Initial Penetration Process in Powdery Mildew Infection of Susceptible Barley Leaves

W. E. McKeen and S. R. Rimmer

Professor and graduate student, respectively, Department of Plant Sciences, The University of Western Ontario, London N6A 3K7, Canada.

This work was supported in part by Grant No. A0752 from The National Research Council of Canada.

The authors thank R. Smith for technical assistance.

Accepted for publication 16 February 1973.

ABSTRACT

The chronological events in the primary penetration process of germinating conidia of powdery mildew (*Erysiphe graminis* f. sp. *hordei*) into epidermal cells of susceptible barley (*Hordeum vulgare*) are presented. The barley leaves induced the conidial germ tubes to grow longitudinally on the leaves and stimulated the production of appressoria. The papilla, a result of host reaction to the fungus, began to form in the epidermal cell below the appressorium arm as the appressorial infection pore developed. The tip of the blunt infection peg lacked a cell wall and the host epidermal wall was dissolved only in the region of the tip. Mechanical pressure was required to force the peg through the host cell wall and papilla, and to invaginate the cytoplasm.

Phytopathology 63:1049-1053.

Additional key words: chemical penetration.

The entry of fungal pathogens into healthy plant cells has been a subject of speculation for a long time, but relatively few plant pathologists have researched this problem. More recently, host-parasite relations have been studied by electron microscopy. Still, the mechanism of penetration and the reaction and interaction of host and pathogen, remains unresolved.

In 1966, McKeen et al. (8), while reporting electron microscopy studies on powdery mildew on sunflower, suggested that changes in the epidermal host wall were probably due to enzyme(s) produced by the infection peg. Akai et al. (1), reported in 1967 that barley infected with *Erysiphe graminis* had chemical modification in the cellulose walls around the penetration tubes. This work was confirmed by McKeen et al. (7) in 1968.

Two research groups have studied infection of barley by powdery mildew by means of electron microscopy. Stanbridge et al. (9), in 1971, carried out experiments on sections of leaves which were placed on water agar containing 200 μg/ml of benzimidazole and Edwards & Allen (4), in 1970, conducted studies on leaves 48 to 72 hr after inoculation.

This paper describes the initial penetration process in powdery mildew infection of susceptible barley leaves 8 to 15 hr after inoculation. By electron microscopy, we show that a papilla forms 8 to 11 hr after inoculation before the infection peg enters the host wall, and that penetration is achieved by chemical dissolution and mechanical pressure.

MATERIALS AND METHODS.—Susceptible barley plants (*Hordeum vulgare* L. 'Keystone') were placed 8 days after planting in a controlled-environment chamber with a day (15 hr) temperature of 20 C and relative humidity of 65%, and a night (9 hr) temperature of 15 C and relative humidity above 95%. The primary leaves of these plants were inoculated 3 hr before the temperature dropped to 15 C and 4 hr before darkness, with *Erysiphe graminis* f. sp. *hordei* Marchal race C R 3 conidia by gently touching them with barley leaves covered with 7-day-old mildew colonies. Some conidia were placed on glass slides. Growth was examined at hourly intervals and most observations were conducted 8 to 15 hr after inoculation. Germination was over 80% and development was synchronous.

For electron microscopy, leaf disks supporting conidia were excised with a No. 17 hypodermic needle with its tip sharpened to resemble a cork borer.

Leaf disks were placed in glutaraldehyde-osmium tetroxide which was made by mixing ice-cold stock solutions of glutaraldehyde (4%) and osmium tetroxide (2%), both buffered with 0.1 M sodium cacodylate, to a pH of 6.8 and used immediately (5). The material was fixed for 30 min at 5 C before being rinsed in cold 0.1 M sodium cacodylate buffer followed by postfixation in buffered 2% osmium tetroxide (5 to 10 min). Then it was rinsed in distilled water and warmed to ambient temperature before being stained for 20 min in 5% magnesium uranyl acetate.

After fixation, disks were dehydrated in a graded ethanol series and infiltrated with Epon-Araldite (6). The disks were then spread in a thin layer of embedding medium on carbon-coated glass microscope slides before being placed in a 60 C oven to polymerize the plastic for 24 hr.

Later, disks with germinated conidia were selected with the aid of the light microscope and cut out and re-embedded on the tip of plastic blocks. The blocks were trimmed with a glass knife until the cutting edge was about 1 μm from desired material. Thin serial sections were then cut on a Porter-Blum (Sorvall MT-1) ultramicrotome with a diamond knife, mounted on 200-mesh copper grids, and stained with lead citrate. Sections were examined in a Phillips EM-200 using 60KV and an objective aperture of 40 μm.

RESULTS.—*Erysiphe graminis* conidia germinated within 2-4 hr after inoculation, and their germ tubes, which swelled apically to form appressoria, frequently grew longitudinally on the uneven surface of the barley leaf to which they were attached by an adhesive slime. The flattened underside of the appressoria mirrored the surface topography of the epidermal cells.

On glass surfaces, the germ tubes usually became greatly elongated (up to 300 μm) and appressoria were seldom formed.

After 7 to 11 hr, the appressorium produced a short appendage, the appressorial arm, and an infection pore formed in the central contact-surface of this arm (Fig. 1). A pore in the fungal wall was the first indication that an infection peg would soon form. By the time the infection pore had developed, the host cell was reacting and an electron-dense, homogenous or globular, dome-shaped deposit was forming between the inner surface of the host wall and plasmalemma, directly below the pore in the appressorial arm (Fig. 1). The amount of the deposit rapidly increased in size and formed a wide base, 5 to 10 m diam, adjacent to the epidermal wall. The center of the dome was up to 2 μm in height, prior to penetration. The deposit continued to change, frequently being homogenous at first, later becoming globular and usually changing to two layers of varying thickness by the time the infection peg had passed through it (Fig. 2). The layer adjacent to the plasmalemma was usually composed of an electron-lucent matrix and completely covered the outer layer. After the peg passed through the papilla, the deposit developed along the neck of the haustorium for a short distance and the plasmalemma was sandwiched between the neck and the deposit in this region. The deposit lacked membranes and fibrils and appeared to lack rigidity.

By the time the first deposit was detected, the normal layer of cytoplasm adjacent to the deposit had increased in thickness 20- to 100-fold and varied in thickness from 1 to 10 μm. Usually the cytoplasm which lined the walls of the epidermal cell was about 0.05 to 0.2 μm thick, except where it surrounded mitochondria or other organelles, and it did not decrease in amount at the time of infection. The cytoplasm by the papilla contained many mitochondria, vesicles, dictyosomes, and some endoplasmic reticulum. A few unidentified bodies, 1- to 5- μm in diam, found only in papillar cytoplasm, were present. Some were homogenous and slightly electron-opaque, whereas others contained membranes, vesicles, and sometimes crystals (Fig. 5). A thin layer of cytoplasm surrounded the body of the young haustorium and its appendages.

Occasionally short germ tubes, lacking appressoria, stimulated and infected the host. Infrequently, the cytoplasm flowed out through the appressorial pore onto the epidermal cuticle and infection never occurred.

Normally, the infection peg (about 0.3 μm in diam) emerged from the pore in the appressorial arm with a blunt tip and without a wall (Fig. 4). The infection peg enlarged somewhat within the host wall and frequently penetrated within 3 hr at a slightly oblique angle. The peg grew through the host papilla which continued to develop and change until the body of the haustorium was initiated. The peg penetrated the papilla during 11 to 15 hr. Often the tips of the pegs were observed in the epidermal wall but few were found in the papillae. Figure 3 shows that the fungal infection peg completely filled the hole in the epidermal wall.

When the tip of the peg was 3-5 μm beyond the papilla and in the lumen of the epidermal cell, it enlarged to form a globular structure, the incipient haustorium. The globular structure then elongated at its distal end and usually turned longitudinally in the epidermal cell. Finger-like branches began to develop about 18 hr after inoculation.

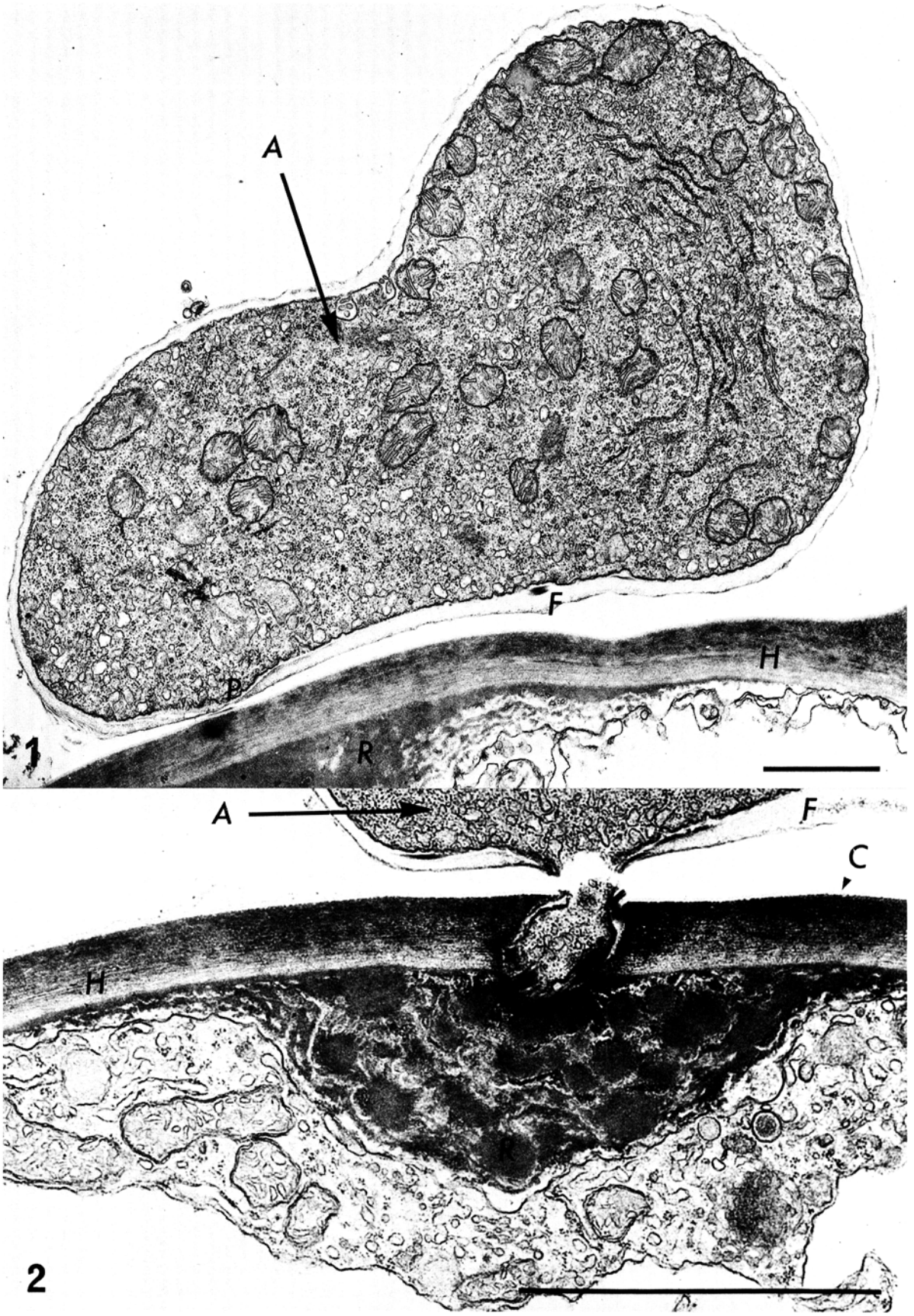
DISCUSSION.—This is the first time that serial sections of early stages of primary infection (8 to 15 hr) on naturally growing hosts have been studied. Edwards & Allen (4) sectioned barley leaves 48 to 72 hr after inoculation and they believed that primary epidermal wall penetration was occurring during the third day after inoculation. Either they were examining material containing haustoria, or the temperature they used (15 C) greatly retarded development. The latter seems unlikely because they reported that under similar conditions a dense mat of sporulating mycelium was present in 9 days (3). It seems more plausible that they were examining material containing haustoria; not only because of the long time (48 to 72 hr) from inoculation to examination but also because we have frequently observed material containing haustoria which, if sectioned obliquely, resulted in sections of infection pegs which appeared to be only part way through the epidermal wall. Also, we have neither observed an appressorial arm with a large vacuole prior to penetration, as they have shown, nor have we observed conidia germinating later than 24 hr after inoculation.

Stanbridge et al. (9) studied early stages of infection in leaves which had been inoculated after being placed on water agar containing 200 ppm (μg/ml) of benzimidazole. We have found that leaves floating on water containing 200 μg/ml benzimidazole have a significantly altered resistance.

This study shows that the germ tubes respond to the host leaf by producing appressoria soon after formation, and that the host reacts to the mildew, prior to entrance of the infection peg into the epidermal wall, as early as 8 hr after inoculation. A chemical irritant must have emerged from the appressorial pore and stimulated the epidermal protoplast. This action in advance of the peg causes the protoplasm to react very quickly and lay down a barrier or set up a healing mechanism in advance of the invader.

Stanbridge et al. (9) found a similar development except that the events occurred about 50% more slowly. This may have been due to environmental conditions such as temperature and presence of the chemical benzimidazole.

Fig. 1-2. The scale lines are 1 μm. **1)** An electron micrograph of a section through an appressorium, appressorial arm (A), barley epidermal wall (H), and part of a papilla (R). Many ribosomes, mitochondria, vesicles and endoplasmic reticulum are present in the appressorium. The appressorial arm has a pore (P) in its lower wall (F), directly over the papilla. The deposit between the plasmalemma and host wall is electron dense and homogenous except in the layer adjacent to the plasmalemma. (× 20,200). **2)** An electron micrograph of a section through part of an appressorial arm (A), young infection peg (11 hr) and a papilla (R) in a barley epidermal cell. The infection peg which has broken away during embedding, emerges through a pore in the fungal wall (F). The infection peg has enlarged somewhat after passing through the cuticle (C) and contains ribosomes and vesicles. The deposit contains electron-dense globular bodies. There is a very thick layer of cytoplasm over the deposit and many mitochondria and vesicles are present. (× 63,500).


A
F
H
P
R
1
A
F
C
H
2

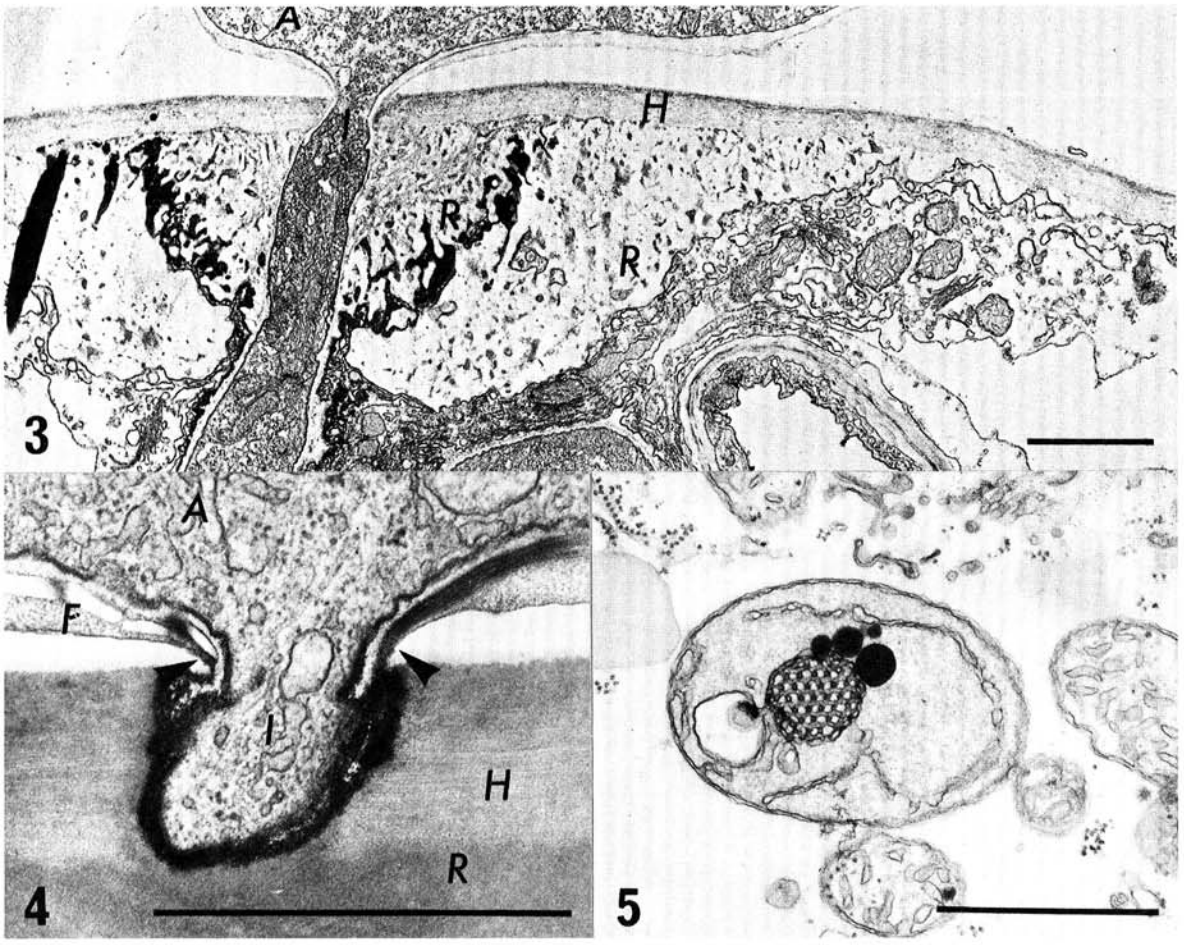


Fig. 3-5. The scale lines are 1 μm. **3)** An electron micrograph of a section through a papilla (R), infection peg (I) and part of an appressorial arm (A). The peg fits tightly in the hole through the epidermal wall (H) and papilla (R), and contains many ribosomes and mitochondria in its dense cytoplasm. The deposit has two layers, one being somewhat more opaque than the other. (× 16,600). **4)** An electron micrograph of a section of a young infection peg (I) (11 hr). Observe that the tip of the peg is blunt and does not have a wall. The arrows point to the tapered edge of the fungal wall (F) around the pore in the appressorial arm (A). The peg contains several vesicles (× 54,200). **5)** An electron micrograph of a section of papillar cytoplasm showing mitochondria and two unidentified bodies (μ). The large one, in the center, contains membranes, vesicles, lipid and a crystal (× 28,800).

The enormous increase in cytoplasm below the infection site would seem to result mainly from synthesis rather than accumulation from other regions of the protoplast because the amount of cytoplasm at the papilla was about equal to that which is normally present in a cell and there were no noticeable decreases in other regions. Also, new large bodies up to 5-μm in size appeared. It is interesting that dictyosomes and vesicles are present in the region around the papilla and may well be responsible for the deposition of this material. Clowes & Juniper (2) state that there is much indirect evidence from both animals and plants that the Golgi are concerned with secretion and, particularly in plants, with the formation of cell walls and extracellular structures.

The extra cytoplasm and deposit is probably synthesized from reserve material in the vacuole.

Penetration through the epidermal wall must result in part, from chemical dissolution, because the tip of the infection peg has no wall, is blunt and no mechanical fracturing was observed. We believe that it is inconceivable that a penetration peg without the rigidity that a cell wall would confer, could achieve penetration solely by mechanical means.

It is likely that the penetration peg passed through the papilla more rapidly than the epidermal wall, because the peg was frequently found in the epidermal wall but seldom in the papilla.

The peg fits tightly in the hole in the epidermal wall. This along with the papilla around the infection peg prevents breakdown of cellular integrity and leakage of cytoplasmic contents from the epidermal cell, thus making a compatible relationship possible.

LITERATURE CITED

I. AKAI, S., M. FUKUTOMI, N. ISHIDA, & H. KUNOH.

1967. An anatomical approach to the mechanism of fungal infections. p. 1-20. *In* C. J. Mirocha & I. Uritani [ed.]. The dynamic role of molecular constituents in plant-parasite interaction. Amer. Phytopathol. Soc., Inc., St. Paul, Minnesota.

2. CLOWES, F. A. L., & B. E. JUNIPER. 1968. Plant cells. Blackwell Scientific Publ. Oxford and Edinburgh. 546 p.
3. EDWARDS, H. H., & P. J. ALLEN. 1966. Distribution of the products of photosynthesis between powdery mildew and barley. Plant Physiol. 41:683-688.
4. EDWARDS, H. H., & P. J. ALLEN. 1970. A fine-structure study of the primary infection process during infection of barley by Erysiphe graminis f. sp. hordei. sp. *hordei*. Phytopathology 60:1504-1509.
5. FRANKE, W. W., S. KRIEN, & R. M. BROWN, JR. 1969. Simultaneous glutaraldehyde osmium tetroxide fixation with postosmication. An improved fixation procedure for electron microscopy of plant and animal cells. Histochemie 19:162-164.
6. MCKEEN, W. E. 1971. Woronin bodies in Erysiphe graminis DC. Can. J. Microbiol. 17:1557-1560.
7. MCKEEN, W. E., R. SMITH, & P. H. BHATTACHARYA. 1968. Alterations of the host wall surrounding the infection peg of powdery mildew fungi. Can. J. Bot. 47:701-706.
8. MC KEEN, W. E., R. SMITH, & N. MITCHELL. 1966. The haustorium of Erysiphe cichoracearum and the host-parasite interface on Helianthus annuus. Can. J. Bot. 44:1299-1306.
9. STANBRIDGE, B., J. L. GAY, & R. K. S. WOOD. 1971. Gross and fine structural changes in Erysiphe graminis and barley before and during infection. p. 367-379. *In* T. F. Preece & C. H. Dickinson [ed.]. Ecology of leaf surface microorganisms. Academic Press. London and New York.