The Etiology and Histopathology of Helminthosporium Blister Canker of Pear

Gregoria N. Acedo and G. N. Agrios

Formerly Graduate Assistant and Associate Professor of Plant Pathology, respectively, Department of Plant Pathology, University of Massachusetts, Amherst 01002.

Based on a portion of a Ph.D. thesis by the senior author. Appreciation is expressed to Margaret E. Bigelow and E. S. Luttrell for assistance in the identification of the pathogen, and to J. Taylor for supplying a culture of *H. papulosum*.

Accepted for publication 1 October 1970.

ABSTRACT

A species of *Helminthosporium* was isolated from the bark of pear trees showing blister canker symptoms. Inoculations of pear seedlings with mycelial suspensions of the fungus proved it to be the cause of this disease on pear. The fungus from pear is similar to *Helminthosporium papulosum* from apples in cultural and morphological characteristics, but the isolate from pear does not infect apple and vice versa. The fungus on pear is therefore considered to be a distinct pathological form of *H. papulosum*, and is therefore designated *Helminthosporium papulosum* f. sp. *pyri*.

Helminthosporium papulosum f. sp. *pyri* is a slow-growing fungus attaining a colony diameter of about 2.5 cm after 3 weeks of growth on nutrient media. Growth is favored by media rich in carbohydrates. The fungus rarely sporulates on nutrient media, but exposure of cultures to continuous fluorescent light enhances sporulation. Sporulation of the fungus in the field occurs in July and August, and coincides with the period of new infections in the field.

Helminthosporium papulosum f. sp. *pyri* penetrates pear bark directly and invades the cortex both inter- and intracellularly. The mycelium also invades the phloem fiber cells, but its presence in the sieve tubes and xylem vessels was not demonstrated. In older lesions, browning and discoloration extend to the cambium and underlying wood. Hyperplasia and slight hypertrophy were observed in parenchymatous cells away from the invading hypha. Phytopathology 61:312-316.

Additional key words: Pyrus communis.

A bark canker disease of pear (*Pyrus communis* L.) was observed in Massachusetts for the first time in 1966, and a description of the disease was reported recently (1). Although a similar disease on apples, called black pox, has been reported in the USA from the area bounded by New Jersey, Kansas, Texas, and Georgia, and has been studied extensively (2, 5, 8), the disease on pear was only noted once (2) in Mississippi.

The disease affects the trunk, branches, and twigs, and symptoms appear as distinct necrotic areas, about 5-12 mm in diam, surrounded by circular or semicircular cracks. Heavily infected trees show coalescent lesions on the bark, defoliated branches, and poor growth. In time, cankers may cause decline and death of affected trees. This work was undertaken to study the etiology of the disease and the histopathology of the infected host.

MATERIALS AND METHODS.—*Etiological studies.*—Sections of diseased bark, about 3 × 3 mm, were cut from the margins of canker lesions, sterilized by dipping them for 3-5 min in a 0.53% sodium hypochlorite solution, washed with several changes of sterile distilled water, drained, and placed on potato-dextrose agar (PDA). Pathogenicity tests were carried out on Bartlett pear seedlings, obtained from Mount Arbor Nurseries, Shenandoah, Iowa, and grown in 6-inch pots in the greenhouse, by brushing the stems and leaves with a mycelial suspension from a 20-day-old fungus culture. The fungus colonies and the agar medium on which they were growing were homogenized in distilled water in a Waring Blendor. The control plants were brushed with bits of agar in distilled water. Both the treated and control plants were covered with plastic bags for 48-72 hr after inoculation.

In the laboratory, six replications of sets of five Bartlett pear twigs, five detached leaves, and both five young and five mature fruits were inoculated. The twigs, leaves, and fruits were incubated in Stender dishes lined with moist paper towels, and were observed for symptom development.

The fungus on pear was identified as a species of *Helminthosporium*. For species identification, comparative studies were made with an isolate of *H. papulosum* Berg from apples and with two isolates of *H. asterinum* Cooke, a species similar morphologically to *H. papulosum* and to the isolate from pear. One of the *H. asterinum* isolates had been isolated from dead oak (*Quercus nigra*) and the other from dead sumac (*Rhus glabra*). The four *Helminthosporium* isolates were grown on different media at room temp, and the weekly growth increments, colony characteristics, and the frequency and abundance of sporulation were compared.

Cross-inoculation experiments with the four *Helminthosporium* isolates were conducted on Bartlett pear and Delicious apple (*Malus sylvestris* Mill.) seedlings in the greenhouse. Ten pear and 10 apple seedlings were each inoculated with isolates from pear, apple, oak, and sumac. The inoculation procedure was the same as that described in the pathogenicity test. The cross-inoculation experiments were repeated 6 times.

The cultural and morphological characteristics of the fungus on pear were studied at room temp on 11 agar media, namely: potato-dextrose, cornmeal, water, lima bean, malt extract, oatmeal, mycological, Czapek-Dox, V-8 juice, pear decoction, and apple decoction. All media except V-8 juice agar and the pear and apple decoctions were obtained from Difco. The decoctions

were prepared by boiling 200 g of chopped Bartlett pear or McIntosh apple twigs and later straining through cheesecloth. The volume of the decoction was made to 1 liter with distilled water and to this, 15 g of agar were added. V-8 juice agar was prepared by adding 200 ml of V-8 juice to 800 ml distilled water, then adding 3 g of calcium carbonate and 20 g of agar, and autoclaving. The other media were prepared according to standard procedures. The perimeters of the colonies were marked weekly, and the diam were measured and recorded at the end of the 7th week. The increment in colony growth, the presence or absence of spores, and other colony characteristics were noted.

Preliminary studies indicated that light influenced sporulation of the *Helminthosporium* isolate from pear. The effect of light on sporulation was determined by exposing 7-day-old cultures of the fungus on different media to (i) continuous light; (ii) intermittent light; (iii) no light; and (iv) ordinary room conditions. The intermittent light treatment was 12 hr light and 12 hr darkness. The light source was two 15-w fluorescent bulbs, 45 cm from the fungus.

The time of sporulation of *H. papulosum* f. sp. *pyri* in the field was studied in the summers of 1967, 1968, and 1969. Infected twigs were collected at weekly intervals from April to September from the orchard, and the scrapings from the cankers were examined microscopically for the presence of spores.

Histopathological studies.—The infected twigs used for histopathological studies were obtained from inoculations made in the laboratory and in the greenhouse. The mechanisms of penetration and infection were studied through the use of frozen and paraffin-embedded sections. Specimens sectioned in the freezing microtome were stained with acid-fuchsin in lactophenol and mounted in plain lactophenol. Paraffin-embedded sections were fixed in formalin:acetic acid: alcohol, cut at 10 μ on a rotary microtome, and stained with either Pianeze III-B according to Conn et al. (3) (except that acid-fuchsin was increased to 0.5 g), or with thionin and orange G (7).

RESULTS.—*Isolation of causal organism.*—A fungus belonging to the genus *Helminthosporium* was consistently found associated with the diseased bark, and was isolated and cultured on nutrient media. Infected, washed twigs when kept moist also developed surface mycelium and spores identical with those isolated on PDA.

Pathogenicity tests.—In the greenhouse, 94% of the inoculated pear seedlings showed symptoms which first appear on the twigs as reddish, elevated portions about 1-3 mm in diam. The reddish, blisterlike lesions gradually enlarge and turn purple to dark brown in color. Wide cracks, often circular, soon develop around the blisters, and the areas delimited by the cracks become dry and slightly sunken. As infection progresses and the number of lesions increases, some of the cracks coalesce and give the bark a netlike appearance. The dried, central portion of the lesion may slough off, exposing the fiber tissues beneath. Slight chlorosis, poor growth, and sometimes death of the seedlings follow. The first symptoms start to develop 14 to 30 days after inoculation. No leaf infection was observed in the greenhouse.

In laboratory inoculations, twigs, leaves, and mature fruits became infected 7 days after inoculation. Symptoms on twigs consisted of distinct, reddish elevations similar to those observed on greenhouse-grown plants, but cracks seldom developed around these elevations. Leaf infections appeared in the form of a few minute, dark lesions surrounded by yellow zones. These lesions did not enlarge even after prolonged observation. Likewise, mature fruits developed small dark lesions on the lenticels with superficial mycelial strands radiating from the lesions.

Identification of the causal fungus.—The four *Helminthosporium* isolates studied showed no marked differences in morphological and cultural characteristics except that the sumac isolate produced small colonies and more abundant sporulation on most of the media tested. On the other hand, the pear isolate produced slightly larger colonies and more abundant sporulation than the apple and oak isolates.

In cross-inoculation experiments, infection on pear seedlings was obtained only with the pear isolate and infection on apple seedlings developed only from inoculations with the apple isolate. Infection was not obtained on pear and apple seedlings inoculated with either the oak or sumac isolates.

The results of the cultural, morphological, and cross-inoculation studies indicate that the pear fungus may be a specialized form of *H. papulosum*. The results of the cross-inoculation experiments are further supported by observations in the orchard where apple trees of several cultivars were growing side by side with heavily infected pear trees but were unaffected by the disease. Another point of difference in their pathogenicity is that, whereas *H. papulosum* infects bark, fruits, and the leaves of apple in nature, the fungus isolated from pear can only infect the bark of the pear trees and has never been observed to infect fruits and leaves in the field. In view of these differences, the fungus isolate from pear is hereby designated *Helminthosporium papulosum* f. sp. *pyri*. The morphological and cultural characteristics of the special form (*H. papulosum* f. sp. *pyri*) are nearly identical with those of the species (*H. papulosum* Berg), a Latin description of which already exists (2). Cultures and specimens of pear twigs infected with *H. papulosum* f. sp. *pyri* are being deposited in the herbaria of the New York Botanical Garden and the American Type Culture Collection.

Cultural and morphological studies.—The fungus grows very slowly in culture with colonies only an inch in diam after 3 weeks. *Helminthosporium papulosum* f. sp. *pyri* sporulates seldom and poorly on artificial media in the absence of additional light.

Light had a marked effect in inducing sporulation in *H. papulosum* f. sp. *pyri*. Cultures exposed to continuous light produced more spores than those exposed to intermittent light. This was especially evident on the V-8 juice agar medium. Sporulation was also enhanced by continuous light on apple decoction agar,

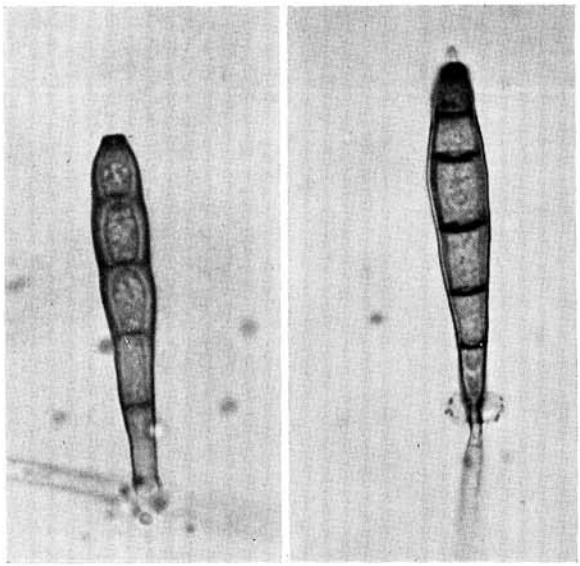


Fig. 1. Typical conidia of *Helminthosporium papulosum* f. sp. *pyri*. Note papilla at the base of spore at right. (×2100)

pear decoction agar, mycological agar, malt extract agar, and oatmeal agar. On the other agar media (lima bean, Czapek-Dox, water, potato-dextrose, and cornmeal), the fungus sporulated sparsely under continuous light.

In intermittent light, the fungus sporulated fairly abundantly on malt extract agar and oatmeal agar, but produced only a few spores on each of the other nutrient media. In continuous darkness the fungus sporulated, although sparsely, only on lima bean agar. Cultures of the fungus kept under room conditions produced a few spores when grown on lima bean agar, water agar, potato-dextrose agar, and cornmeal agar, but not on any of the other media.

Morphological characteristics.—The hyphae of *H. papulosum* f. sp. *pyri* are hyaline when young but become brown, thick-walled, septate, branched, and often densely granular with age.

The conidia are usually clavate, thick-walled, 3-4 septate, and grayish to dark brown in color (Fig. 1). Spores produced on diseased bark measure 23-50 μ long and 4.6-8.1 μ wide, with an average of 35.4 × 6.3 μ. The conidia are produced singly on short conidiophores or at the tips of common, elongate hyphae. A conidium may arise through the apical pore of another conidium. A vesiclelike structure is sometimes present at the attachment of the conidium to the conidiophore. The conidiophores are dark brown and arise singly at

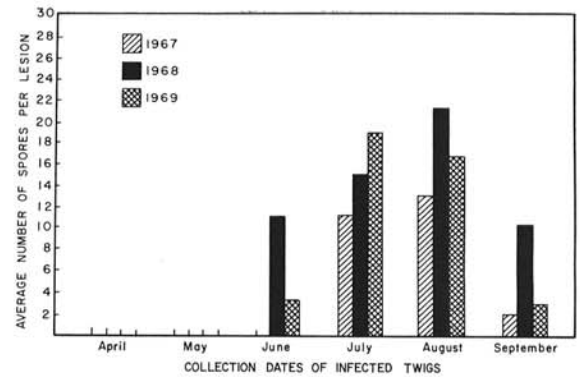


Fig. 2. Time and relative abundance of sporulation of *Helminthosporium papulosum* f. sp. *pyri* on blister canker-infected pear twigs in the orchard.

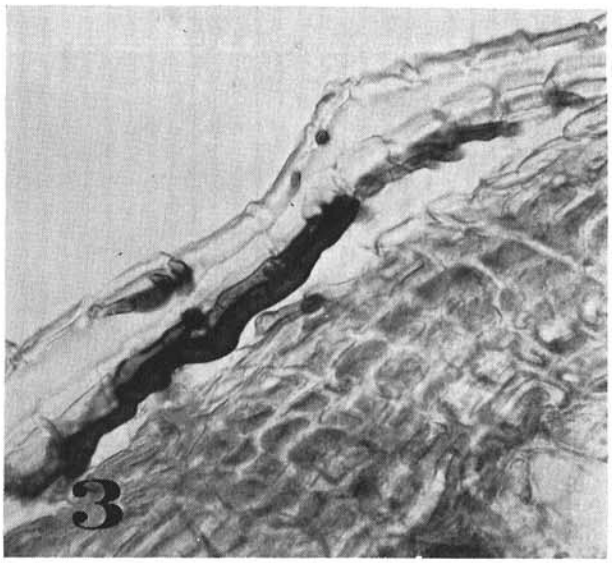
right angles along a hyphal strand, or in clumps from stromatic hyphae. Spore germination most commonly occurs by formation of a germ tube at the basal-end cell of the spore. Apical and bipolar germination was also observed.

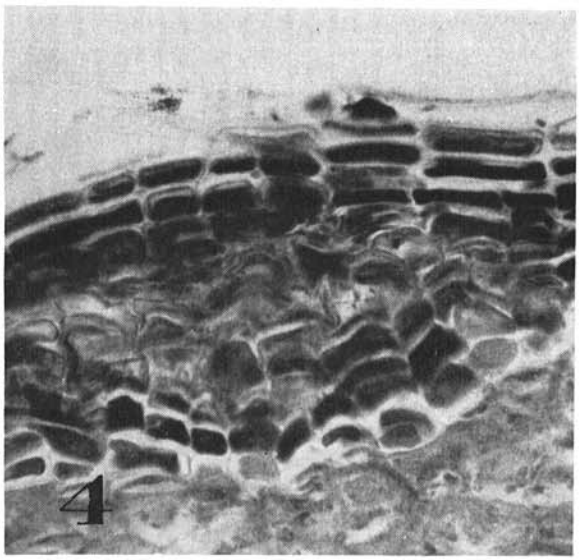
Sporulation in nature.—Figure 2 shows that sporulation in 1967 occurred in late July and August. In 1968, spores started to appear during the last week of June, with abundant sporulation occurring in July and August. For the 1969 season, sporulation started in the late part of June, reached its peak in July and early August, then gradually declined.

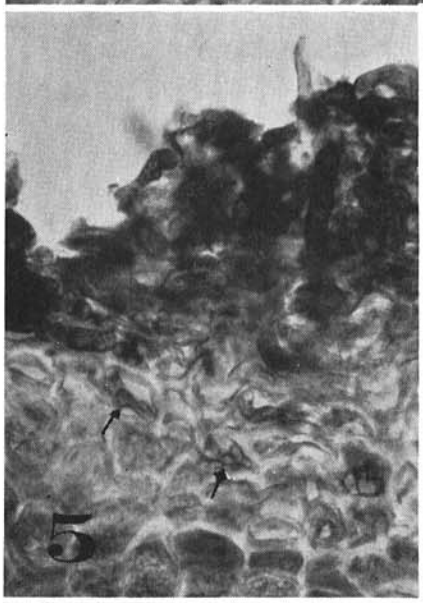
Histopathological studies.—The germ tubes or mycelium of *H. papulosum* f. sp. *pyri* penetrate the bark of pear twigs directly through the intact epidermis within 24-36 hr after inoculation. In some instances, the hyphae form compact masses of rounded, thick-walled mycelium on the surface of the bark before penetration, but eventually they produce small hyphal pegs that penetrate the cuticle and grow into the intercellular spaces of the epidermis. Infection also takes place through the lenticels and through wounds. After penetration, the mycelium usually spreads in all directions just below the epidermis (Fig. 3). In response to the infection, several layers of cork cells form just below the epidermis, and another one or two series of cork cell layers form deeper in the cortex (Fig. 4). Some hyphae, however, are able to penetrate the cork barriers below and spread down into the cortex (Fig. 5). The cells immediately below and those several cells away from the invading hyphae undergo extensive hyperplasia and slight hypertrophy. In addition to these reactions, several layers of cork cells form that delimit the affected area. At this stage, the infected portions of the bark appear as reddish blishers about 2 mm in

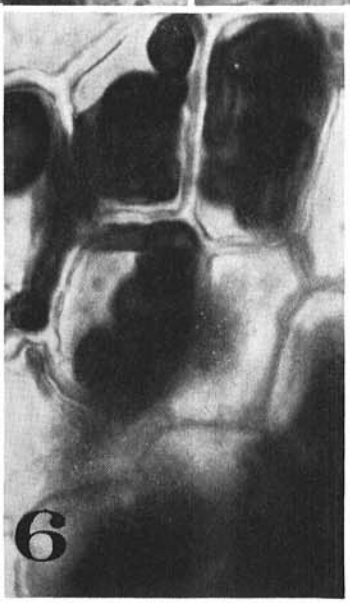
→

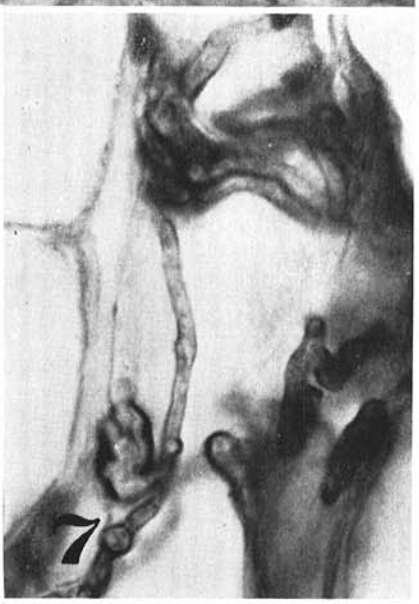
Fig. 3-9. *Helminthosporium papulosum* f. sp. *pyri* in tissue of *Pyrus communis*. **3)** Hyphae growing tangentially between the epidermis and the cork layer of the bark of twigs. (×800) **4)** Two groups of cork cell layers formed by the host in response to the infection. (×800) **5)** Hyphae (arrows) passing through the cork cells of an infected twig. (×800) **6, 7)** Cross sections of cortical cells of the stem of a seedling showing inter- and intracellular growth of the hyphae. (×1,500) **8)** Hyphae growing along the longitudinal axis within phloem fibers. (×1,000) **9)** Disintegrated and discolored portions of wood separated from nonaffected wood tissues by a thick layer of hyperplastic cells. Notice that the discoloration has diffused below the hyperplastic cells. (×200).

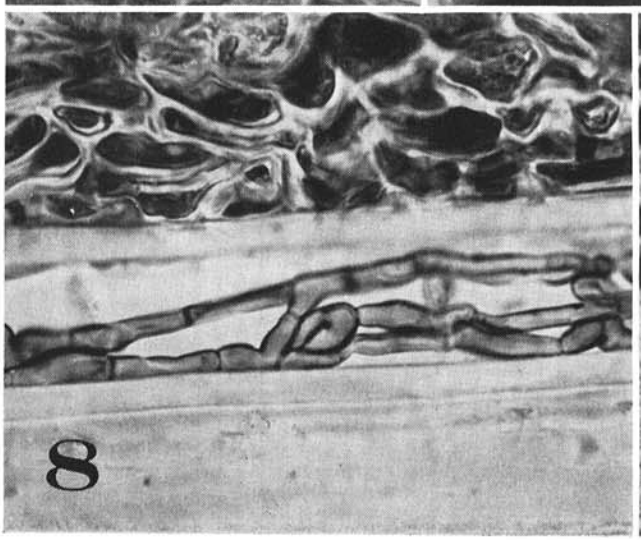

3

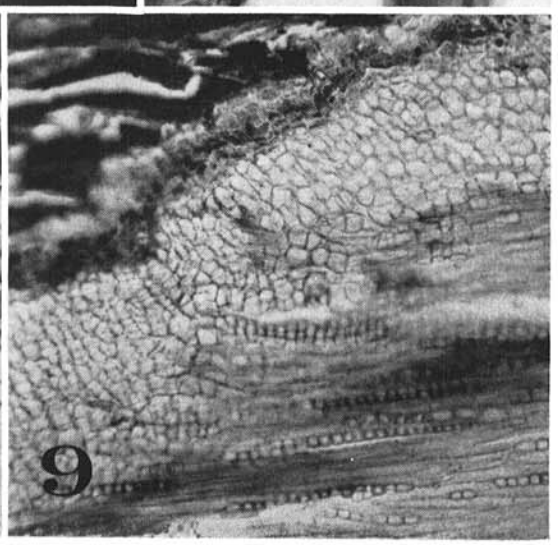

4


5


6


7


8


9

diam. The mycelium seems to be able to pass through the cork layer mostly by growing between the cork cells. Once the mycelium has advanced beyond the cork cells, it continues to invade the cortex both inter- and intracellularly (Fig. 6, 7). In intracellular invasion, the hypha becomes constricted as it passes through the wall and resumes its normal diam upon reaching the lumen of the invaded cells.

From the cortex, infection spreads to the area of the phloem, although it has not been determined whether the mycelium enters sieve elements. Mycelial growth has been observed in the phloem fiber cells (Fig. 8). Often, isolated necrotic areas develop near fiber cells at places far from the original point of infection. As infection progresses, discoloration and browning extend to the cambium and into the underlying wood. No mycelium, however, was seen either in the xylem vessels or in the ray parenchyma of the brown areas of the wood which are separated from the healthy portions by a thick mass of hyperplastic cells (Fig. 9).

DISCUSSION.—The constant association of the *Helminthosporium* fungus with blister canker disease of pear, its isolation from diseased tissue, its reinoculation on pear twigs and seedlings with subsequent development of the same disease, and the reisolation of the fungus from infected tissues provide sufficient evidence that blister canker of pear is caused by that organism. The size of cankers on pear seedlings in the greenhouse was smaller than those produced in the field, but the difference was probably due to the influence of the environmental conditions and the age, size, and vigor of the twigs.

The fungus on pear has the taxonomic characters of a *Helminthosporium* sp. and does not differ markedly in morphological and cultural characteristics from *H. papulosum* on apples and from the saprophytic isolates of *H. asterinum*. Luttrell (*personal communication*) accepts Hughes' (4) reduction of *H. papulosum* to synonymy with *H. asterinum*, but the type of *H. asterinum* was not described. The taxonomy of *Helminthosporium* is still unsettled, and Luttrell (6) classifies *H. papulosum* with *Corynespora* because it has acrogenous conidia. For the present, we place the fungus under *Helminthosporium*, and since the fungus on pear is similar in cultural and morphological characteristics to *H. papulosum* on apples but differs in pathogenicity, the fungus on pear is designated *H. papulosum* f. sp. *pyri*.

Helminthosporium papulosum f. *pyri* is capable of inciting cankers on the bark of pear trees, the symptoms being similar to those of the black pox disease of apples described by Berg (2). The damage caused by the fungus may be negligible as long as the cankers are superficial, but the situation becomes more serious once the injuries extend to the cambium and the vascular tissues of the plant. No mycelium was demonstrated in the sieve elements and in the vessels, but there was considerable browning and subsequent disintegration of the tissues in the bark and in the wood.

The formation of layers of cork cells around the locus of infection is apparently a defense mechanism of the pear tissues against the invading mycelium and possibly against toxic substances secreted by it. The cork barriers are, generally, effective in checking the further spread of the fungus, but since the mycelium has been observed to pass between the cork cells and to penetrate directly, it is apparent that the fungus is able to break through the barrier and to start new infections, as is evident from the zonate appearance of some lesions.

The histological changes observed in the bark and wood are probably caused either by the presence of the mycelium in these areas, by some biologically active substance, or a combination of both. On apples, the mycelium of *H. papulosum* was found mostly on the surface of the lesions, while necrosis, hypertrophy, and increased density of the cells occurred quite deep in the tissues, which led Lewis (5) to suspect that it was a result of toxic action.

The fungus does not infect fruits and leaves in nature, but will do so in the laboratory where warm temp and high relative humidity are maintained during penetration and infection. In the field, the period of max sporulation was in July and August, which are the warmest summer months and the periods of max rainfall. Berg (2) and Lewis (5) also found that the period of max sporulation of *H. papulosum* on apples was in July and August.

LITERATURE CITED

1. ACEDO, GREGORIA N., & G. N. AGRIOS. 1970. Helminthosporium blister canker of pear. Plant Dis. Reptr. 54: 296-299.
2. BERG, A. 1934. Black pox and other apple bark diseases commonly known as measles. W. Va. Agr. Exp. Sta. Bull. 260:1-31.
3. CONN, H. J., MARY A. DARROW, & V. M. EMMEL. (ed.) 1960. Staining procedures [2nd ed.] The Williams and Wilkins Co., Baltimore. 289 p.
4. HUGHES, S. J. 1958. Revisiones Hypomycetum Aliquot cum appendice de nominibus rejiciendes. Can. J. Bot. 36:725-836.
5. LEWIS, G. D. 1953. A study of black pox of apples caused by Helminthosporium papulosum. M. S. Thesis. Purdue University. 51 p.
6. LUTTRELL, E. S. 1964. Systematics of Helminthosporium and related genera. Mycologia 56:119-132.
7. STOUGHTON, R. H. 1930. Thionin and orange G for the differential staining of bacteria and fungi. Ann. Appl. Biol. 17:162-164.
8. TAYLOR, J. 1963. An apple leaf spot caused by Helminthosporium papulosum. Plant Dis. Reptr. 47:1105-1106.