ฮิสโตวิทยาของลูกมะเขือยาวที่เป็นโรคเกิดจากเชื้อรา *Phytophthora parasitica*

Histopathology of Eggplant Fruits Infected by *Phytophthora parasitica*.

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This study is a portion of the author' M.S. Thesis entitled "Phytophthora Fruit Rot of Eggplant" prepared under the advisorship of Dr. Gill G. Divinagracia in the University of the Philippines.

ABSTRACT

Approximately 3-week old eggplant fruits of Dumaguete and Long purple strains were inoculated with drops of zoospore suspension of *Phytophthora parasitica*. In Dumaguete strain of eggplant fruits, zoospores germinated on the surface after 25 minutes and entered the hosts after 2 hours. The fungus infected all tissues, except the xylem vessels. It spreads inside intra- and intercellularly and did not kill cells in advance of the hyphae. No histologic defense reaction was observed. The first symptom of infection on young fruit became evident 18 hours after inoculation.

In Long purple strain of eggplant fruits, a corky layer was formed below the infected region in response to infection by the pathogen.

A fruit rot disease of eggplant *(Solanum melongena* L.*)* caused by *Phytophthora parasitica* Dastur was reported from Japan by Tanaka in 1917 as *P. melongenae* Sawada (10). It was reported from the Philippines in 1918 (6), from the United States in 1924 (5), from Java in 1949 (7), and from Greece in 1925 (8). It may be present in all eggplant-producing countries of the world. The disease infects the fruit in all its stages of development and causes considerable damage. Fruits of eggplant near or in contact with the soil become readily infected.

Research conducted so far have not probed in detail the histopathology of *Phytophthora*-infected eggplant fruit. Several authors reported that the fungus penetrated freely the cell wall of uninjured eggplant fruits (3,6,10). The only report on the anatomy of the host-parasite relationship was made by Tanaka (10) who stated that hyphae were intercellular in the host tissue.

Zoospores may be one of the agents of infection of fruits above the ground and spread of the pathogen in the soil. In order to have a better understanding of the disease and to have a better basis for effective control measures, a study on the mode of penetration and development of *Phytophthora parasitica* in eggplant fruit with zoospores as inocula is imperative.

This work was conducted to determine the mode of penetration and the histopathological development of *P. parasitica* in eggplant fruit.

Materials and Methods

Pathogenicity test. Eggplant fruits (Dumaguete strain) were surface sterilized and inoculated with drops of zoospore suspension (50,000 zoospores per ml.) of the4isolates of *P.parasitica*. The zoospores were produced by chilling sporangia at 8 to 16 C in a few ml. of distilled water for

20 minutes and transferring them at room temperature (26 to 30 C). Sporangia were induced by growing the fungus on plantes of oatmeal agar for 4 to 7 days. The resulting mycelial growth was stripped off directly from the medium. Portions of the mycelium were placed inside sterile Petri dishes containing to 3 ml. sterile distilled water and incubated for 2 to 5 days at room temperature (26 to 30 C) for sporangial formation. The plates were exposed to continuous white light during the incubation period. The inoculated fruits were kept in stender dishes lined with moist tissue paper for 3 days at room temperature (26 to 30 C).

Mode of ingress and internal spread. Approximately 3-week old eggplant fruits of Dumaguete and Long purple strains were inoculated in moist chambers by the method just described under pathogenicity test. Fruit disc samples were taken after inoculation at 1 hour interval for the first 12 hours, 6-hour interval for the next 24 hours and 1-day interval up to the first appearance of the symptom.

Whole mount sections were prepared by cutting very thinly longitudinal sections of the inoculated fruit surface. They were decolorized in a saturated chloral hydrate solution for 2 days and stained for 30 minutes in lactophenol containing 0.1 % aniline blue. Afterwards, they were rinsed and mounted in clear lactophenol.

Cross, tangential and longitudinal sections were cut by the freezing and rotary microtomes. Sections on the freezing microtome were cut 25 to 35 microns thick and then stained with lactophenol cotton blue. Tissues cut in paraffin were fixed in FAA, dehydrated in a tertiary butyl alcohol series, and embeded in paraffin. Sections were cut 12 to 16 microns thick, stained and mounted in Canada balsam. Several stain combinations were used. They were safranin-fast green, tannic acid-ferric chloride safranin (4), Pianeze 111 B (11) and thionin-orange G (9).

Results

Pathogenicity test. Eggplant fruits inoculated with the different isolates produced typical fruit rot symptoms (Figure 1). The first symptoms appeared 18 hours after inoculation on 7 to 9 day old fruits and 36 hours on 14 to 16 day old fruits.

Mode of ingress and internal spread. Lactophenol containing 0.1 % anilin blue was best suited for demonstrating penetration in whole mount sections, and safranin-fast green and thionin-orange G stain combinations for demonstrating penetration and internal spread of *P. parasitica* in paraffin sections.

Zoospores germinated on the fruit surface 25 minutes after inoculation. They penetrated the eggplant fruit as early as 2 hours after inoculation. Penetration was direct and occurred either on the walls between two adjacent cells or through the outer walls of cells themselves (Figure 2). Penetration by long germ tubes was usually preceded by the formation of appresorium at the point of entry. Penetration without an appresorium or with a slight swelling was observed mostly on short germ tubes. The point of entry of the germ tube was surrounded by a yellowish to brownish discoloration. Hyphae spread intra- and intercellularly from the epidermal cell to the sub-epidermal tissues (Figure 3A). They colonized cortical tissue as well as parenchyma cells near xylem vessels. The fungus formed prominent vesicles especially in the cortical cells (Figure 3B), but usually only a slight swelling of the hypha was evident where wall contact was made. The diameter of the intracellular hyphae was usually greater than intercellular ones. The size of the latter varied depending upon the size of the intercellular spaces they occupied. The nuclei of the host cells were usually located close to the point of contact with the hyphae (Figure 4).

The fungus killed the cells only after it had penetrated them. Three hours after inoculation

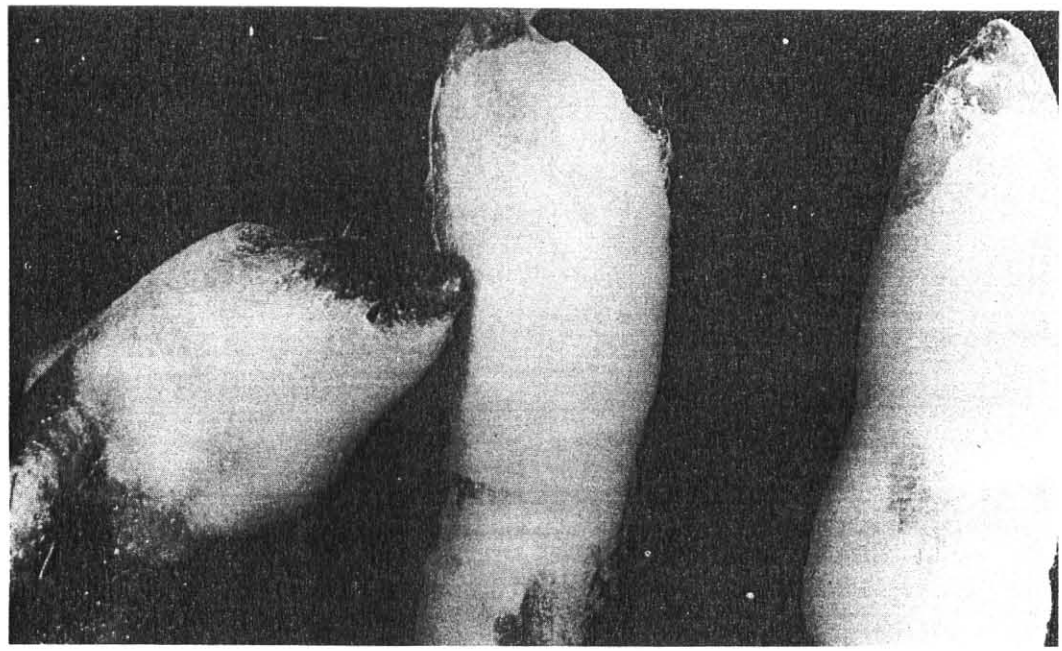

Figure 1. Typical fruit rot symptoms incited by *Phytophthora parasitica* on about 8-day old eggplant fruits (Dumaguetes strain) 2, 3 and 4 days (left to right) after inoculation (approximateiy 1x).

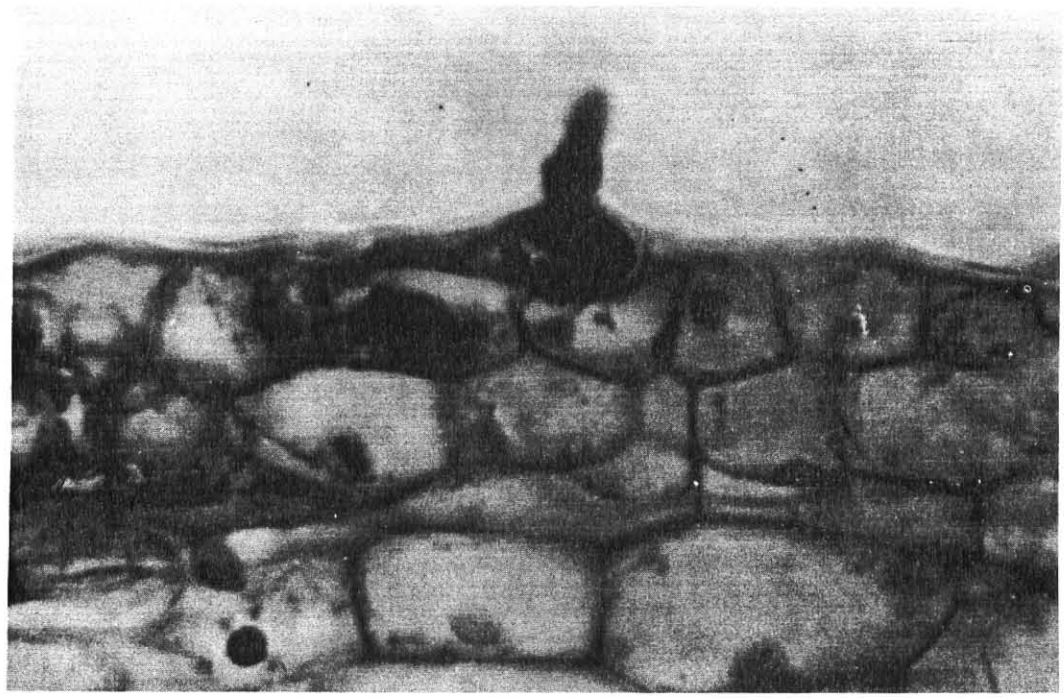

Figure 2. Portion of a cross section of an eggplant fruit showing direct penetration of a germinating zoospore of *Phytophthora parasitica* 2 hours after inoculation (approximately 800x).

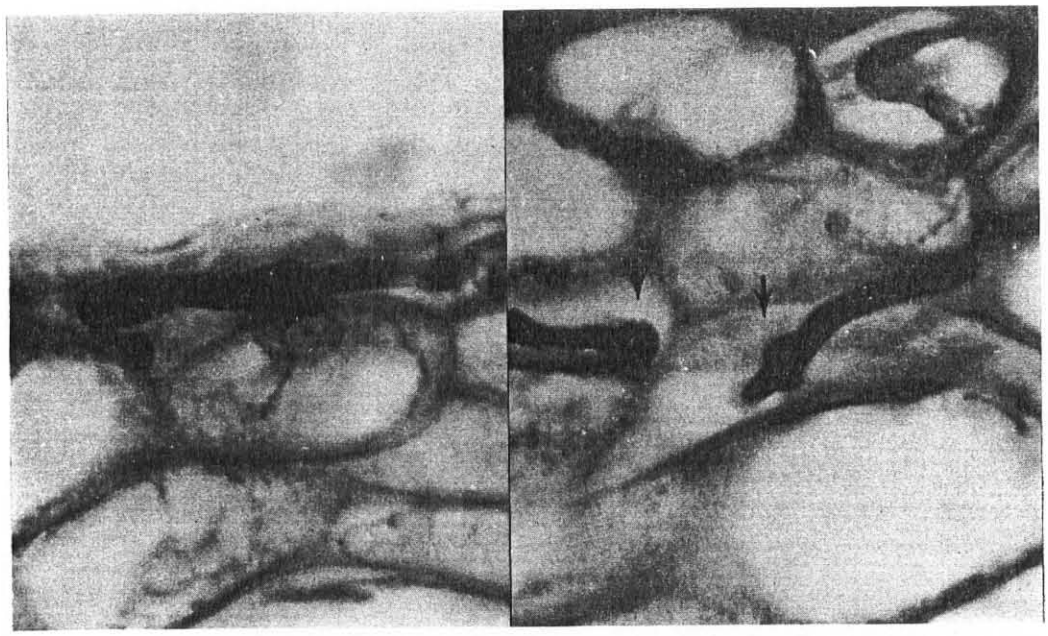

Figure 3. Portion of cross section of an eggplant fruit infected by *Phytophthora parasitic* showing A) Hyphae inside epidermal cells and B) Hyphae inside cortical cells showing (arrows the formation of vesicles (approximately 800x).

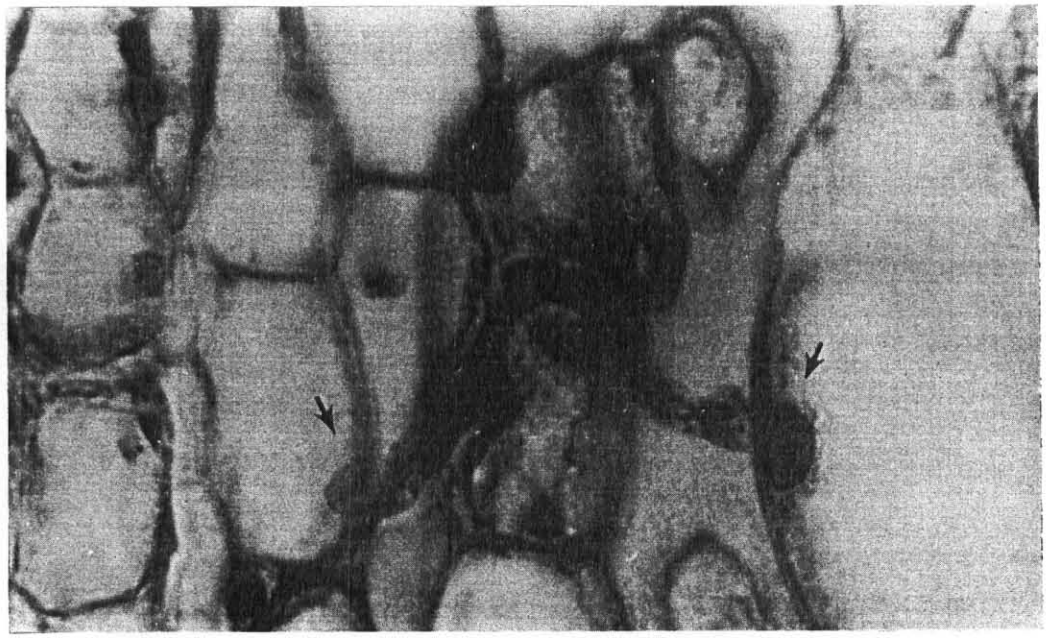

Figure 4. Portion of a cross section of an eggplant fruit infected by *Phytophthora parasiti* showing nuclei (arrows) the host located at the point of contact with the hyphae and the granul contents of infected cells (approximately 800x).

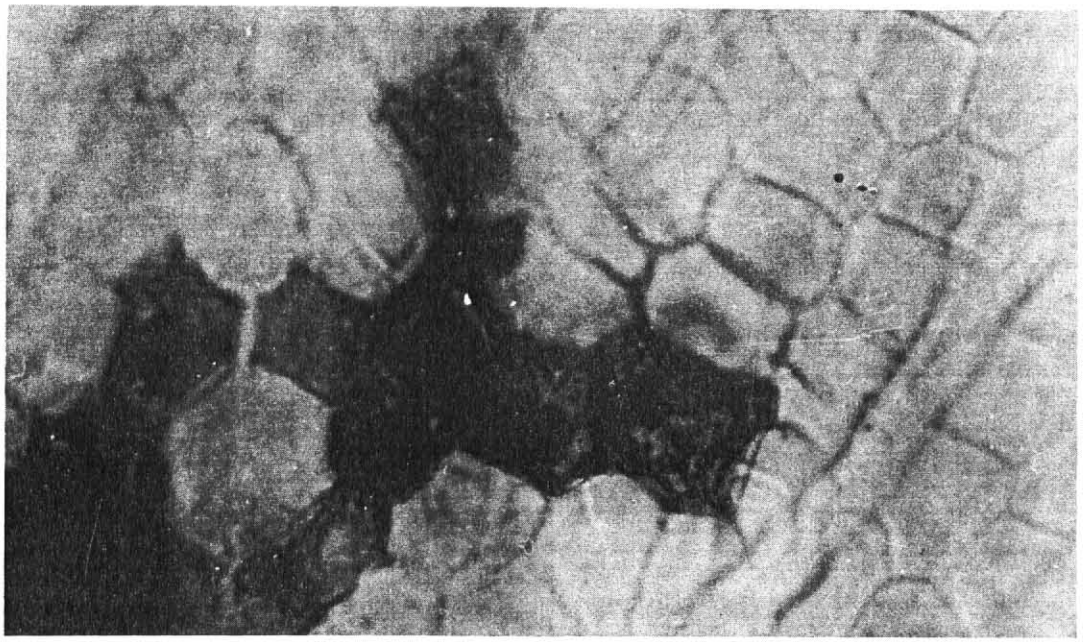

Figure 5. Whole mount section of epidermal tissues of infected eggplant fruit showing surface lesion 6 hours after inoculation (approximately 320x).

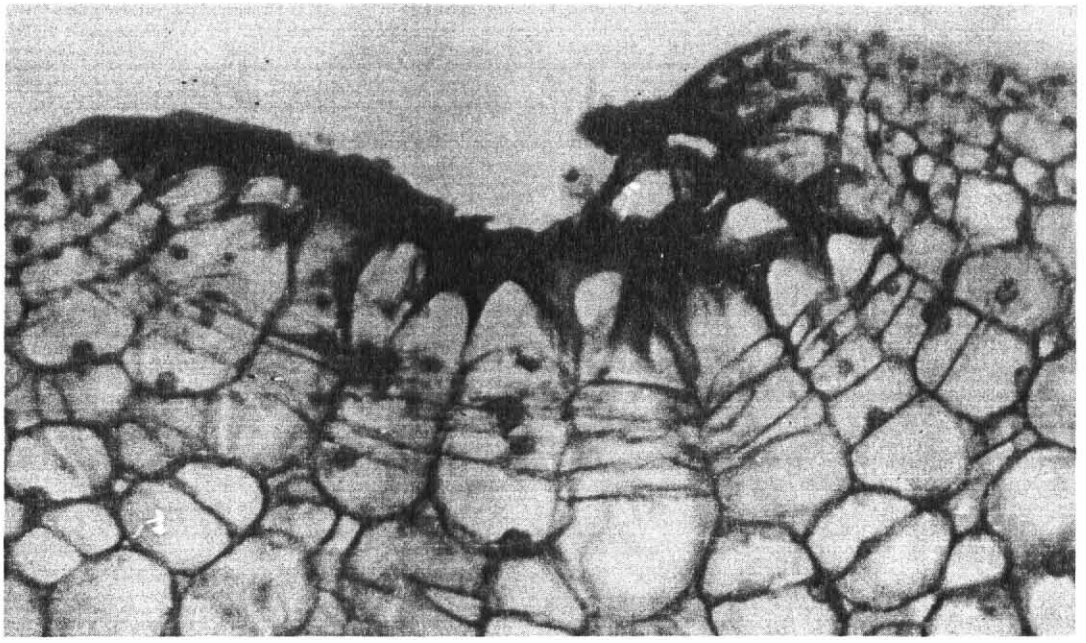

Figure 6. Portion of a cross section of an eggplant fruit (Long purple strain) infected by zoospores of *Phytophthora parasitica* 6-hour after inoculation, showing the corky layer formation separating the affected tissues from the subjacent normal tissues (approximately 500x).

the infected cells turned brown and their contents became granular (Figure 4). The discoloration became more intense after 6 hours (Figure 5). Later the cell walls lost their integrity, the cells collapsed, and the contents apparently dissolved.

Hyphae became abundant 36 hours after inoculation. At this time the first symptoms consisting of a brown spot was observed at the point where the inoculum was introduced. Infected cells stained red in safranin fast green and violet purple in thionin-orange G, whereas the healthy cells were green and unstained, respectively.

In the Long purple strain of eggplant fruit, sections taken 6 hours after inoculation, showed that the cells collapsed resulting in the formation of a depression which marks the site of lesion. Corky layers were formed beneath the collapsed cells and completely separated the invaded tissues from the subjacent normal tissues (Figure 6).

Discussion

The pathogenicity tests of uninjured fruits by 4 isolates of *P. parasitica* showed that the 4 strains were equally virulent to the Dumaguete strain of eggplant. This was similar to the report of Cabaccang, et al. (2) on the infectivity of 4 *Phytophthora* isolates from eggplants collected from Batangas and Laguna. Evidence has been obtained that the disease symptom showed earlier on younger than older fruit. The fungus penetrated directly through the cuticle. This shows that the cuticle did not provide a mechanical barrier to penetration by zoospores of *P. parasitica*. Host cells were killed only after the fungus had penetrated them.

The histologic response of the Long purple strain of eggplant to infection by *P. parasitica* was the initiation of hypertrophy and hyperplasia of subjacent affected tissues. This response may by stimulated by some substances produced by the fungus that diffuse to the other cells and induce cell division and enlargement. The histologic response was similar to the report of Bach and Wolf (1) who found that the band of corky layers in citrus fruit infected by *Diaporthe citri* was due to hyperplasia. The response inhibited the further invasion by the pathogen. This strain showed resistance to the pathogen after penetration has been effected.

This strain should be studied more thoroughly. It may be useful in a breeding program to improve the level of resistance of eggplant to Phytophthora rot.

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