm. The death of the cells must be due to the secretion of a material by the fungus hyphae, either enzymatic or toxic in nature, which diffuses through the cell wall and into the cytoplasm, the least dense portion of the protoplasm and the first to

show any effect, and finally into the plastids and the nucleus. The cell walls do not appear to be affected. Under natural conditions the center of the leaf spot becomes dry and brittle. This region corresponds to the area of the collapsed and shrunken cells. Whether there is a loss of water due to evaporation, or due to its absorption with soluble nutrient material by the fungus can not be said. Probably both of these are responsible in part for the collapse of the cells and the drying of the tissue.

The hyphae may continue to grow through the mesophyll, forming larger or smaller lesions as the case may be. The size of the lesion depends largely on the number of infections in a given area, there being usually several small ones or one large one. There seems to be a repellent influence between the mycelia of adjacent lesions preventing further growth of the hyphae. The host tissue does not show any modification in the healthy cells surrounding the lesion which would prevent the further growth of the hyphae. Cunningham (1928a, 1928b) describes the formation of a cicatrice in the host tissue around the invaded area which prevents the further spread of the hyphae. Such is the case in lesions caused by Coccomyces sp. Sphaceloma fawcettii, and Cercospora beticola. Such layers were not formed by any of the host plants infected with Septoria petroselini, S. podophyllina, S. acerina, and others of this genus.

The hyphae seem to be more numerous at the margin of the collapsed area, due to the greater number of branches from the first hyphae growing in the leaf. These may be traced

back from section to section and their connection with those in the center of the lesion shown. The number of hyphae present in the collapsed area increases prior to the formation of the pycnidium, when several adjacent hyphae branch forming a knot, the pycnial primordium. The vegetative hyphae, and those which will later make up the pycnidium, may be easily distinguished between since the latter are coarse, often more septate and stain more deeply with haematoxylin than do the vegetative hyphae. A physiological change must occur which results in these differences.

The primordium of the pycnidium of Septoria lycopersici is formed from the intercalary division and increased branching of several adjoining hyphae. This places it, in regard to its method of initiation, in a class intermediate between the two groups as described by de Bary (1887). He classifies them in two groups as those arising meristogenously, that is arising from the intercalary division of an hypha; and those arising symphyogenously, that is arising by the branching of adjacent hyphae. Kempton (1919) finds the intermediate condition the more prevalent. He also shows that members of the same genus, and also of the same species, do not all follow the same plan. This is the case in the genus Septoria. Pycnidia of S. polygonorum may arise either meristogenously or symphyogenously, or may show a combination of the two. Those of S. helianthi and of S. scrophulariae were found to arise meristogenously. Levin (1916) describes the intermediate condition for S. lycopersici with which the evidence presented here agrees.

The subsequent development of the pycnidium follows the plan as outlined by Levin (1916) for S. lycopersici, and by Dodge (1923) for Phyllostictina carpogena, Sclerotiopsis concava, and Schizoparmi straminae. The first stage is the formation of the undifferentiated ground tissue; the second an increase in the complexity of this tissue with the formation of the peridium, the central cavity, the sporogenous tissue, and the ostiole when present; and the third the formation of the spores. Septoria lycopersici differs from this plan in that the ostiole is not formed until after the spores are formed, since it is due to the pressure of the spores against the peridium which causes it to break, thus forming the ostiole. In Schizoparme straminea as described by Dodge the ostiole is the result of a disorganization of the central tissue of a knob formed on the top of the primordium, which with the surface buffer tissue burrows its way through the host tissue, finally reaching the surface. The disintegration of the central tissue of the pycnidium of Schizoparme straminea progresses downward from the top, beginning in the knob which is to be the ostiole. In Septoria lycopersici the disorganization appears to begin in the center and to progress outward, leaving a narrow layer next to the peridium which becomes the sporogenous tissue.

The spores are formed by budding from the sporophore even before the cavity is completely empty. Dodge found that in Phyllostictina carpogena the spores were rarely formed before the cavity was complete. The central cavity in these forms appears very early in the development of the primordium,

while it is still little carbonized. In Septoria lycopersisi the peridium is mature before disorganization begins. In Phyllostictina sporophores, called protosporophores, may be formed before the cavity is complete, but usually they disintegrate. The spores are formed in the pycnidium of Septoria lycopersici by budding from the sporophores. Often three are produced simultaneously by one sporophore. They are apparently pinched off when they have become mature. Another layer of spores is then formed. In small pycnidia two distinct layers of spores are often evident.

SUMMARY

Spores of Septoria lycopersici germinated by forming several germ tubes, often one or two from each cell. The germ tubes grew for a longer or shorter distance over the epidermal cells of the tomato, finally forming appressoria.

The penetration of the fungus into the host tissues occurred by the entrance of an infection hypha, rising from the under side of the appressorium, through the wall of the epidermal cell. The germ tubes did not enter the leaf tissue through the stomata.

The first hypha within the host tissue was shown to be intracellular, within the epidermal cell. Branches arising from this intracellular hypha penetrated the cell wall and continued to grow intercellularly, through the intercellular spaces. The formation of haustoria was not observed.

Infection was shown to occur from either surface of the leaf. Lesions formed from infections occurring on the lower epidermis were at first broad and shallow, whereas those formed

on the upper side of the leaf were narrow and extended more deeply into the tissue. This is due to the arrangement of the host cells, since the growth of the hyphae in the spongy parenchyma may extend farther than in the palisade parenchyma in the same period of time. As the leaf spots increased in size, the mesophyll and epidermal layers were affected.

A mature leaf spot was shown to be made up of a central area of collapsed cells and fungus hyphae, a margin of semi-affected cells and hyphae, surrounded by an area in which the hyphae were present but the host cells were not affected. A cicatrice was not formed by the host tissue.

Death of the host cells occurred after the hyphae were established in the leaf tissue, not ahead of their growth or coincident with their passage through the mesophyll. The first sign of injury to the cell was a granular appearance of the cytoplasm. Later the cytoplasm, plastids, and nucleus showed a greater affinity for stains, appearing as a black undifferentiated mass.

The primordium of the pycnidium was formed by the intercalary division and branching of several adjacent hyphae. These continued to branch and interweave, forming a large mass of undifferentiated tissue. When about one third mature size the hyphal knot differentiated into a well defined peridium and central cavity, filled with hyphae which later disintegrated.

The sporogenous layer was formed as a continuous layer within the peridium, the sporophores forming a palisade-like layer perpendicular to the peridium. Spores were formed by

budding from the sporophores. One to three may be formed at one time from each cell.

The ostiole was formed by the pressure of the spore mass against the peridium causing it to break, thus allowing the exudation of the spores.

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Explanation of Plates

Figures 1, 2, and 3 were made with a Spencer 1.9 achromatic objective and 10X ocular; figures 4, 9, 15, 17, 18, 19, 21, 22, and 23 with a Zeiss 2mm. apochromatic objective and Spencer 10X ocular; figure 12 with a Zeiss 2mm. apochromatic objective, and 10X compensating ocular; figures 11, 13, 14, and 20 with a Spencer 4mm. achromatic objective and 10X ocular; figures 5, 6, 7, 8, and 10 with a Zeiss 2mm. apochromatic objective and 15X compensating ocular; figure 16 with a Spencer 16 mm. achromatic objective and 10X ocular. All drawings were made with the aid of the camera lucida. All photomicrographs were made with a Zeiss photomicrographic camera.

Plate 1.

Figure 1. A germinating spore of Septoria lycopersici lying on the epidermis of tomato with the germ tubes growing away from the stoma. X 1425.

Figure 2. A germ tube growing across a stoma, without entering. X 1425.

Figure 3. The face view of a stoma with three germ tubes, which have grown across without entering. X 1425.

PLATE I

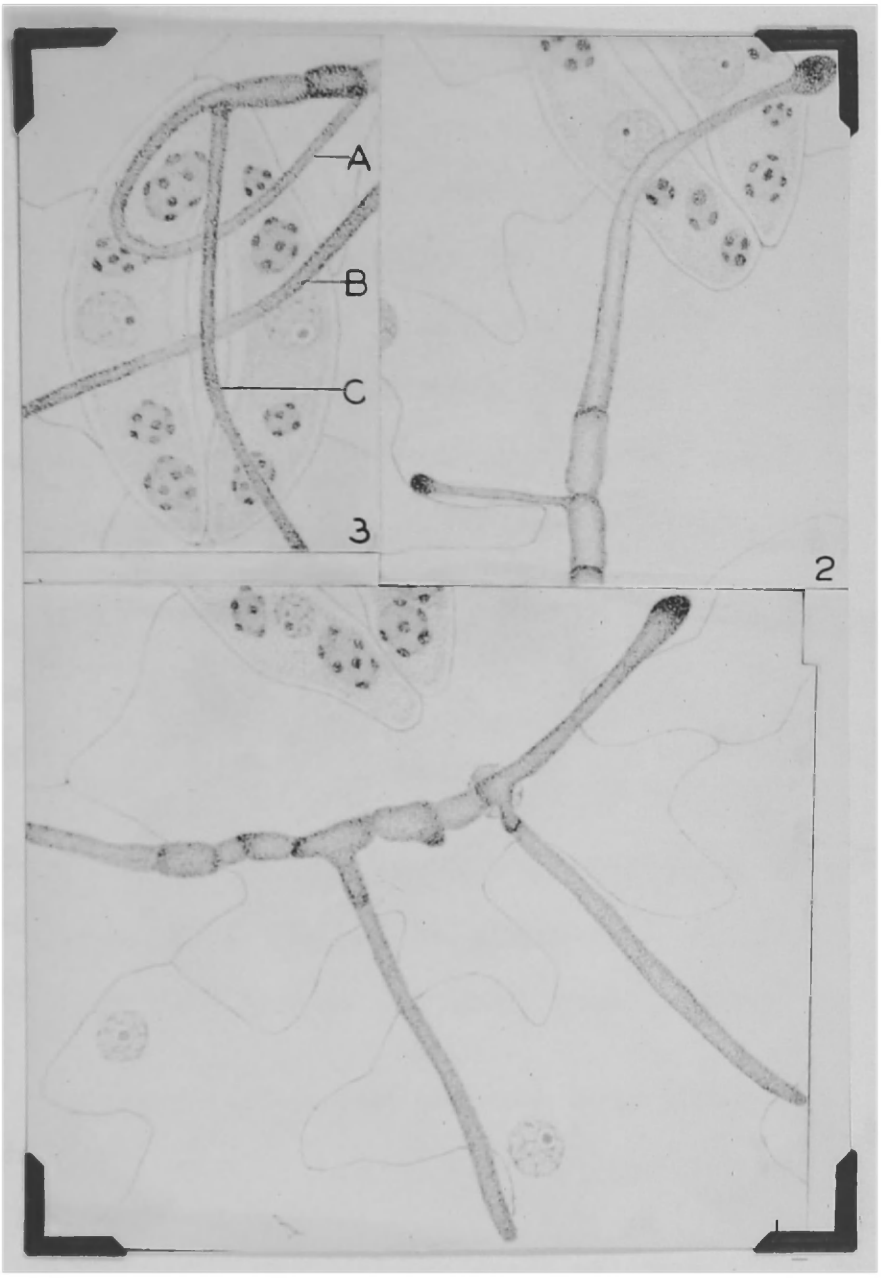


Plate 2.

Figure 4. A germ tube and appressorium lying on the surface of an epidermal cell. X 1467.

Figure 5. Face view of an appressorium lying on the tomato epidermis. X 2109.

Figure 6. An appressorium formed on a short germ tube from the spore. X 2109.

Figure 7. A penetration hypha arising from the appressorium, passing through the wall of the eipdermal cell of tomato. X 2109.

Figure 8. An appressorium with its penetration tube, which has passed through the cell wall and extended into the cytoplasm. X 2109.

Figure 9. An intracellular hypha with a branch which has penetrated the wall of the eipdermal cell and entered the underlying intercellular space. X 1467.

Figure 10. An intracellular hypha with a branch which has penetrated the tomato cell wall and is growing into the underlying intercellular space. X 2109.

PLATE 2

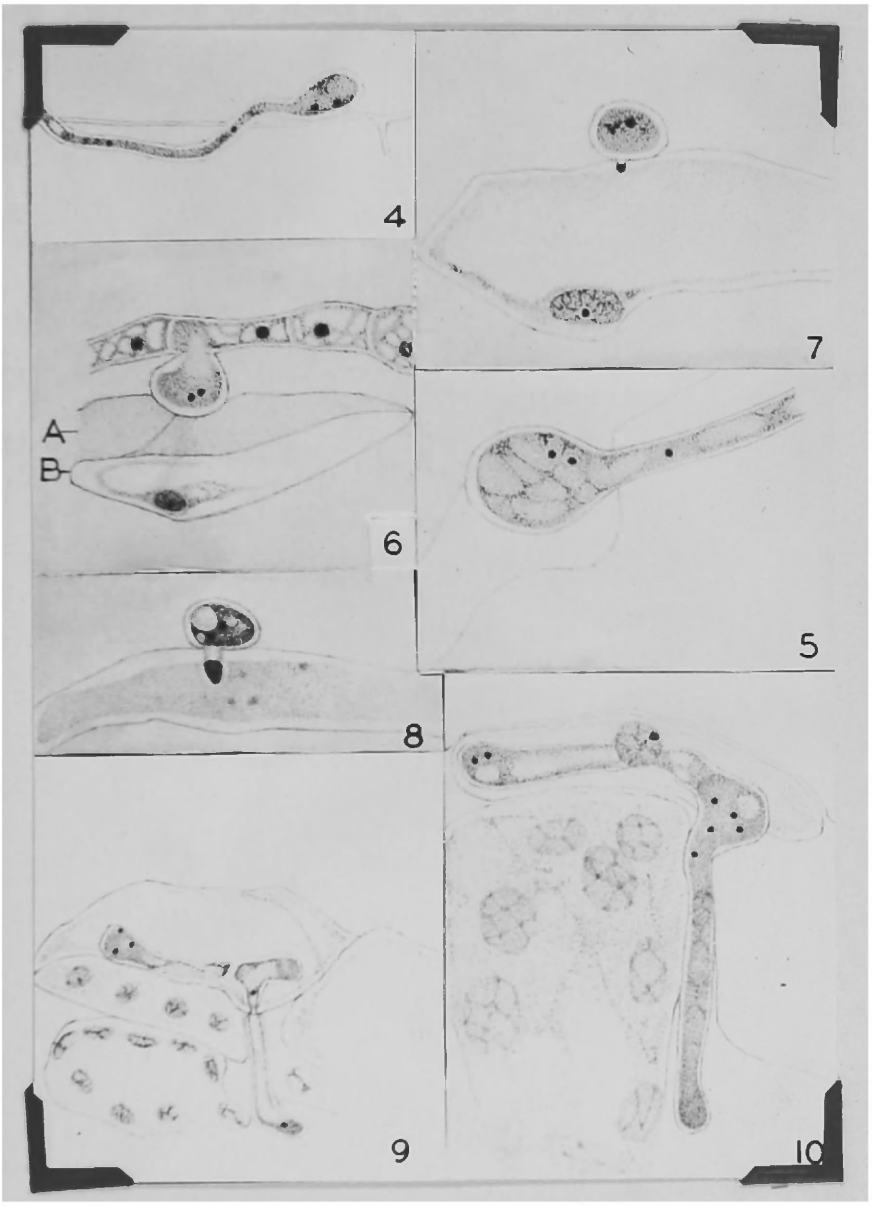


Plate 3

Figure 11. The infection of the tomato leaf occurring at the lower epidermis showing the collapse of the lower epidermal cells and the adjacent spongy parenchyma cells, with the hypha of Septoria lycopersici extending to the upper epidermis. X 485.

Figure 12. An intercellular hypha entering the spongy parenchyma from an infection of the lower epidermis. X 952.

PLATE 3

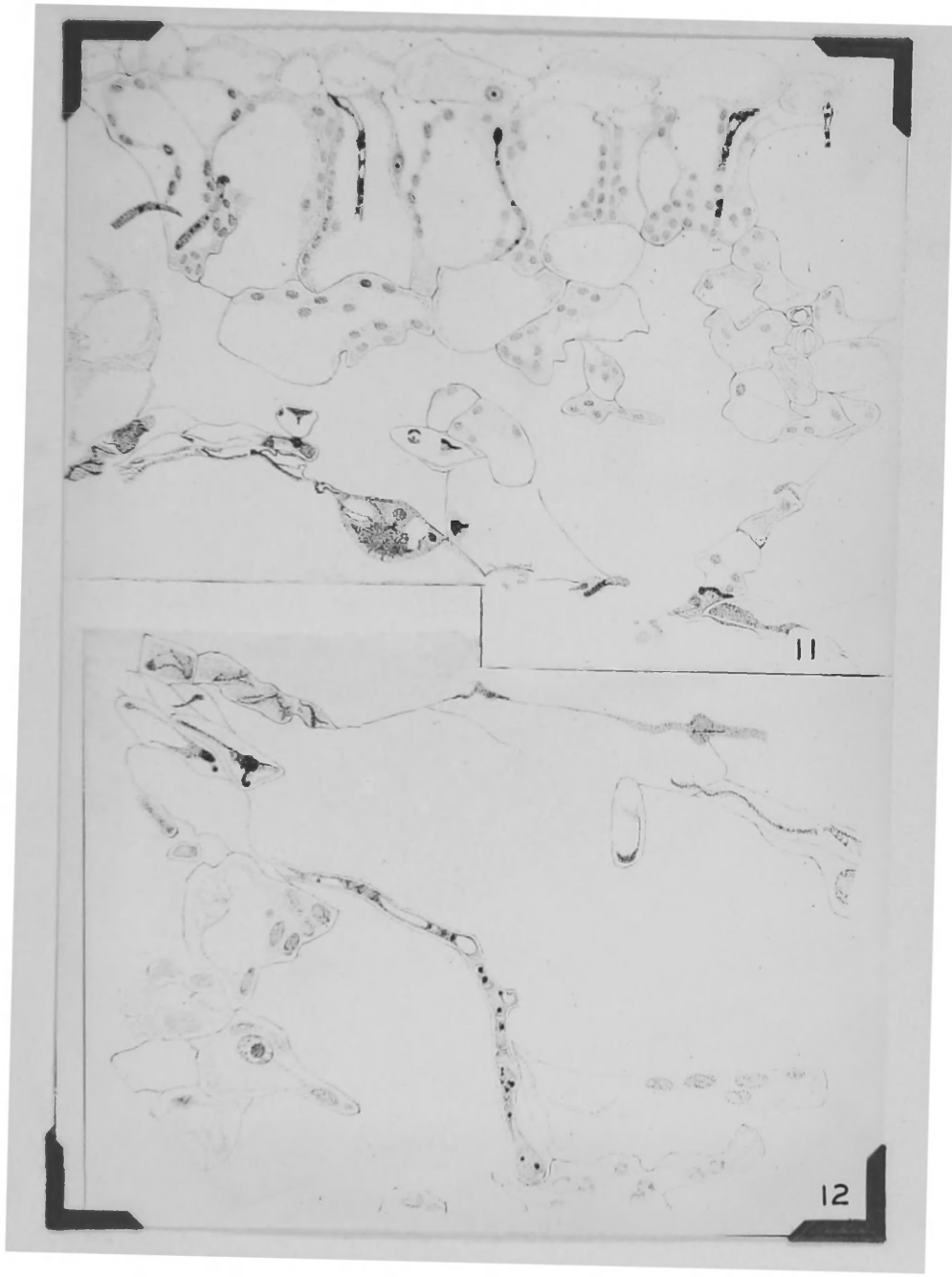


Plate 4.

Figure 13. Infection of the leaf occurring at the upper epidermis with the collapse of an epidermal cell and the underlying palisade and spongy parenchyma cells. X 485.

Figure 14. Young leaf spot with the mesophyll and epidermal layers involved in the collapse of the cells. X 485.

Figure 15. An epidermal and palisade parenchyma cell showing an intermediate stage in the death of the cell. X 1067.

PLATE 4

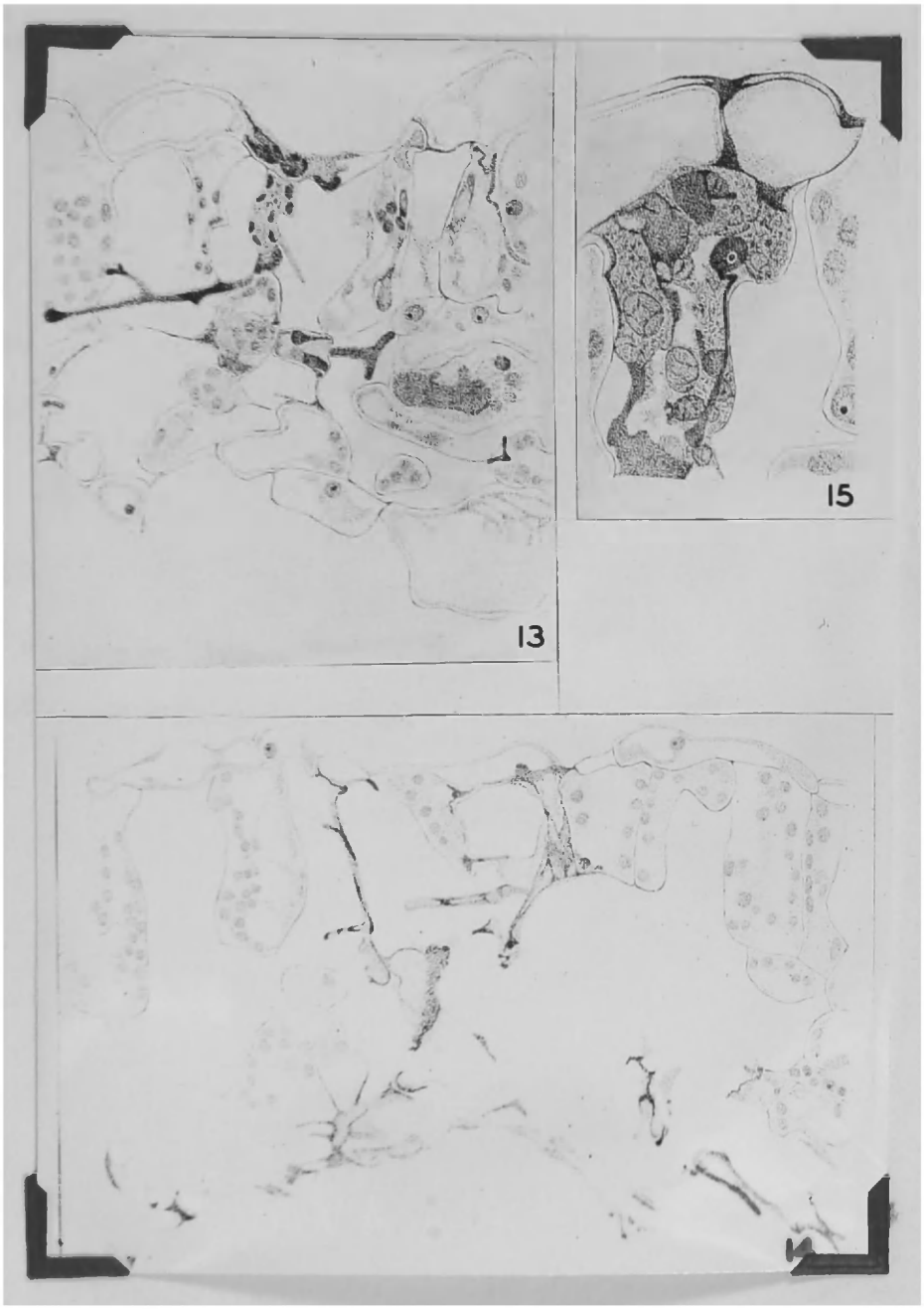


Plate 5.

Figure 16. A diagram of a mature leaf spot from the center of the collapsed area out to the farthest extent of the hyphae, showing the position of figures 17, 18, and 19. X 106.

Figure 17. Section A from the collspsed area of a mature leaf spot showing fragments of leaf cells, fungus hyphae, and a pycnial primordium. X 976.

Figure 18. Section B. from the margin of the collapsed area showing affected, semi-affected, and uninjured host cells and the fungus hyphae. X 976.

PLATE 5

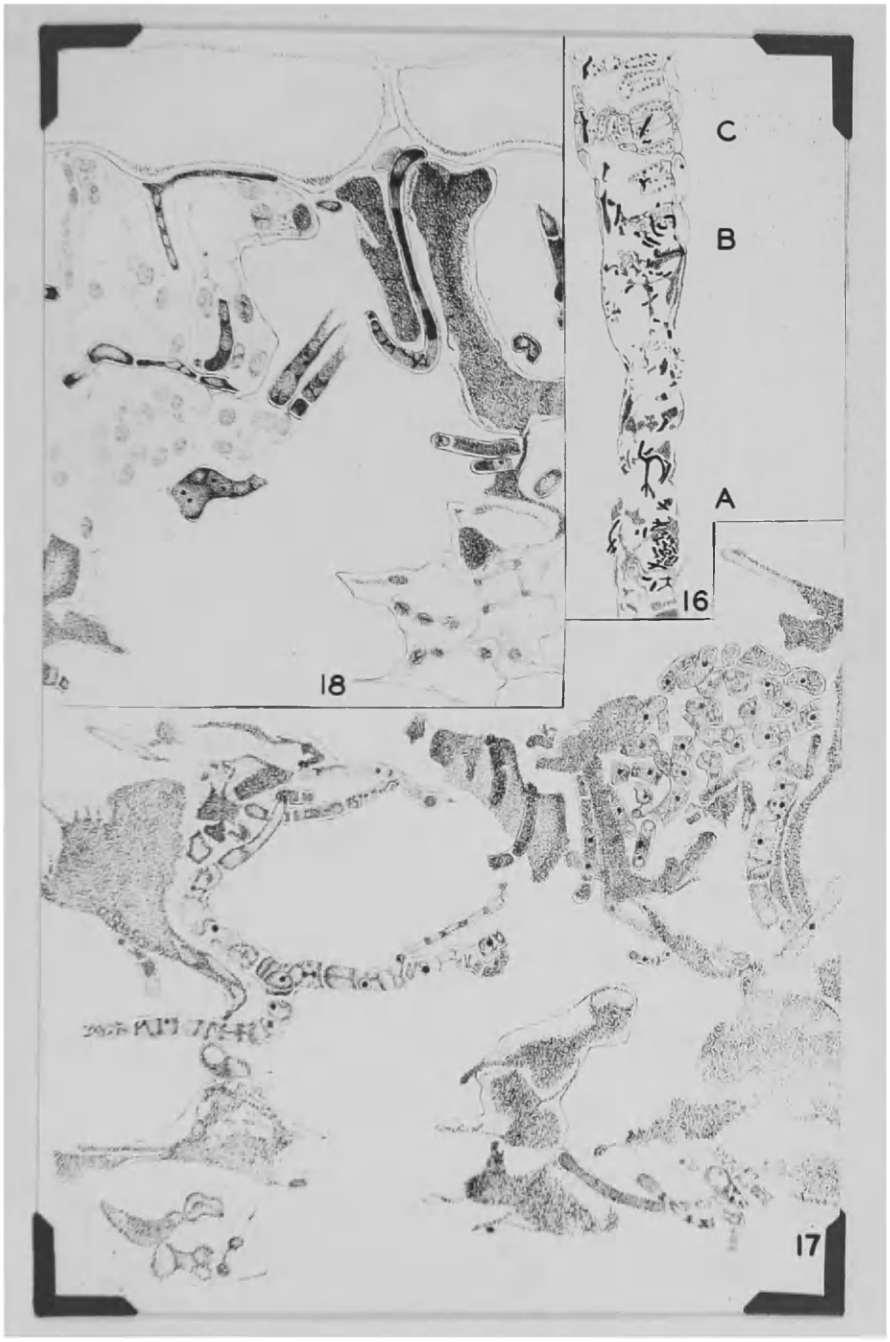


Plate 6.

Figure 19. Section C showing the farthest extent of the hyphae from the collapsed area and the uninjured host cells. X 1198.

Figure 20. Cross section of a young leaf spot showing the collapse of the mesophyll and epidermal layers and the presence of the fungus hyphae. X 485.

PLATE 6

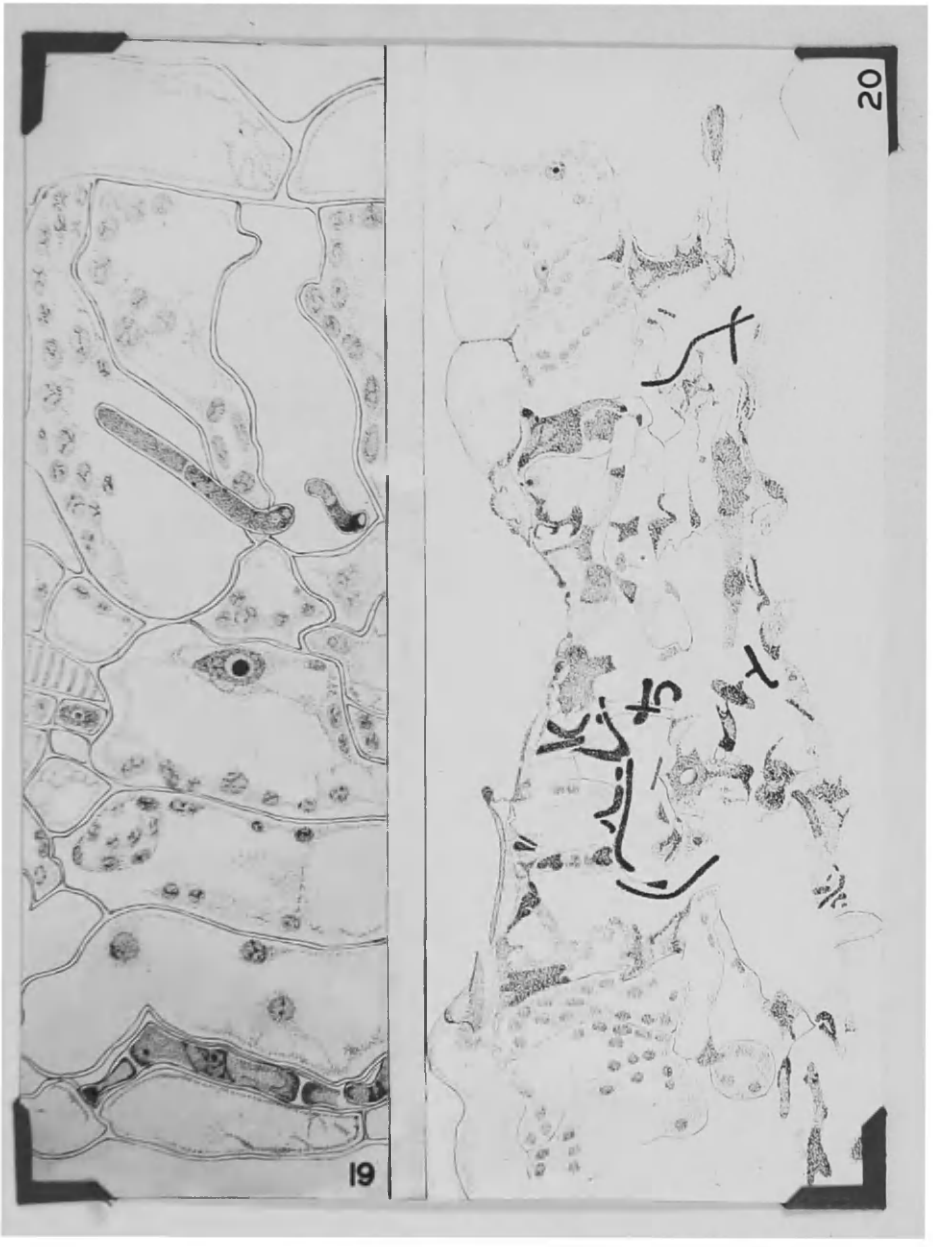


Plate 7.

Figure 21. A pycnical primordium. X 1280.

Figure 22. Pycnical primordium showing the increase in ground tissue. X 1280.

Figure 23. Section from a mature pycnidium showing the peridium and hymenium. X 1280.

PLATE 7

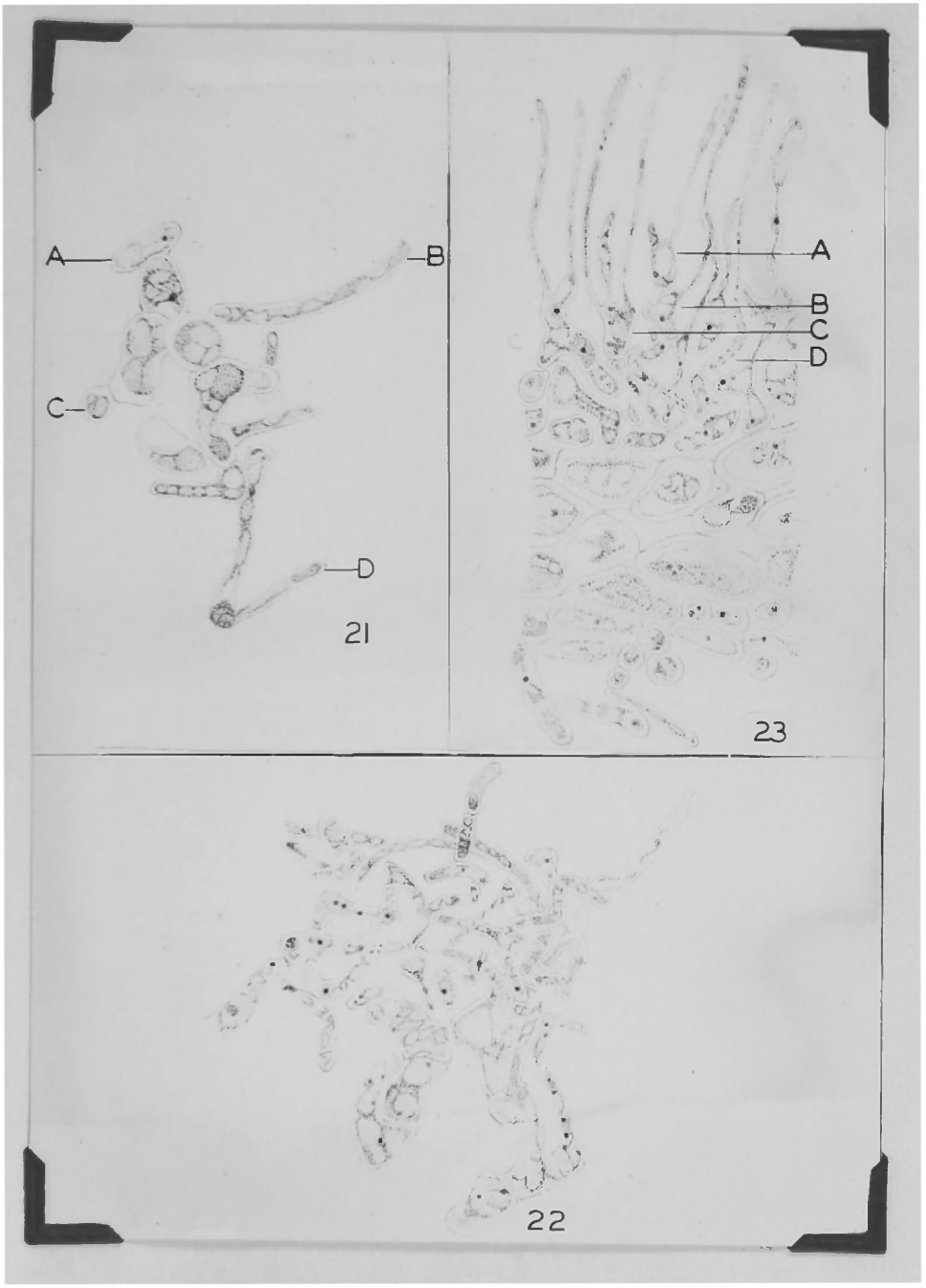


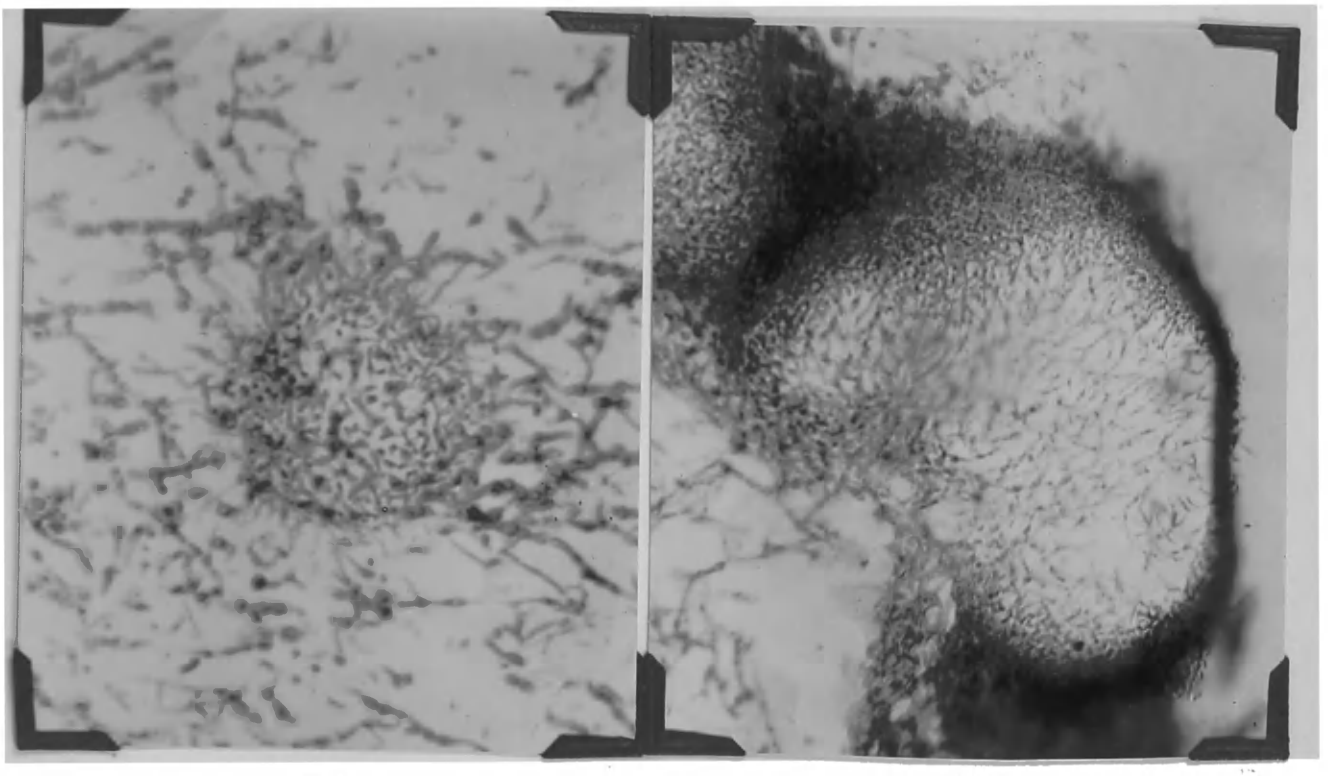
Plate 8.

Figure 24. A primordium showing an increase in the ground tissue and the presence of the hyphae which will form the peridium. X 362.

Figure 25. Young pycnidium showing a well defined peridium and central net work of hyphae. X 362.

Figure 26. Pycnidium showing an early stage in the disorganization of the central tissue. X 274.

PLATE 8



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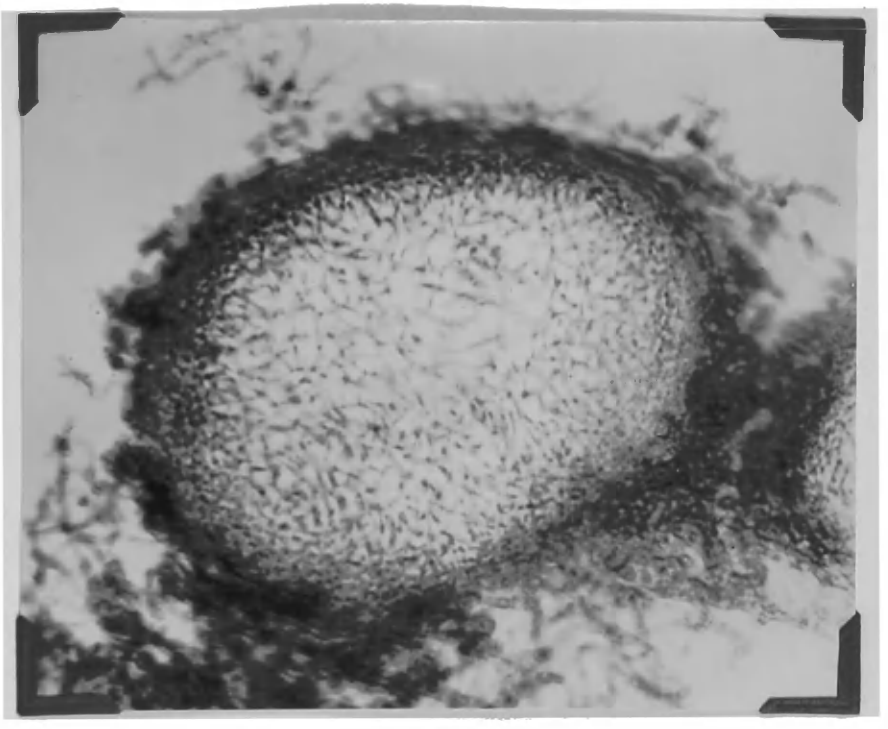

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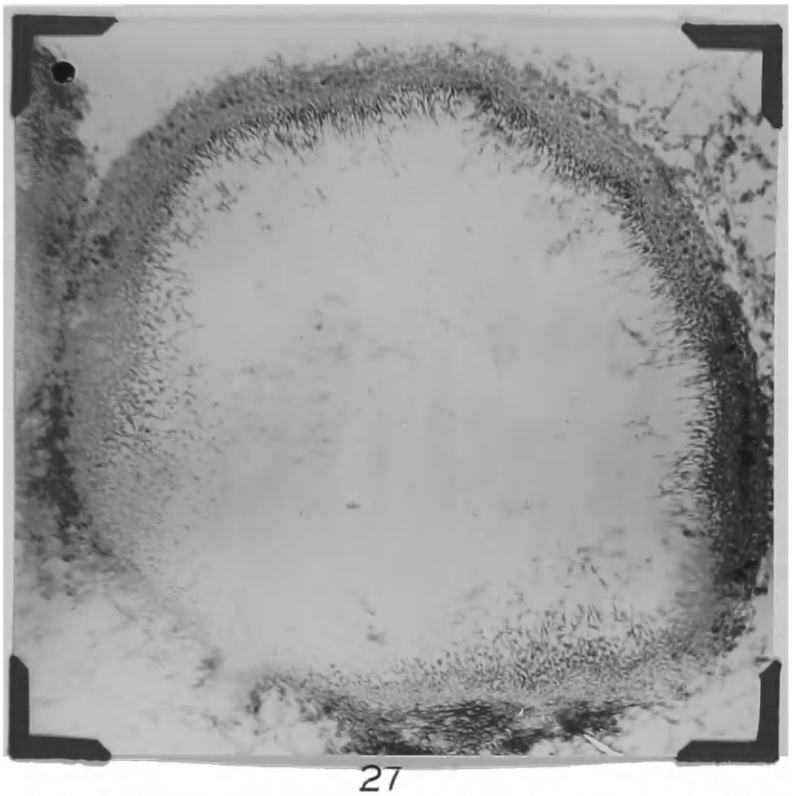
Plate 9.

Figure 27. Pycnidium showing the disorganization of the central tissue. X 183.

Figure 28. A pycnidium showing spore formation. X 183.

PLATE 9



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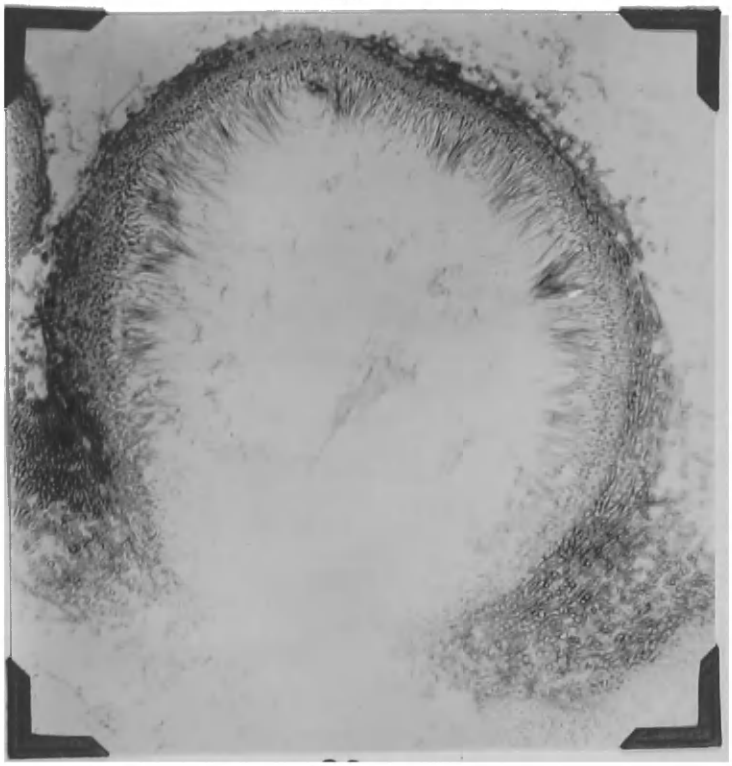

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Plate 10.

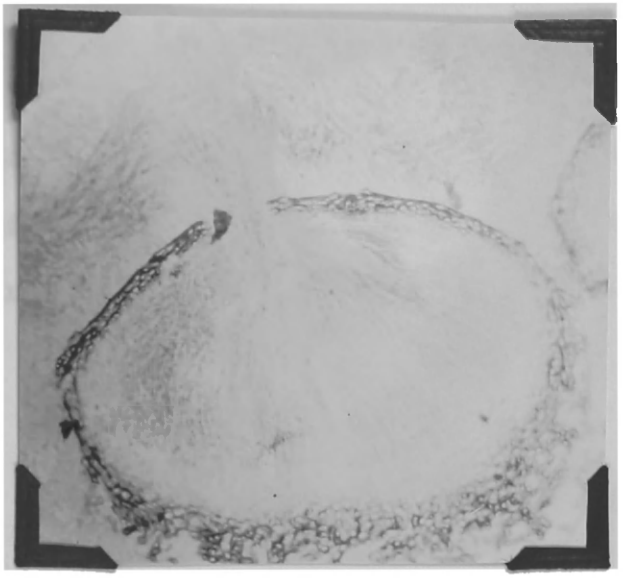
Figure 29. Mature pycnidium with spores exuding from the ostiole. X 200.

Figure 30. Pycnial primordium in the tomato tissue. X 362.

Figure 31. Mature pycnidium in the leaf tissue showing the formation of the ostiole. X 362.

Figure 32. Mature pycnidium in the leaf tissue showing the exudation of spores. X 362.

PLATE 10



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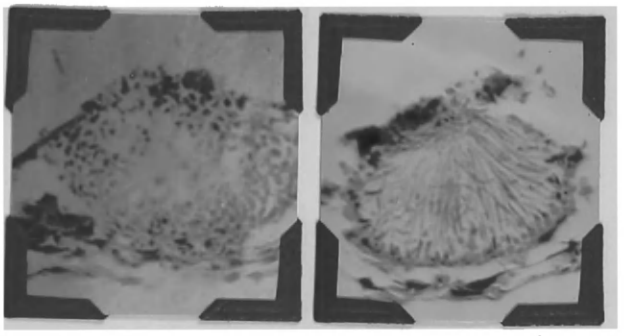

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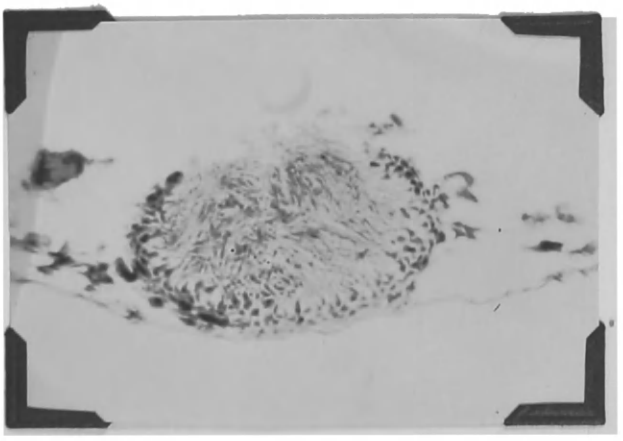

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Acknowledgment

The writer wishes to acknowledge her indebtedness and appreciation to Dr. J. B. S. Norton and Dr. Ronald Bamford for their criticism, suggestions, and constant interest throughout the progress of this work.