A Fine-Structure Study of the Primary Infection Process during Infection of Barley by Erysiphe graminis f. sp. hordei

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ABSTRACT

The chronological events in the primary penetration process of germinating powdery mildew conidia (*Erysiphe graminis* f. sp. *hordei*) into the epidermal cells of barley (*Hordeum vulgare* 'Traill') primary leaf tissue are described. Observation of this process by electron microscopy shows that the penetration consists of (i) the enzymatic digestion of the cuticle and cellulose portion of the epidermal wall by enzymes apparently secreted by the developing mildew infection peg; and (ii) a mechanical pushing of the infection peg through a layer of material which has been deposited on the underside of the epidermal wall. This layer (papilla) is more electron-dense than the cellulose portion of the cell wall, and contains membranous material embedded in an amorphous matrix. The papilla is formed after the appressorium attaches to the outer surface of the host epidermal wall, but prior to the formation of the infection peg. Phytopathology 60:1504-1509.

Two major mechanisms have been suggested to explain the penetration process of pathogenic fungi into healthy host cells. One mechanism indicates that penetration is accomplished through the mechanical puncture of the host cell wall by a fine, threadlike infection peg formed in the center of the underside of an appressorium. The appressorium is tightly bound to the surface of the host cell wall, and thus provides the needed anchorage against which the infection peg can generate the necessary force to effect penetration (6). Experiments have shown that fungal infection pegs can penetrate such substances as gold foil (5). Mechanical penetration has been the most popular theory, and as late as 1967 it was mentioned as the most probable mechanism involved (10). A second mechanism interprets the process as an enzymatic digestion of the host cuticle and cell wall. Smith (18) in 1900 indicated the possibility of chemical degradation in the penetration of powdery mildew infection pegs into geranium epidermal cells. This work was little appreciated, and only in the last few years has the enzymatic mechanism gained some experimental support (1, 2, 13, 14, 15). Recently, several workers demonstrated that pathogens are capable of producing the necessary enzymes for cuticle and cell wall digestion (3, 9, 12). Also, histochemical techniques have shown that chemical changes occur in the host cell wall in the area of penetration (1, 2, 4, 7, 14, 15).

Still, the problem is not resolved, primarily because the process has not been analyzed in a systematic, chronological sequence. This paper describes such a study of the initial penetration process in powdery mildew infection of barley with the aid of the electron microscope. We show that initial penetration in this host-parasite combination is a two-stage process. The first stage is an enzymatic digestion of the primary cell wall (and possibly the cuticle) by the infection peg, the second, a mechanical penetration by the peg through a structure (papilla) formed on the underside of the host cell wall in response to the formation of the appressorium.

MATERIALS AND METHODS.—Barley seedlings (cultivar Traill) were used as the host material. They were grown as previously described (8). The primary leaves of 9-day-old seedlings were inoculated with conidia of the powdery mildew fungus (*Erysiphe graminis* f. sp. *hordei* Marchal; race unidentified). Inoculation was accomplished by applying spores to marked segments of the leaf with a camel's-hair brush and by shaking infected, sporulating leaves over the seedlings.

Tissue for examination of the primary penetration process was obtained by cutting pieces of leaf tissue approximately 2 mm^2 from primary leaves inoculated 48-72 hr previously. Tissue containing small colonies was sampled for several days after inoculation to observe later stages in the infection process. These squares were immediately fixed for 2 hr at room temp in 3% glutaraldehyde containing 0.05 M phosphate buffer at pH 6.8. The tissue was washed in 0.05 M phosphate buffer and postfixed in 2% osmium tetroxide (0.05 M phosphate buffer) for 1.5 hr. It was then dehydrated in an acetone series and embedded in a mix-

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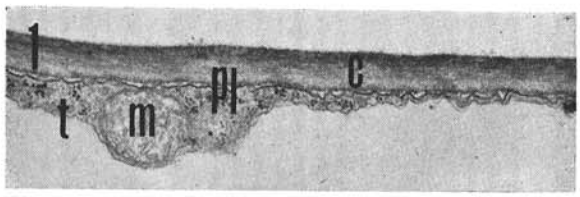
Fig. 1-7. **1)** Portion of the outer epidermal cell wall from uninfected barley leaf tissue. Note the thin layer of cytoplasm consisting of plasmalemma (Pl), mitochondrion (M), and tonoplast (t) which is irregularly attached to the electron-dense inner layer of the cell wall (C) (×19,000). **2-7)** Various stages of primary penetration (×19,000). **2)** Appressorium (A) and well-developed papilla (P) prior to formation of the infection peg; the cellulose portion of the cell wall is shown (C). **3-6)** Serial sections of penetration in which the infection peg (I) has digested through the cellulose portion of the host cell wall (C) with the tip of the peg just beginning to push into the papilla. Note granular substance (Gr) between appressorium and host cell wall in Fig. 3. The infection peg is entering at an angle to the plane of sectioning. Arrow indicates tip of infection peg. **7)** Tip of infection peg (arrow) pushing partially through the papilla. Infection peg is entering at an angle to the plane of sectioning; thus, only the tip is visible in this section.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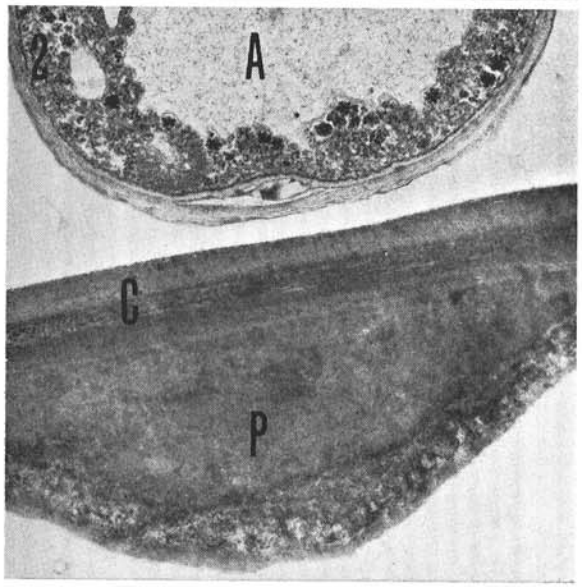
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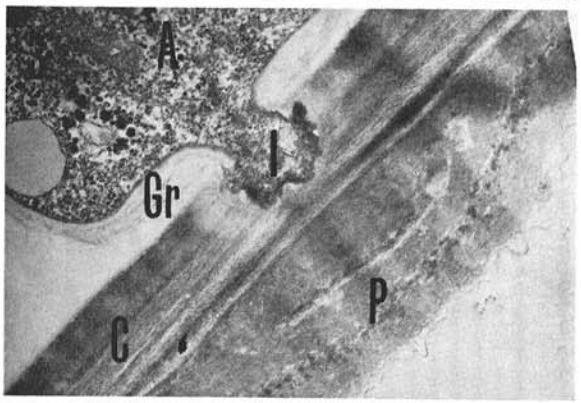
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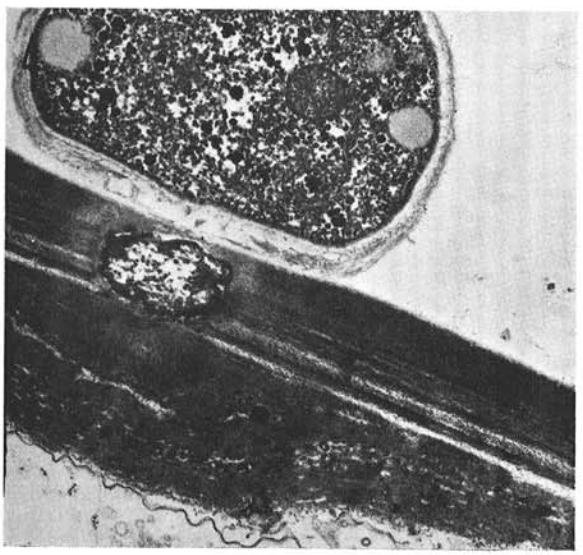

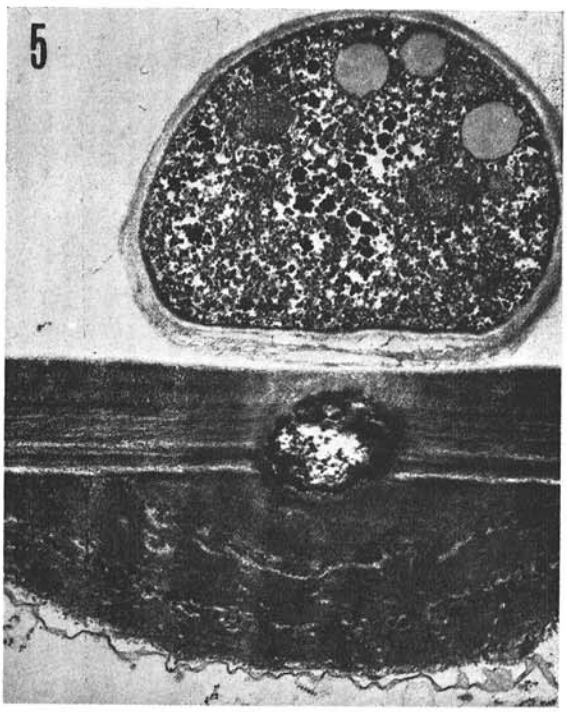
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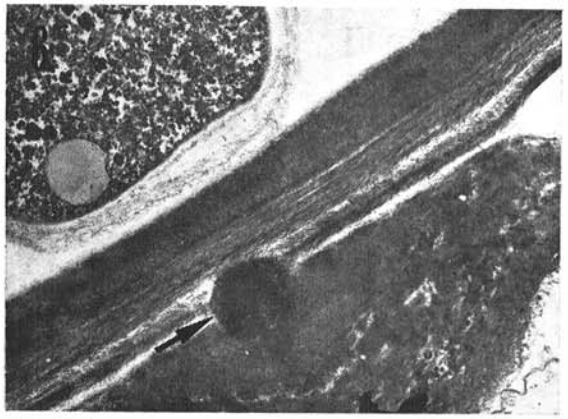

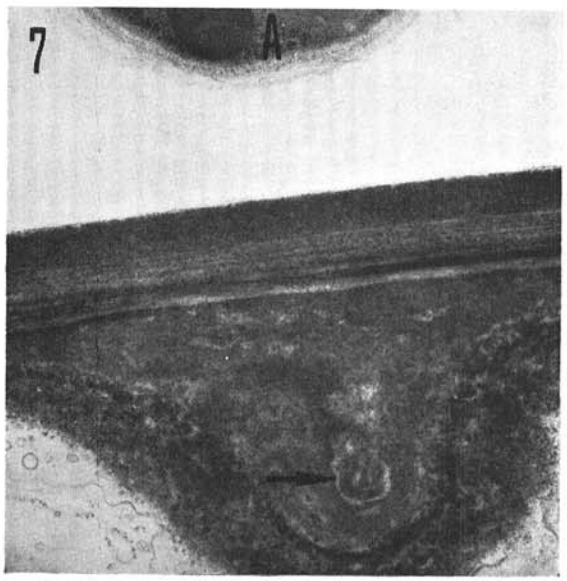
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ture of Araldite-Epon (16). Sections were cut on Sorvall MT-1 and 2 Ultramicrotomes using diamond knives. Thin sections were mounted on grids and stained with aq 2% uranyl acetate followed by lead citrate (17). Examination of the grids was made in a Hitachi HU-11A electron microscope.

RESULTS.—An attempt was made to determine the sequence of events which takes place in the initial penetration process of a healthy host epidermal cell. Consequently, the majority of the micrographs were made from sections obtained from primary barley leaf tissue inoculated 48-72 hr previously (Fig. 2-7). Care was taken during sectioning to collect thin sections of epidermal cells which were being infected by single germinating conidia. Micrographs of older infected tissue are also included to demonstrate either differences between primary and secondary infections or to present aspects of the infection process which were not as clear in sections from the primary infected tissue (Fig. 8-11).

In electron micrographs, the outer epidermal cell wall of the healthy host leaf tissue ranges between 0.2-0.5 μ in thickness (Fig. 1). This does not include the cuticle, since it is dissolved during the fixation and embedding procedure. The wall consists of several layers of fibrillarlike (microfibrils) material with the strands oriented in different planes. The innermost layer of the wall consists of a thin layer of dark, amorphous material. The plasmalemma is irregularly attached to this inner layer. The cytoplasm consists of a very thin layer, with the tonoplast almost immediately adjacent to the plasmalemma. Very few structures or organelles occur in the cytoplasm, although most cytoplasmic components except chloroplasts can be found infrequently in the epidermal cells.

The general appearance of the appressorium is similar to that of the aerial hyphae. A major difference, however, is the occurrence of a loose granular material between the appressorium and the epidermal cell (Fig. 2-6). This material is an extension of the outer portion of the appressorial cell wall, and may be a portion of the sticky substance produced by the appressorium which helps bind the appressorium to the host's surface (6).

Sometime after the appressorium attaches to the host cell wall and prior to the formation of the infection peg, a noticeable change begins to occur in the host cell wall directly beneath the appressorium. An electron-dense amorphous material is deposited on the inner surface of the cell wall, and forms a pad which is an extension of the inner amorphous layer of the wall (Fig. 10) and corresponds to the structure designated the papilla (4, 18). This material appears to be formed as the result of biosynthetic activity of the host cyto-

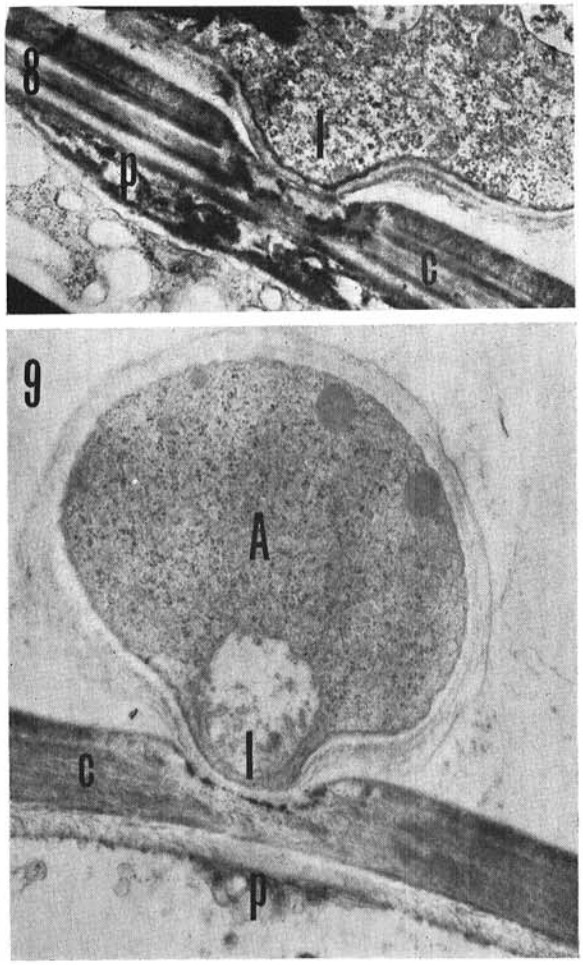

Fig. 8-9. Enzymatic digestion stage of penetration in previously infected host epidermal cells. Note complete lack of any signs of stress in the microfibrillar portion of the host cell wall (C) and the limited area of digestion around the tip of the infection peg (I). Previously infected epidermal cells produce a much smaller papilla (P) (×19,000).

plasm. The amount of host cytoplasm increases and contains much rough endoplasmic reticulum and numerous polyribosomes (Fig. 2-7, 10). Mitochondria are present, and Golgi apparati have been observed frequently. Both the plasmalemma and tonoplast become highly convoluted. Many small vesicles occur between the plasmalemma and the papilla. These appear to be formed in the host cytoplasm, and their deposition on the host cell wall aids in the buildup of the papilla. Besides containing electron-dense amorphous material, the papilla also contains membranous structures (Fig. 3-6). The thickness of the papilla may be as great

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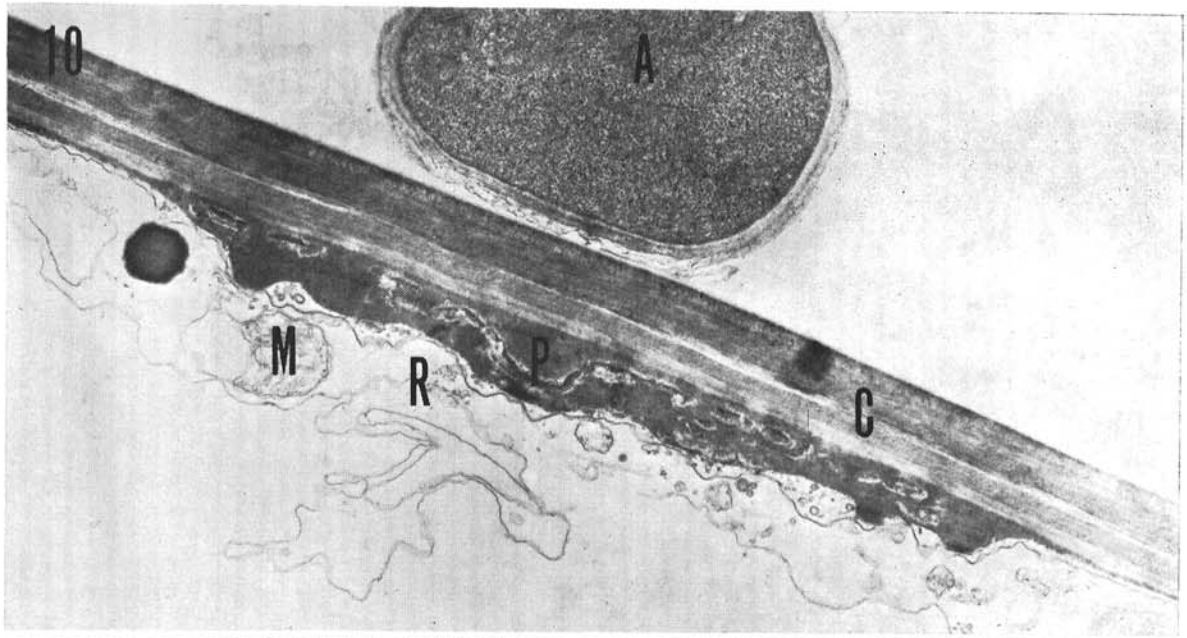
Fig. 10-11. Previously infected cells. **10)** Appressorial (A) stage prior to formation of infection peg. Note that papilla (P) appears to be a buildup of the inner electron-dense amorphous layer of the host cell wall. Note also the presence of cytoplasmic constituents such as mitochondria (M) and polyribosomes (R) beneath the papilla (×19,000). **11)** Infected epidermal cell containing a well-developed haustorium (H). Parts of the appressorium (A) and infection peg (I) as well as host plasmalemma (Pl) and mitochondria (M) are visible. The structures designated plate and collar by Bracker (4) are all portions of the papilla (P) which has been partially thrust into the cell lumen by the penetrating infection peg. Note darkly staining blotches in the cellulose portion of host cell wall (C) (×10,000).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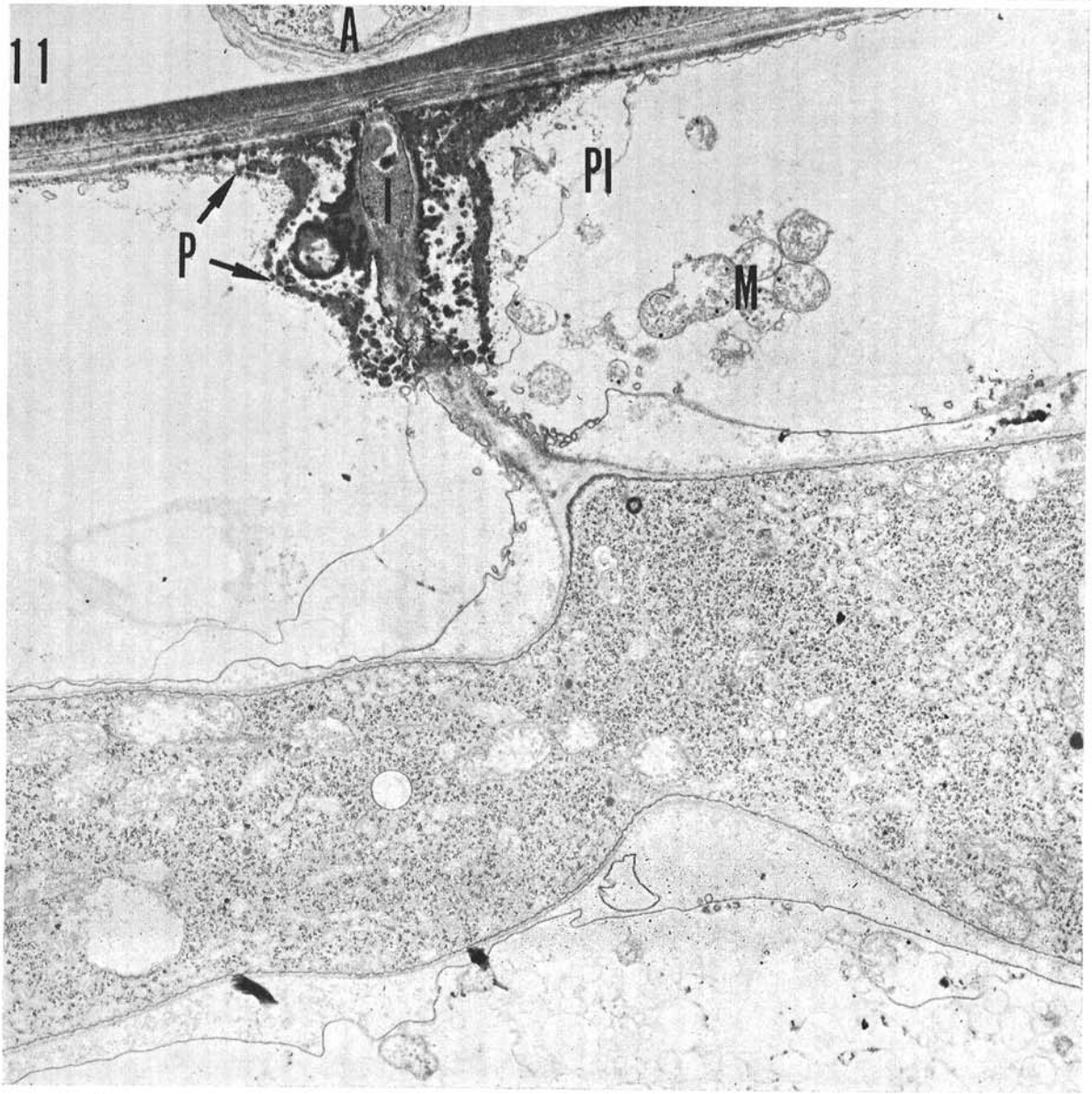
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as 1.5 μ, but varies depending upon the history of the infected cell. Previously noninfected epidermal cells produce the thickest papillae as shown in Fig. 2-6, whereas cells which have already been infected form thinner papillae (Fig. 8-10). The papilla is well formed prior to the formation of the infection peg.

The penetration by the infection peg through the host cell wall is a two-stage process. The first stage of the penetration consists of the apparent enzymatic digestion of the microfibrillar strand portion of the cell wall which was present prior to the formation of the papilla. Appressoria with penetrating infection pegs are shown in Fig. 8, 9. The peg has digested away about one-half to three-fourths of the microfibrillar portion of the host cell wall. The area of the apparent digestion is limited, and there is a complete lack of signs of stress. Serial sections of a penetrating infection peg (entering at an angle to the plane of sectioning) which has completely digested through the microfibrillar portion of the host cell wall are shown in Fig. 3-6. The tip of the infection peg in Fig. 6 is just beginning to enter the papillar portion of the host cell wall. A small halo of lighter-staining cell wall material is shown around the infection peg with no signs of stress in the microfibrillar portion.

A mechanical pushing of the infection peg through the papillar portion of the host cell wall occurs in the second stage. The tip of an infection peg (the peg is at an angle to the plane of sectioning and was cut tangentially) which has pushed part way through the papilla is shown in Fig. 7. In contrast to the first stage, apparent lines of stress occur in the papilla near the tip of the infection peg.

Once the infection peg has pushed through the papilla, it enters the epidermal cell lumen where it swells or expands and begins to form the primary haustorium. A portion of an epidermal cell, after penetration has taken place, can be seen in Fig. 11. Parts of the infection peg and haustorium are included in the micrograph. The structures designated as the plate and collar by Bracker (4) can be seen to be portions of the papilla which has been partially thrust into the epidermal cell lumen by the penetrating infection peg. The microfibrillar portion of the host cell wall extending several μ on either side of the infection peg now contains areas of darker stain. In advanced stages of infection, the entire epidermal cell wall will possess these dark-staining blotches.

Discussion.—From the preceding observations, penetration of the leaf epidermal cell wall of barley by a powdery mildew infection peg appears to be a two-stage process. The first stage of the process is probably facilitated by enzymatic hydrolysis, since no signs of stress are evident in the microfibrils below the developing infection peg (Fig. 8, 9). The portion of the host cell wall which is digested is confined to a small area immediately adjacent to the infection peg. The enzymes which are secreted by the peg do not appear to be active at distances greater than 0.1 μ from the surface of the peg. Their secretion appears to be confined only to the tip of the peg. The initiation of the infection peg penetration process therefore may be the formation of these digestive enzymes in a small area of the underside of the appressorium. Their digestion of the cuticle and microfibrils of the host cell wall would provide not only an opening for passage through the wall but also might make a portion of the appressorial wall pliable so that the infection peg could begin growing into the developing opening. Some fungi possess the necessary enzymes for cuticle digestion (12), and there is some indication that fungi are capable of producing the necessary enzymes for host cell wall digestion (3). One study has indicated that the induction or repression of these cell wall enzymes may form the basis of susceptibility or resistance in that particular host-parasite combination (3, 9).

The second stage is a forceful puncture of the host papilla by the infection peg. The papilla appears to have an amorphous matrix composition in which is embedded much membranous material. The membranous material may add structural strength to the papilla. According to others, the papilla contains the polysaccharide callose (P. H. Williams, *personal communication*). Penetration of the papilla must be a rapid process, since this stage was rarely encountered in viewing thin-sectioned material. The digestion of the microfibrillar portion of the wall appears slower, as it was frequently encountered.

Formation of a papilla in response to infection peg formation may be a common phenomenon, as it has been reported in *Plasmodiophora brassicae* infection of crucifers (P. H. Williams, *personal communication*) and in other host-parasite combinations (13, 15, 19, 20).

Additional research on the function of the papilla is needed. It may function in the formation of a mechanical barrier through which the infection peg must penetrate to gain access to the cell lumen (20). It has been shown experimentally that infection pegs are capable of generating sufficient force to puncture various materials, including gold foil (5). Since this force is not needed for passage through the cuticle or microfibril parts of the host cell wall, it may be required for puncturing the papilla. Older leaves of barley are more resistant to powdery mildew infection (11), possibly because papillae formed in older leaf tissues are thicker or structurally stronger, and thus inhibit penetration of the peg.

The papilla may also serve as an accumulation site for substances produced by the host which inhibit the development of the infection peg. These substances could come in direct contact with the peg outside the host protoplasm in a highly concd form. A basic staining substance which may be associated with resistance occurs initially in the papillae of the invaded host cells of powdery mildewed barley (7).

Recently, several studies of powdery mildew infections in which histochemical approaches were employed indicate that chemical changes occur around the penetration site in the host cuticle and cell wall (1, 2, 7, 14, 15). Some of these reports are difficult to interpret, as it is not clear whether it is the microfibrillar portion of the cell wall or the papilla which is giving the response to the histochemical stain. In future studies this distinction should be made.

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