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Fine-structural Studies Of Major Gene Resistance Of Barley To The Powdery Mildew Fungus Erysiphe Graminis Dc

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FINE STRUCTURAL STUDIES OF MAJOR GENE RESISTANCE OF BARLEY TO THE POWDERY MILDEW FUNGUS *Erysiphe graminis*. DC.

by

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ABSTRACT

A comparison of the development of Erysiphe graminis f. sp. hordei (race CR3) on the leaves of near-isogenic susceptible and resistant lines of barley was made using light and electron microscopy techniques. The lines of barley used were Manchurian (mla/mlk) which is susceptible to the race of the fungus used, Kwan (mla/Mlk) and Algerian (Mla/mlk), which are resistant varieties. Another susceptible variety, Keystone, was also used for primary infection studies.

Penetration of the epidermal cell wall and the development of the fungus up to the formation of the primary haustorium occurred similarly on all genotypes. Penetration was effected by a penetration hypha extruded from a lobe of the appressorium. Evidence is presented that penetration involves chemical alteration of the cell wall local to the penetration peg. The papilla, the host deposit formed by the epidermal cell cytoplasm beneath the infection site, had usually started to form before penetration commenced. This structure is shown to be an important defense mechanism in preventing haustorial formation on the susceptible and resistant hosts alike. Thus the papilla functions as a genetically non-specific determinant of fungal development. It is composed of an inner electron-

dense layer, where the penetration peg is usually stopped, and an outer electron-lucent layer. About 50-70% of attempted infections are stopped by papillae. Appressoria associated with papilla-stopped penetrations usually re-attempt infection by forming a secondary appressorial lobe.

The Algerian and Kwan varieties expressed resistance after primary haustorial formation, 20 hr and 72-96 hr after inoculation respectively. A hypersensitive reaction on the Algerian host was manifested by a sudden collapse and necrosis of the infected epidermal cell. Necrosis and collapse of the haustorium and appressorium occurred concurrently with epidermal cell collapse. This reaction, observed by electron microscopy, is related with a change in epidermal cell permeability to trypan blue and other dyes, as seen in light microscope preparations. Necrosis was confined to the penetrated epidermal cell but adjacent cells reacted also and formed wall deposits between the plasma membrane and the cell wall adjoining the necrotic cell. Hypersensitive reactions occurred in about 45% of the penetrated epidermal cells in the Algerian host.

Hypersensitivity on the Kwan host commenced 72-96 hr after inoculation and was initially confined to mesophyll cells beneath the developing colony. Epidermal cells containing haustoria started to collapse 96-120 hr after inoculation. The first fine structural change associated with both mesophyll and epidermal cell necrosis was the deposition of lipoidal material on the plasma membrane and

tonoplast. This was followed by disorganisation of chloroplasts in mesophyll cells and the formation of wall depositions between the plasma membrane and the cell wall in both mesophyll and epidermal cells. Eventually cells were completely collapsed and contained a mass of electron-dense material with no recognisable cell constituents. Fungal haustoria and hyphae also became necrotic and disorganised in association with epidermal cell necrosis. Sporulation on this host was sparse compared to normal sporulation on the Manchurian host.

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ABBREVIATIONS

A	Appressorium
AL	Appressorial lobe
C	Chloroplast
Cm	Chloroplast membrane
Cu	Cuticle
D	Papilla
D_1	Primary deposition of papilla
D_2	Secondary deposition of papilla
EC	Epidermal cell
Edm	Electron-dense material
Ehm	Extrahaustorial membrane
EM	Electron microscopy
ER	Endoplasmic reticulum
ESH	Elongating secondary hypha
EW	Epidermal cell wall
Fam	Fungal adhesive material
GCW	Guard cell wall
Go	Golgi apparatus
gr	Chloroplast grana
Gt	Germ tube
Gy	Glycogen particles
H	Haustorium
Ha	Halo
Hl	Haustorial lobe
Hn	Haustorial neck
Hpm	Haustorial plasma membrane
HR	Host response (In text Hypersensitive reaction)
Hy	Hypha
Ics	Intercellular space
L	Lipid body
M	Mitochondrion
mb	Microbody
ml	Middle lamella
MW	Mesophyll cell wall
N	Nucleus
NC	Necrotic cytoplasm
NH	Necrotic haustorium
NMC	Necrotic mesophyll cell
Nu	Nucleolus
P	Penetration peg
pa	Plasmodesmata
Pd	Plastid
pg	Plastoglobuli
pm	Plasma membrane
Po	Pore
PR	Polyribosomes
R	Ribosomes
RER	Rough endoplasmic reticulum
S	Conidium
SAL	Secondary appressorial lobe
Se	Septum
SEM	Scanning electron microscopy
Sg	Starch granule
Sm	Sheath (matrix)
SV	Small vesicles
SW	Conidial wall

T	Tonoplast	Ve	Vesicle
V	Vacuole	WL	Wall deposit
Vb	Vesicular body		

CHAPTER I

INTRODUCTION

1.1. General

Erysiphe graminis DC. ex Merat is a biotrophic fungus of the family *Erysiphaceae*. The *Erysiphaceae* are ascomycetous fungi which cause the plant diseases called powdery mildews. These are aptly named because the ectoparasitic fungi of this group produce enormous numbers of conidia which give the surface of the host the appearance to the unaided eye of being coated with a fine white powder.

Some of the plant diseases caused by members of this family are among the most destructive ones known, whereas others appear to be very mild and cause little damage. Among the most serious parasites are *Uncinula necator*, the cause of powdery mildew of the grape vine, *Sphaerotheca mors-uvae*, powdery mildew of gooseberries and *Erysiphe cichoracearum*, powdery mildew of cucurbits and other plants (Alexopoulos, 1962).

Powdery mildew diseases of cereals caused by *E. graminis* are diseases of major economic importance, and, in cool humid regions of the world, may be the main cause of reduced yields, especially of barley. Schaller (1951)

in the USA in a study comparing the yields of resistant (Atlas) varieties of wheat concluded that mildew could decrease yield by 25%. Last (1955) studied the yield of spring sown varieties of barley in field experiments and found that loss due to mildew could be as high as 22%.

Barley, Hordeum vulgare L., is one of man's oldest food crops, possibly even the first crop that mankind cultivated at the beginning of agriculture (Darlington, 1969). The wild 'relatives' of our cultivated barley are to be found in the Middle East and the parasitic fungus E. graminis f. sp. hordei probably coevolved with its host in this part of the world (Koltin and Kenneth, 1970). Anthropological evidence for the early culture of barley may be inferred from the myths of Demeter - the barley mother - a prototype of corn goddesses throughout the world (Frazer, 1922). Presumably one of her functions was to protect the barley from any natural disasters which might befall it including powdery mildew disease.

Erysiphe graminis DC. ex Merat has been studied in recent years not only because of its importance as the causal agent of powdery mildew disease of cereals but also because, in conjunction with its hosts, it affords a convenient system to study the physiology of parasitic symbioses. The reasons for this suitability as a research tool lie in the fact that it has an ectoparasitic growth habit and that its penetration of host tissue is restricted to the outer epidermal layers of the plant. This facilitates

light microscopic observation of the infection process and the subsequent development of the fungus on the host. It enables one to separate almost completely the host from the fungus for physiological and biochemical studies simply by swabbing off the external hyphae of the fungus or by coating the leaf with plastic films and peeling off the plastic.

Other advantages of this system are that the genetics of the host parasite systems of E. graminis on wheat and E. graminis on barley have been thoroughly investigated and isogenic lines of wheat and barley for resistance and susceptibility have been produced so that the physiology of disease resistance can be studied in controlled systems. In addition the dynamics of the infection process have been thoroughly investigated.

1.2. Life cycle of Erysiphe graminis

The life cycle of powdery mildews is initiated by conidia or ascospores both of which germinate similarly (Diagram 1). Germination usually begins within two hours of seeding (Brodie, 1945) and is favoured by light, suitable temperature and the absence of free water.

Under favourable conditions, one or more germ tubes are produced from the conidium (Corner, 1935; Hirata, 1967). One of the germ tubes differentiates to form an appressorium in contact with the host surface. On water, agar or glass surfaces, germination is abnormal in that the germ tubes may grow away from the substrate, they may grow un-

usually long and do not, commonly, form appressoria (Corner, 1935).

Penetration and infection is effected by the formation of a fine penetration peg emerging from an appressorial lobe. This peg grows through the epidermal cell wall into the lumen of the cell, at the same time as a papilla of host material is deposited beneath and around the penetration site (Smith, 1900). Within the lumen of the cell, a haustorium is formed and expands to its characteristic shape (Diagram 3). While the primary haustorium is becoming established, hyphae grow out from the primary appressorium and the conidium along the epidermis of the leaf. Branching is acute and fairly regular. Secondary appressoria are formed as a lateral extension, commonly on alternate hyphal cells.

The mother spore of the colony remains a living integral part of the colony and does not collapse after the fungus has established a symbiosis with the host. As the colony develops, conidiophores are formed, at first, at the centre of the colony and later farther away as the colony expands. Colonies from single spores rarely become larger than a few millimeters in diameter. The mycelium of most genera of the Erysiphaceae, including Erysiphe sp. is entirely superficial. It consists of a network of abundant hyaline hyphae on the epidermis of the infected parts of the host and is firmly adfixed by the numerous haustoria which develop in the epidermal cells and obtain nourishment

from their protoplasts. Species of Leveillula and Phyllactinia are exceptional because their hyphae penetrate through the stomata and grow between the mesophyll cells. Although most of the hyphae of Phyllactinia corylea are superficial, they do not develop haustoria (Alexopoulos, 1962).

When the colony is 4 or 5 days old, conidiophores begin to form as swellings from the vegetative cells in the centre of the colony. Conidia are cut off at the tips of the conidiophores (oidium type of conidiogany) to form a chain of conidia as shown in Diagram 1. In Leveillula taurica the mycelium is endophytic, the conidiophores grow out of stomata and produce their conidia outside the leaf. The conidia of the Erysiphaceae are hyaline and single-celled. They vary in shape from species to species but, generally, they may be described as oval. Conidia are the asexual propagating units of the powdery mildews and are normally wind-disseminated and by chance come in contact with a suitable host surface. The conidia are usually short-lived but if they are deposited on a suitable host substrate they develop and repeat this process of the asexual cycle within seven days.

The perfect stage of the life history of powdery mildews involves the formation of a cleistothecium or fruiting body (Diagram 1). Cleistothecia are produced regularly, occasionally or not at all, varying from species to species. They are usually first formed after the

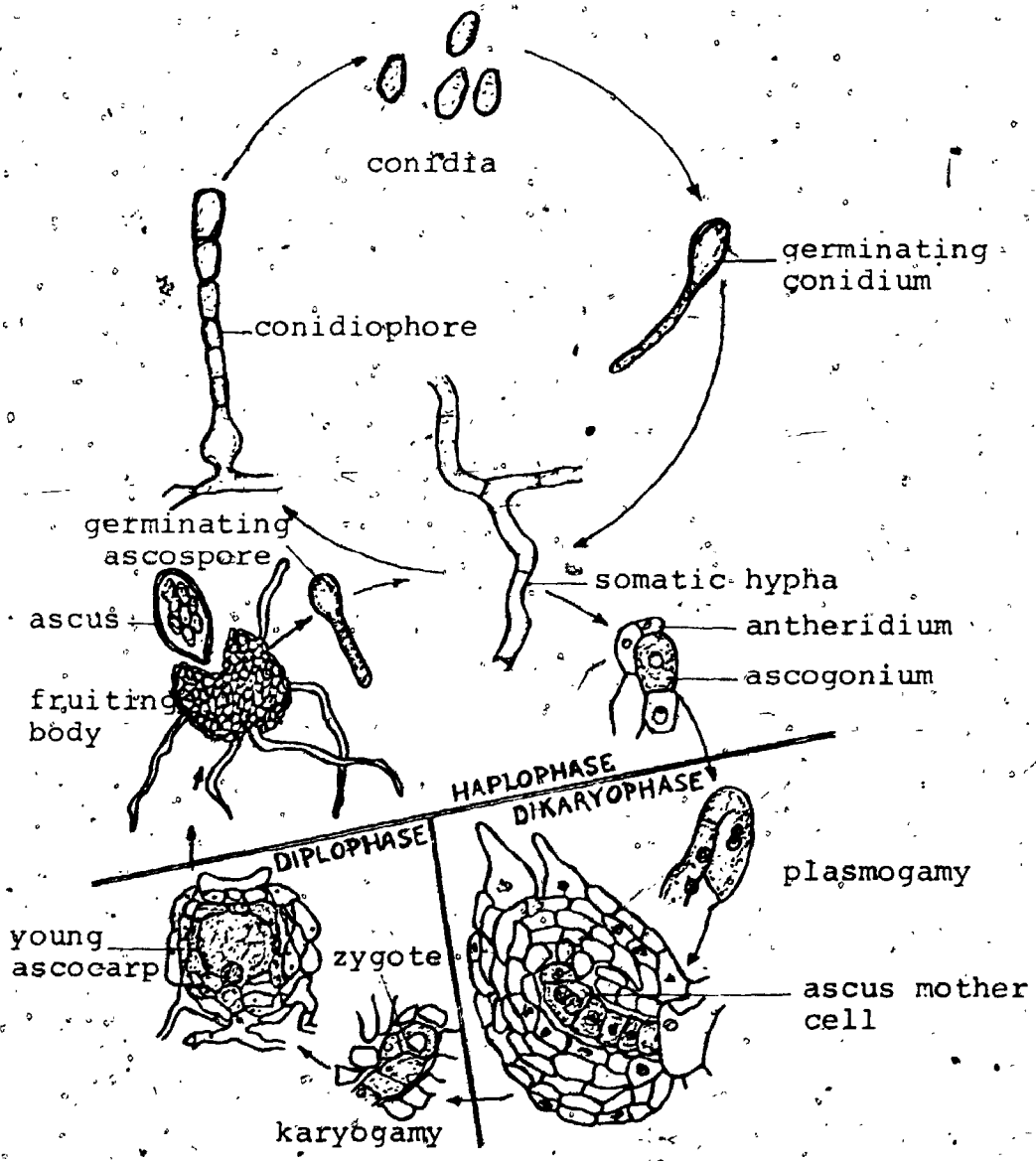
asexual stage has existed for several weeks, normally in the late summer (Yarwood, 1957). The nutritive condition of the host and sexuality are considered the major factors determining cleistothecial formation, which is usually preceded by a decrease in conidial production.

The cleistothecia at first appear white, then change through orange, reddish brown to black when finally mature. Asci and their ascospores are formed within the cleistothecia and the development of these structures requires a long period of time. In many species the asci mature late in the fall and sometimes not until the following spring. Overwintering takes place usually in the cleistothecial stage but sometimes colonies may overwinter as mycelia in the dormant buds of their host. For E. graminis f. sp. hordei the cleistothecial stage is important for oversummering in the hot desert climates of the Middle East where barley and its mildew evolved (Koltin and Kenneth, 1970). The mature cleistothecia of most of the Erysiphaceae have characteristic appendages which may vary considerably in length and shape. The asci are globose to ovoid and, when mature, burst to release the ascospores. Ascospores are generally hyaline, unicellular and oval. Two or four celled ascospores are also known in some genera. The number of ascospores in each ascus varies between species and within the same species. Uncinula aceris regularly contains eight, rarely six ascospores per ascus but E. cichoracearum has two, rarely three ascospores

per ascus.

The mature ascospores, when dispersed, may germinate and infect their plant host. Few life cycles have been experimentally completed through the cleistothecial stage probably because of the long period of maturation necessary before some ascospores germinate. Because the cleistothecia can survive up to thirteen years (Moseman and Powers, 1957) in contrast to the conidial life span of only a few days, it is believed that they may function to maintain the fungus over long periods of adverse conditions.

Diagram 1.



Life cycle of Erysiphe graminis. Redrawn from Alexopoulos (1962).

CHAPTER 2

REVIEW OF LITERATURE

The amount of work reported in the literature on the physiology of host-parasite relations, disease resistance and the fine structure of host-parasite interactions is vast and an exhaustive review of these broad fields is not attempted here. In any case much of this work has been reviewed in recent years so only literature of immediate relevance to the work presented here is included.

In recent years excellent reviews on the physiology of host-parasite relations, have been published (Brian, 1967; Scott, 1972; Shaw, 1963; Smith et al., 1969; Yarwood, 1956). Lewis (1973) has examined the terminology employed in classifications of fungal nutrition and has suggested a return to the broad use of the term symbiosis as originally intended by de Bary when he coined the word. The terminology proposed by Lewis will be used in this study. The term 'obligate parasitism', a term usually applied to fungi that are not culturable on artificial media (these include the powdery mildew fungi), is rendered ambiguous by recent work in the culture of some species of rusts (Scott and Maclean, 1969) and is replaced by the term obligate bio-

troph, which includes all the organisms which, in nature, are capable of growing only in association with their hosts. This group includes both mutualistic and parasitic fungi, embracing the fungi of vesicular, arbuscular and sheathing mycorrhiza, lichens, smuts, the Taphrinales, rusts, powdery mildews and the Plasmodiophorales. It includes some members of other groups such as Phytophthora infestans, Venturia inaequalis and Claviceps purpurea.

Recent reviews on the fine structure of fungi, haustoria and the host-parasite interface include those of Bracker (1967), Bracker and Littlefield (1973), Calonge (1969), Ehrlich and Ehrlich (1971), and Hawker (1965). Various aspects of disease resistance have been reviewed, eg. Cruikshank (1963) on phytoalexins, but probably the most thorough survey is that of Wood (1967).

The establishment of a functional host parasite relationship depends on certain critical stages. These stages will be examined more fully later in this chapter but it is obvious that one of the stages concerns the initial penetration of the host by the fungus. Powdery mildews, like rusts and other pathogens, are able to penetrate not only their hosts but also plants on which they will make no further growth (Corner, 1935; White and Baker, 1954). Though penetration itself does not ensure the success of the mildew in establishing a symbiosis, it is an essential step in this regard. Various students of powdery mildew infections have described this stage as they observed it in

light microscope studies. Smith (1900) and Corner (1935) have described the germination of the conidium to form an appressorium and the fine penetration peg, which emerges from this structure, and penetrates through the cell wall to form a haustorium within the lumen of the epidermal cell. Corner (1935) observed penetration beginning 24 hr after inoculation at 20° C and 100% R.H. In many instances after penetration and haustorial formation, no further development occurs on incompatible hosts. Although the role of the haustorium as an organ of nutrient absorption for the fungus has never been properly established, it is reasonable to assume that this is the case. The formation of a functional haustorium must then be another crucial step in the establishment of a compatible host-parasite relationship. This draws attention to the importance of studying the primary penetration and the invasion of the epidermal cell by the fungus during the early stages of infection.

Ellingboe and his students were the first to study in detail the initial interaction of E. graminis on susceptible and resistant varieties of wheat and barley. Masri and Ellingboe (1966b) considered the primary infection of barley by E. graminis to be a multicomponent process, each phase distinguishable on the basis of morphology or differential sensitivity to environmental factors or both. They considered these stages to be germination, formation of appressorial initials, maturation of appres-

soria, penetration, formation and development of the haustorium and formation of a functional, elongating secondary hypha.

They demonstrated that there is a good correlation between the number of elongating secondary hyphae on the leaf surface and the number of primary haustoria in the epidermis of the same leaf. The formation of elongating secondary hyphae can be used therefore as a good criterion for the establishment of a successful host-parasite relationship. They found that as early as the 11-12 hour after inoculation a few infection pegs have formed in the epidermal cells. The tips of the infection pegs are often swollen as they emerge from the cell wall. Two hours later, after further elongation and enlargement, the penetration hypha starts to turn toward a position parallel to the leaf surface. The haustorial sheath can be observed at this time. 18 hr after inoculation the haustorial body is fully formed with a distinct sheath, a nucleus and a vacuole at each side of the nucleus. Later development consists only of the elongation of the finger-like projections on both ends of the haustorial body. By 34-36 hr after inoculation the haustoria have reached their mature size of 64-70 µm.

Hirata (1967), in his monograph summarizing the results of many years of study on the parasitic relations of E. graminis on barley, found the development of the mildew to be much the same as that observed by Masri and Ellingboe.

However, he said that the haustorium requires 4-5 days to mature with about ten lobes at each end of the body and attains a total length of 150-160 µm. He found that the primary haustorium begins to develop 10 hr after inoculation, whereas the secondary and subsequent haustoria begin to develop at about midnight of the following day. 20 hr after inoculation, when the haustorial growth is somewhat advanced, one or two hyphae grow out from the appressorium. In support of the idea that hyphal development from the appressorium is due to or highly related to the activities of the primary haustorium, he found that hyphae from the appressorium generally do not grow out for more than 10 hr after establishment of the appressorium and also do not grow out from germ tubes formed on substrates which permit no haustorial formation, such as glass slides or epidermal strips of onion bulb scales which have been immersed in alcohol, washed with water and then floated on water.

It seems very probable then that the haustorium is required for the development of hyphae and that the conidium can only supply enough nutrients to form the appressorium, penetrate the defences of the epidermal cell and form the primary haustorium.

Pathogenic fungi invade host plants by direct penetration through the epidermal cell wall or by intrusion through natural openings such as stomata, lenticels or wounds. Whether the former method of penetration is accomplished by mechanical or chemical means or both has

been discussed for a long time. Erysiphe graminis, as do other powdery mildew fungi, penetrates directly through the cell wall.

McKeen et al. (1969) have pointed out that in the past many plant pathologists speculated that entry through the cell wall was mechanical. Opinions varied, however, from the idea that penetration depends on total enzymic dissolution of both cuticle and cellulose wall (Caporali, 1960; Woodward, 1927) to its being totally mechanical (Peries, 1962). Between these two extremes were those who believed that penetration of the cuticle is mechanical whereas penetration of the cellulose layer is both chemical and mechanical (Blackman and Welsford, 1916; Corner, 1935; Leong, 1971; McKeen et al., 1966; Mitchell, 1967; Stanbridge et al., 1971).

Woodward (1927) working with Podosphaera leucotricha reported that chemical dyes showed an enlargement of the passage through the cuticle. His objection to mechanical penetration was that some means of attachment of the fungus hyphae to the host epidermis would be necessary to produce the relatively great force required for a purely mechanical penetration. He could find no evidence of such attachment with this organism. Mitchell (1967), however, in his electron microscopy study found no evidence for degradation of the cuticle by three species of powdery mildew fungi, namely S. macularis, E. polygoni, and E. cichoracearum. He found that the passage made through the cuticle of

strawberry, clover and sunflower by the penetration peg is always the same diameter as the peg. No difference in electron-density of the cuticle was observed at the penetration site or elsewhere. Thus he considered that cuticular penetration is purely mechanical. In addition he observed a mucilaginous coat surrounding the hyphae that he thought would aid the firm attachment of the fungus to the host cell during penetration. Leong (1971) confirmed these findings.

The classical work in support of mechanical penetration is that of Brown (1915, 1916) who showed that extracts of mycelium of Botrytis cinerea were not capable of dissolving the cuticle of their hosts, even though they had been shown to have a powerful maceratory activity on exposed parenchyma. Blackman and Welsford (1916) supported this argument by establishing that the cuticle over the epidermal cells is penetrated by infection hyphae before the cells are killed. They also claimed that the mucilaginous coating held the spores of B. cinerea to the leaf even before penetration. Brown and Harvey (1927) demonstrated that fungal hyphae can penetrate relatively hard substances such as various thicknesses of paraffin wax membranes and films of gelatin of different hardness obtained by treatment with alcohol followed by formaldehyde.

Flentje (1957) showed that penetration of host plants by Pellicularia filamentosa is preceded by a firm attachment of the fungus to the host by a mucilaginous sheath and

that failure to achieve this attachment on the host results in failure to infect the host. Consequently, he thought penetration by this fungus to be mechanical.

Transmission electron microscope studies of penetration have supported the idea that penetration of the cuticle by powdery mildew fungi is mechanical. Besides Mitchell's observation on hyphal penetration, Leong (1971) studied primary penetration of clover by E. polygoni and considered that penetration of the cuticle is mechanical because no change in electron density or erosion of cuticle was observed. Similarly Stanbridge et al. (1971) and McKeen and Rimmer (1973) observed no indication of chemical degradation of the cuticle layer of barley around the penetration zone of E. graminis.

Martin (1973) remarked that, considering the chemical nature of cuticular waxes and cutin, it would be unlikely that these soft substances should provide much of a barrier to penetration. It is also interesting that scanning electron microscope (S.E.M.) studies have shown that the waxy outer layers of the leaf are excavated beneath the conidium and appressorium of E. graminis and an indentation conforming to the shape of these structures is left where they have been removed from the leaf. Schwinn and Dahmen (1973) in an S.E.M. study reported that during the infection process prior to the penetration of the cell wall the wax layer of the leaf is dissolved by the appressorium and beneath this structure disappears completely.

Direct evidence for degradation of the cuticle by powdery mildews has been obtained from analyses of the surface layers of healthy and mildewed leaves of apple and turnip. Whereas the wax content of mildewed leaves was higher than that of symptomless leaves, the cutin content was considerably lower. Rose leaves infected with S. pannosa had only a quarter of the cutin found in healthy leaves, and there was also a marked reduction in the cutin of leaves of Malus sp. heavily infected by Venturia inaequalis (Roberts et al., 1960).

Linskens and Haage (1963) reported cutinase activity in vitro for Botrytis cinerea, Rhizoctonia solani, Cladosporium cucumerinum and Pyrenophora graminea all of which are plant parasitic fungi.

One of the most challenging problems of plant pathology today is the elucidation of the mechanism(s) which determine whether a plant is resistant or susceptible to a particular pathogen. Resistance to a plant pathogen has its basis in the genetic composition of the host, the expression of which may be modified by nutritional and other environmental factors. There appears to be a very close relationship between varieties of a given host and pathogenic races of a given fungus. The gene-for-gene hypothesis was first formulated as the genetic basis for host-parasite compatibility for the flax rust disease (Flor, 1946, 1947) and, since then, similar relationships have been found for many other diseases involving biotrophic fungi

(Person, 1967; Day, 1974). Such a relationship exists in the barley powdery mildew disease (Moseman, 1971; Table 1) where specific genes for resistance and susceptibility in barley and specific genes for virulence and avirulence in the powdery mildew fungus have been identified.

Low infection types result from an interaction between hosts with a resistant genotype Ml/Ml or Ml/ml and pathogens with the corresponding genotype A. High infection types result either from interactions between hosts with resistant genotypes Ml/Ml or ML/ml and pathogens with a corresponding virulence genotype V or from interactions between hosts with susceptible genotypes ml/ml and pathogens with either the corresponding virulent (V) or avirulent (A) genotypes (see Table 1). The expression of these genes during the course of the infection of barley by E. graminis and the subsequent growth into a sporulating colony have been studied with light microscope techniques by Masri and Ellingboe (1966a, b), Lin and Edwards (1974), McKeen and Bhattacharya (1970), Stanbridge et al. (1971), and most recently, by Bushnell and Bergquist (1975).

For E. graminis hordei, Masri and Ellingboe (1966b) have described five mechanisms by which barley containing various resistant genes is resistant to attack by the CR3 race of the fungus. The resistant genes they examined are Algerian (Mla), Goldfoil (Mlg), Kwan (Mlk) and Psaknon (Mlp). They considered all these genes to be pleiotropic because they all exhibit more than one effect in their mode

Disease			Host-parasite Interaction			
Organism	Host				Pathogen	
Character	reaction		Infection type		pathogenicity	
Phenotype	Resistance	Susceptibility	low	high	Avirulence	Virulence
Genotype	M1/M1 M1/m1	m1/m1	M1/M1A M1/m1A	M1/M1V M1/m1V m1/m1V m1/m1A	A	V

Table 1. Diagrammatic representation of gene interactions between host (barley) and pathogen (E. graminis hordei) resulting in the disease (barley powdery mildew). (After Moseman, 1971).

of action. The manner in which these genes conferred resistance were:

" i) exclusion of the pathogen from the host
ii) delay in early haustorial development
iii) distortion of some haustoria
iv) destruction and distortion of the majority of the haustoria five days after inoculation and
v) suppression of fungus sporulation after the establishment of primary infection, thereby preventing the occurrence of repeating disease cycles."

For the Algerian gene (Mla) the various mechanisms by which resistance of the host was expressed were identified as follows:

" i) exclusion of 95% of the applied conidia from entering the host
ii) delay in early haustorial development
iii) reduction in final size of haustoria
iv) distortion of a small portion of haustoria
v) suppression of fungal sporulation even after a successful host-parasite relationship was established."

They considered the first and last of these mechanisms to be most important in conferring a high degree of resistance in this host.

McKeen and Bhattacharya (1970) studied the effect of the Algerian gene (Mla) in the light and electron microscope. They used coleoptiles in their study because of the difficulties in observing mildew on leaf tissue. They found about 80% of spores germinate to form haustoria on susceptible plants and only 14% on the resistant host. They thought that two types of resistance occur, one which

is effective during or immediately after host wall penetration and another which is effective 2-4 days later. They had great difficulty in obtaining EM pictures through the peg region because of the large numbers of sections which had to be cut before a peg was obtained.

The use of coleoptiles by Bushnell et al. (1967), McKeen and Bhattacharya (1970), Bushnell (1971) and Bushnell and Bergquist (1975) in the studies of the primary penetration process of the mildew on barley and the resistance mechanism of the various genes, undoubtedly has certain advantages in that with Bushnell's microculture system an in vivo study of the infection process can be made. Nonetheless it is confusing to attempt to compare the infections of coleoptiles, which are small, non-photosynthetic and would hardly ever be infected naturally with powdery-mildew, with those of leaves. To assume that the susceptibility of coleoptiles is similar to that of the photosynthetic leaves may be unwarranted, and in this regard Bushnell et al. (1967) state that 'the coleoptiles may have been slightly resistant to E. graminis in contrast to fully susceptible foliage leaves' because the hyphal growth pattern was similar to that of the fungus on incompatible hosts as reported by Hirata (1967).

Edwards (1970) provided evidence that a 'basic staining material' is present in the infection site of unsuccessful attempts at penetration. Lin and Edwards (1974) extended the work and studied the primary infection process

of E. graminis on four isogenic lines of barley. They found that the unidentified 'basic staining material' is associated with papillae when penetration by appressoria is unsuccessful but not with penetrated papillae. They found that the Algerian, Goldfoil and Psaknoh genes for resistance delay or reduce the frequency of infection but that the Kwan gene has no effect during penetration. The frequency of infection on all varieties, including the susceptible Manchurian control, was less than that observed by Masri and Ellingboe. They observed that the resistance of leaves to penetration increases in both resistant and susceptible hosts with cell age and also that different types of epidermal cells exhibit differences in resistance to penetration. Stanbridge et al. (1971) undertook an EM study of the primary penetration. They showed that a small part of the parasite population ceased growth without papilla formation, a larger part with papilla formation and that the incompatibility conditioned by specific genes (Mla, Mla_6 or Mla_6 and Mlg together) was expressed as a halt in fungal development and hypersensitive death of host cells only after a haustorium had formed in the host cells. Bushnell and Bergquist (1975) studied the primary penetration process using living material. They observed the development of cytoplasmic aggregates in the infection site, papillae and haustoria in various compatible and incompatible host-parasite combinations. Aggregates were induced by 92-100% of the observed appressoria on all but

one of the host-parasite combinations. Papillae were induced by 30-70% of the appressoria and haustoria were formed by 39-83%. The frequency of these structures was generally the same in both compatible and incompatible combinations. Papillae were present in many instances in which haustoria failed to form but were also frequently present when haustoria were formed.

Incompatibility was expressed in the Algerian-CR3 combination by the collapse of the infected host cells 16-21 hr after inoculation. Cytoplasmic streaming stopped at an early time and the host cells then lost turgor as the cytoplasm clumped and haustoria degenerated. About half of the appressoria involved also degenerated, whereas the others remained healthy in appearance. They noticed that host cells which were not under attack also collapsed, especially in parts of the tissue that had unusually heavy inoculum loads. Similar to the findings of the present work and that of Stanbridge et al. (1971) they showed that a high percentage of germlings failed to enter the host when a papilla was produced regardless of the host-parasite combination or the presence of specific genes for resistance in the host.

The role of the papilla in non-specific resistance seems unquestionable but how it excludes the fungus has not been determined. The papilla begins to form before an attempt at penetration of the cell wall is made (McKeen and Rimmer, 1973). It is not a simple homogeneous struc-

ture. It contains callose (Bogdan, 1968; Edwards and Allen, 1970), a high accumulation of silicon (Kunoh and Ishizaki, 1975), two distinctive layers and membranous fragments.

Most of the ultrastructural studies on powdery mildews have been concerned with the nature of the host-parasite interface. This boundary, though not easy to define, is the site where host and parasite are most intimately associated and through which transport of nutrients and, probably, exchange of information takes place. A comprehensive survey of such interfaces, and an attempt at a classification of them, has been published by Bracker and Littlefield (1973). The ontogeny of the functional host-parasite relationship may involve more than one type of interface.

Bracker (1968) has given the most complete account of the fine structure of the haustorium of E. graminis but the first study was that of Ehrlich and Ehrlich (1963). The haustorium consists of a central body and several lobes. This main haustorial body is separated from the infection peg by a septum. Bracker showed that the host plasma membrane surrounds the collar (or papilla) and is inserted between the collar and the infection hyphae and encloses the haustorium to form a sheath or extra-haustorial membrane. McKeen (1974) confirmed the sheath membrane to be an invaginated portion of the epidermal cell plasma membrane and showed that, in association with the haustorium, it thickens, becomes more osmiophilic and that

invaginations towards the haustorium are frequently present. Kunoh (1972) demonstrated that the sheath membrane is a unit membrane but McKeen showed that the transparent central stratum, typical of conventional unit membranes, is lost from the plasma membrane when it encapsulates the haustorium.

The hypersensitive reaction (HR) of cells to plant parasites was reported first by Ward (1902) for Bromus spp. parasitised by Puccinia dispersa. It is usually regarded as the local reaction of a resistant plant to infection (Wood, 1967). The extent and characteristics of HR vary in different combinations of host cultivar and pathogen race. White and Baker (1954) have described the hypersensitive reaction of barley to mildew. Infection of epidermal cells of highly resistant plants by a single haustorium is followed by collapse of the adjacent mesophyll cells. In resistant plants this is delayed until three or four haustoria have formed. In contrast, in what are described as semi-resistant plants, there is no collapse of mesophyll cells until up to ten haustoria have formed in the epidermal cells and when this occurs a considerable number of mesophyll cells are involved in the HR.

At first the restriction of biotrophic fungi by the host's HR was explained simply that in these diseases killing of host cells meant that growth of the parasite in the necrotic tissue was limited due to the starvation of the parasite. But eventually it was realized that some of

the characteristic features of hypersensitive responses of tissues attacked by fungi such as the rusts and mildews, also appear in diseases caused by fungi capable of necrotrophic growth habits such as Phytophthora infestans. P. infestans in certain tissues of susceptible hosts does not cause death of the cells in invaded tissues and is capable of forming haustoria although it does not do so readily. P. infestans, however, is not restricted in its growth to living cells. The mere fact that host cells are killed will not explain inhibition of growth of the parasite in such tissues. Furthermore, fungi such as Colletotrichum, Helminthosporium and Septoria typically cause necrotic spots on leaves and other parts of susceptible plants. This necrosis does not contain the fungus but enlarges, and as it does so the parasite continues to spread through the tissues. In resistant plants, however, necrosis is very limited and does restrict further development and there may be little to distinguish the reaction from HR of resistant plants to rusts and mildews.

Muller and his coworkers observed that if potato slices are inoculated with zoospores of a race of P. infestans to which they are resistant and are subsequently inoculated with zoospores of a race to which they are highly susceptible, the virulent race develops freely on the surfaces where no previous inoculation was made but only poorly where the surface has previously been inoculated with the avirulent strain (Muller and Borger, 1941). This and sub-

sequent work led Muller to propose the existence of phytoalexins, which are non-specific fungitoxic substances induced by the avirulent pathogen. Other phytoalexin-like substances have since been characterized and probably play an important role in the resistance of plants to phytopathogenic fungi (Cruikshank, 1963). However, there is little evidence as yet that such compounds are involved in the specificity of plants to rusts and mildews.

In spite of the potential value of electron microscopy to help elucidate the relationship of host and parasite in resistance and in hypersensitive reactions, there have been very few studies on the fine structural changes associated with these phenomena. Most of what has been done has been concerned with rust infections.

Ehrlich and Ehrlich (1962) and Shaw and Manocha (1965b) were the first to incorporate resistant varieties in their studies of the fine structure of host-parasite interactions. Both are studies of Puccinia graminis infection of wheat. Ehrlich and Ehrlich (1962) observed an encapsulation surrounding the haustorium of P. graminis f. sp. tritici. This encapsulation was present on all the varieties examined whether of high or low infection type. In the susceptible 'Little club' variety the encapsulation is rich in particulate material while on the resistant varieties 'Tunstein X' and 'Reliance' only a minimum of particulate material is present. They observed dead haustoria in all the varieties but most frequently in the semi-

susceptible variety 'Chinese' which developed extensive chlorosis and necrosis around infection sites. They found that haustoria in the more susceptible varieties tend to have numerous mitochondria and rather well developed endoplasmic reticulum (ER) but that haustoria in the resistant varieties tend to have fewer mitochondria and less ER.

Shaw and Manocha (1965b) showed that the haustoria of Puccinia graminis f. sp. tritici are characterized by numerous mitochondria, an extensive ER, densely packed ribosomes and a well defined plasma membrane which is often invaginated by lomasomes. They could not see any difference in the number of mitochondria and the amount of ER in the susceptible 'Little club' and the resistant 'Khapli'. Many haustoria on the resistant variety were necrotic. They also observed a granular encapsulation of the haustoria which separated the haustoria from the invaginated host protoplast. The encapsulation, they considered, was mainly secreted by the host and developed more quickly in the resistant varieties than in the susceptible. Presence of haustoria induced in the host the formation of an extensive smooth ER and contraction and fragmentation of the vacuole, an increase in cytoplasmic volume and ultimately the degeneration of the host cells. They considered that the breakdown of subcellular organelles in the wheat cells was similar to that which had been reported to occur in detached senescing leaves.

In a comparative fine structural study of Puccinia

catharmi infection of seedling-rust resistant and susceptible safflower, Zimmer (1970) observed that the fine structure of intercellular hyphae and haustoria of P. catharmi is similar to that of other Puccinia spp, but in contrast to observations on P. graminis the encapsulations (or sheath) of P. catharmi were not of a single amorphic structure but were composed of three distinct regions. He found that the resistance of safflower (Nebraska 1-1-5), resulted from 'cytoplasmic inhospitability of the cells of the perivascular region', and was manifested by cytoplasmic collapse, degradation of the haustorial encapsulation and the apparent deprivation of the haustorium of nutrients essential for glycogen synthesis as glycogen was abundant in haustoria in the perivascular region of the susceptible 'Nebraska 8' but absent in the 'Nebraska 1-1-5' variety.

Crystal-containing microbodies were abundant in cells in the region where exclusionary seedling-rust resistance was operative. These bodies disappeared rapidly in rust-infected cells and Zimmer suggested that they may play a role in the incompatibility of this variety.

Heath and Heath (1971) compared the ultrastructure of the susceptible and immune reactions of Vigna sinensis leaves infected with Uromyces phaseoli var vignae. Signs of incompatibility were detectable in the immune cultivar during the early stages of haustorial formation when a deposit of callose-containing material was formed on the host cell wall around the point of entry of the haustorium.

No such reaction occurred on the susceptible variety. Haustorial formation in the immune variety examined resulted in either the simultaneous necrosis of both host cell and haustorium or the enclosure of the living haustorium in the callose-containing encapsulation which, they speculated, may have been derived from the activity of dilated rough ER. The first observable reaction to occur, however, was that the host plasma membrane around the haustorium became convoluted and deposits of phospholipid-like material aggregated with the membrane.

Heath (1972) extended this work to examine more varieties which exhibited resistance and included the non-host Phaseolus vulgaris. The non-host responded to each infection hypha by the deposition of the electron-opaque material on and within the surrounding host cell walls. These deposits prevented haustorial formation in 95% of the infection sites. In two immune cowpea varieties, however, there was no indication of resistance detected until after the formation of the primary haustorium. One of these varieties showed a similar resistant response to that observed earlier (Heath and Heath, 1971). In the other immune variety haustorial development was retarded and all the host cell membranes were dotted with osmiophilic material. These initial responses were followed in both varieties by a simultaneous necrosis of host cells and haustoria.

She also examined the intermediate variety, which re-

acted to infection by the production of a small necrotic fleck four or five days after inoculation. No signs of resistance were present during the initial stages of penetration or haustorial formation. The eventual slow disorganization of invaded cells involved the fragmentation of host cytoplasm, followed by a disintegration of most of the host cell membranes. Haustorial disorganization did not immediately follow that of the host cell.

Recently Skipp et al. (1975) examined resistance conferred by the temperature sensitive Sr6 gene in near-isogenic lines of wheat. Mesophyll cells of the resistant line which had undergone a rapid necrotic response to haustorial invasion were collapsed and lacked the thin layer of cytoplasm that bounded the vacuole in the susceptible line. Instead, the lumen of the cells was filled with an electron-dense material of mixed cytoplasmic and vacuolar constituents containing organelles in various stages of disorganization. The haustoria within such cells were necrotic as were their mother cells. Infection hyphae proximal to the haustorial mother cell showed little evidence of damage. Cells adjacent to host cells showing necrosis were often vesiculated. A deposit of electron-opaque material was observed between the wall of a necrotic cell and its plasma membrane and a similar deposit was present close to the adjoining wall of an adjacent non-necrotic cell.

An ultrastructural study of resistance of the French

bean, Phaseolus vulgaris, to anthracnose caused by Colletotrichum lindemuthianum, revealed that the hypersensitive death of cells appeared to be the most important cause of resistance (Mercer et al., 1974). Walls of killed cells became granular but this granularity did not extend beyond the middle lamella. Pits between killed and adjacent living cells became occluded. The volume of cytoplasm in living cells of infected resistant hypocotyls increased about three fold, nuclei were convoluted and chlorophyll was not removable by the usual solvents. Hypersensitivity was observed in all resistant combinations examined and in some susceptible combinations. Densely staining particles were observed along the plasma membrane in otherwise normal cells adjacent to cells in which the hypersensitive response was incomplete. Similar membrane responses were observed by Skipp et al. (1975) and Heath (1972) as mentioned previously. Mercer also observed reaction material in cells adjacent to hypersensitive cells deposited between the plasma membrane and the cell wall. This material was also found in non-hypersensitive combinations. This material was largely composed of callose and often contained organelles and possibly other cytoplasmic components which had lost their identity and became electron-dense.

Shaw and Manocha (1965b) attributed the ultrastructural changes that do eventually take place in wheat susceptible to P. graminis to premature senescence and showed that natural senescence in the same plant species is accompanied

by similar responses (Shaw and Manocha, 1965a). Heath (1975) noticed that conspicuous changes in chloroplast ultrastructure in cowpea rust infections, which occur in susceptible hosts after sporulation, more closely resemble chromoplast development in some ripening fruits. As infection by rusts is known to enhance ethylene production (Daly et al., 1970) and as the ability of ethylene to accelerate senescence and its role in natural fruit ripening is well known (Abeles, 1973), she compared the changes in chloroplast ultrastructure induced by rust infection of cowpea leaves with those occurring with natural and ethylene-induced senescence. Ethylene treatment of healthy leaves did induce changes similar to those seen during the later stage of pathogenesis and detached infected leaves released greater amounts of this gas than did controls. Chromoplast-like changes were restricted to the pustule centre and she suggested that most of the increased gas production was associated with this region of high fungal concentration. Naturally senescing leaves contained only one sixth of the ethylene content of infected leaves. The first chloroplast changes involved an increase in the size of plastoglobuli similar to what occurred in natural senescence. Later development, however, involved the development of prolamellar-like bodies and peripheral vesicles in some chloroplasts and thylakoid breakdown and carotenoid crystalloid formation in others, changes which resembled chromoplast development in certain fruits. This

evidence suggests that ethylene may play a role in the change of chloroplast ultrastructure that occurs during rust infection.

Ultrastructural studies on resistance of plants to powdery mildews have been fewer than those of rust infections. Generally these powdery mildew studies have concentrated on primary infection and resistance expressed at this time (Leong, 1971; McKeen and Bhattacharya, 1969; Stavely et al., 1969; Stanbridge et al., 1971).

McKeen and Bhattacharya (1969) examined very few infection sites in the electron microscope of E. graminis on Algerian barley. About two days after inoculation they observed that the penetration of the host cell by the fungus was stopped in the papilla and that the infected epidermal cell and the appressorium were necrotic. Stanbridge et al. (1971) found no difference during the primary penetration process between the susceptible and resistant varieties examined. No resistant mechanisms attributable to specific genes were expressed before haustorial formation.

Stavely et al. (1969) examined resistance of clover to E. polygoni. Degeneration of cellular organelles occurred in the resistant cells soon after infection. In infected resistant cells they observed that not much of the sheath membrane was destroyed within 20 hr after inoculation. They thought that destruction of the host plasma membrane resulted in the elimination of a membrane barrier between the host cytoplasm and sheath matrix and that this re-

sulted in the death of the host cell and the fungus.

Leong (1971) studied the same host-parasite combination as did Stavely et al. He reported that the penetration process was similar in both the susceptible and resistant hosts. The failure of the fungus to establish a compatible relationship with the resistant host, he thought, was mainly due to the lack of response of the resistant cells and the early degeneration of these cells. He observed that the collar of the resistant host was much shallower than in the susceptible cell.

Although in recent years electron microscope techniques have been available their use in the study of the aforementioned problems has been incomplete. This is especially so with respect to the problems of host-parasite interaction and disease resistance. In the earlier studies a developmental approach was lacking, due no doubt in large part to the technical difficulties associated with sectioning through penetration sites, a situation quite analogous to searching for a needle in a haystack!

Bracker (1967) pointed out that most of the studies of host-parasite interactions used material one or more weeks old. It is gratifying to notice that since then the situation has changed and that the more recent studies have attempted to describe the ontogenic aspects of pathogenesis. This approach was adopted in the present study. Because of the paucity of fine structural studies on the effects of resistant genes on the host and pathogen, it was

felt that a useful contribution to our knowledge of this important phenomenon could be made by comparing the development of powdery mildew on susceptible and resistant hosts.

Erysiphe graminis f. sp. hordei was chosen for this study because of its economic importance as a parasite of barley and because of the availability of near-isogenic susceptible and resistant varieties. The Algerian variety was chosen because it is highly resistant and the Kwan variety was chosen because it demonstrates an intermediate resistant reaction. Moreover an ultrastructural study of powdery mildew on these hosts would complement the physiological studies conducted by researchers elsewhere employing these same varieties.

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

In this investigation the fungal pathogen was Erysiphe graminis DC. f. sp. hordei race CR3. The hosts were varieties of barley, Hordeum vulgare L. as listed below.

Keystone	Used for routine maintenance of the fungus
Algerian x	Resistant Mla/mlk.
Manchurian	Susceptible mla/mlk.
Kwan x	Resistant mla/Mlk.
Manchurian	Susceptible mla/mlk.

The fungal pathogen, race CR3 of E. graminis f. sp. hordei and the near-isogenic lines of barley were kindly supplied by Dr. J. G. Moseman, USDA, Beltsville, Maryland, U.S.A.

3.2. Growth and maintenance

Barley plants were grown from seeds in the greenhouse

in three or six inch pots. The temperature ranged from a minimum of 15 C to a maximum of 24 C during the winter months. During the summer, however, the temperature often rose to 32 C or more in accordance with the external temperature. Seedlings were often almost wilting from the effects of high temperature, and grew more quickly but with a limpness and a paler coloration than occurred at lower temperatures. Such plants were not used for experimental purposes but were discarded or used, as were healthy plants from time to time, as indicators to determine that the mildew had not changed its virulence characteristics on the varieties of barley employed.

Seedlings were grown in soil consisting of loams; peat moss; black muck; sand in the proportions 6:3:2:2 and were lightly watered each day. The variety 'Keystone' was used routinely for maintenance of the mildew which was kept in a controlled environment room. This room was programmed for a 15 hr day and a 9 hr night. The day temperature was maintained at 20 C, with 5350 lux illumination and a relative humidity of about 65%.

Mildewed plants were used to inoculate fresh 'Keystone' seedlings every week or two and discarded when young colonies were observed on the fresh plants. The main parasites of barley in the greenhouse and in the controlled environment room were white fly and mites, which were controlled, when necessary, by spraying usually with Malathion and Lindane. Occasionally plants grown in the

greenhouse would be infected by powdery mildew either from outside or from contamination from the separate stock culture. As the origin of the infection of these plants could not be determined, these plants were discarded to prevent unknown races, perhaps with different reaction from the CR3 mildew on the resistant varieties, from contaminating the experimental race.

3.3. Inoculation of experimental plants

Experimental plants were inoculated with fresh conidia from the maintained stock culture. For light microscopy, plants were inoculated simply by shaking pots of infected plants over the uninfected plants and allowing the spores to settle onto the leaves of these plants. This method of inoculation was unsatisfactory for electron microscopy because the density of spores landing on any particular leaf area was low. Leaves that were intended for EM preparation were marked with Indian ink delineating a section of the leaf about 2 cm long and this marked portion of the leaf was inoculated on either the underside or both sides by means of a camel hair brush or by directly pressing a heavily infected leaf against the marked portion of the leaf.

3.4. Incubation of inoculated plants

For best infection and a high rate of germination, the plants were transferred after inoculation to the experimental

cabinet programmed to the regimen of light, temperature and relative humidity shown by Masri and Ellingboe (1966b) to be most effective for synchronous high germination, appressorial differentiation and primary infection. The environmental cycle employed was as follows:

1. 18 C, darkness and 95% R.H. during the first hour after inoculation.
2. 22 C starting the second hour after inoculation.
3. Light (1572 lux) during the 2-6th hour after inoculation. Darkness during the 7-16th hour, light after the 16th hour.
4. 65% R.H. beginning the second hour after inoculation.

This cycle was used for all studies on primary infection. From time to time the growth cabinet was checked with a recording hygrometer to ensure that it was operating in the required manner.

3.5. Light microscopy methods

For light microscope observations of germination of conidia on glass slides, conidia were collected by directly shaking leaves over the slides. For the observation of the development of the fungus on the host the following method was employed.

3.5.1. Lactophenol trypan blue method

This method involved the cold clearing of leaf segments

in a mixture of methanol; chloroform; lactophenol in equal proportions. Leaf segments were immersed in this solution for 2 days, then removed into a dilute lactophenol trypan blue stain (0.005% trypan blue). The leaf segments were immersed in the stain for 1-2 days, then mounted in lactophenol trypan blue and examined as wet mounts under cover slips sealed with Glyceel. This method provided excellent clearing of the leaf and normally only fungal material was stained with this concentration of trypan blue. This method was employed for the experimental studies on primary infection of susceptible and resistant hosts.

3.6. Electron microscopy methods

Healthy and infected portions of whole leaves were cut in 1 mm^2 portions, or punched with a hypodermic needle with the tip squared off to resemble a miniature cork borer of about 1 mm in diameter. For primary infection studies, heavily inoculated leaf areas marked with Indian ink were punched out with the hypodermic needle. Leaf segments and disks acquired in these ways were placed immediately into fixative.

3.6.1. Fixation

a. Glutaraldehyde with post osmification

Material was fixed in 4% glutaraldehyde in 0.1 M sodium cacodylate buffer with 1% sucrose at pH 6.8. Material was fixed for 30-120 min at 4 C or room tempera-

ture. It was then washed with three changes of buffer solution for 3-6 hr or overnight. Leaf segments were post-fixed in 1% osmium tetroxide for 30 min then washed with distilled water. Material was usually stained at this time, after being warmed to ambient temperature, in 5% magnesium uranyl acetate for 20 min.

b. Glutaraldehyde with simultaneous osmium fixation

Leaf disks were placed in a glutaraldehyde-osmium tetroxide mixture which was made by mixing ice cold stock solutions of glutaraldehyde (4%) and osmium tetroxide (2%), both buffered with 0.1 M sodium cacodylate with 1% sucrose at pH 6.8 and used immediately (Franke et al., 1969). Material was fixed for 30 min at 5C before being rinsed in cold 0.1 M cacodylate buffer. Material was post-fixed in buffered 2% osmium tetroxide for 5-10 min. Then it was rinsed in distilled water and warmed to room temperature before staining in 5% magnesium uranyl acetate for 20 min.

3.6.2. Dehydration

Material was passed through ethanol solutions of increasing concentration from 30% through 50%, 70%, 95% to absolute alcohol. Material was left 15 min in each solution from 30-95% followed by two changes of absolute alcohol each for 20 min.

3.6.3. Infiltration

After dehydration, specimens were cleared with propylene oxide, three changes of 15 min each. Specimens were then placed in a 1:3 epon-araldite/propylene oxide mixture. The epon-araldite was prepared by mixing 30 ml of DDSA (dodecenyl succinic anhydride) with 10 ml of Araldite 6005 and 12 ml of Epon 812. These were thoroughly mixed, 0.4-0.8 ml of DMP (dimethyl amino methyl phenol) accelerator was added and the plastic was mixed thoroughly again. The plastic was allowed to settle for a while and then added to the material in propylene oxide in a 1:3 proportion for 1 hr, then with a 2:1 mixture for 1 hr. This was replaced with pure plastic and left on a rotary mixer for at least 24 hr.

3.6.4. Embedding

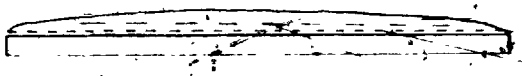
Material was transferred to fresh pure plastic for 2-6 hr. Material was embedded in a thin layer of plastic on carbon-coated glass slides or in flat plastic holders. Slides were coated with carbon in a shadow casting/carbon evaporation unit. The embedding slides were hardened in a 60 C oven for 24-48 hr. The slides were then examined with the light microscope to select and mark disks for sectioning. After stripping the plastic from the slide, suitable disks were cut from the plastic with a pair of scissors and re-embedded in flat plastic holders. In this way the conidia and appressoria could be easily located and orientated in the desired way for sectioning. Blocks were rough

inoculation, a fine peg, which was extruded from the appressorial lobe, penetrates through the epidermal cell wall and the papilla beneath the penetration site. By 15 hr a bulbous pear-shaped haustorium is formed within the lumen of the cell in about 50% of the attempted infections on the non-resistant host, Manchurian (Fig. 1, 2, 3). After 18 hr the haustoria are elongated and are beginning to change their orientation from that of being almost vertical to the external cell wall to a position more or less parallel to it (Fig. 4). After 21 hr, elongating secondary hyphae begin to form as an extension of the main appressorial body and the haustoria are beginning to produce lobes at each of their ends (Fig. 5). The secondary hyphae are elongated after 26 hr to a length of 25 µm or more and branch hyphae are formed originating from the germ tubes which produced the appressoria. The haustoria are enlarged and their lobes well developed (Fig. 6. Diagram 3.1-6 illustrates this sequence of development).

4.1.3. Incompatible interactions

Two different types of incompatible interactions were observed during the first 24 hr after inoculation. The first type was seen on the Manchurian host and on both the resistant hosts. The second type was observed commonly on the Algerian host but only rarely on the Kwan and Manchurian hosts.

Diagram 2.

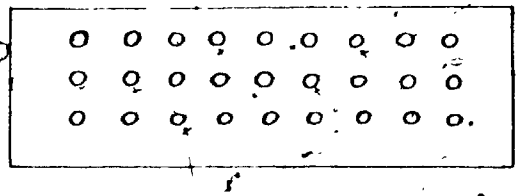


plastic

carbon film

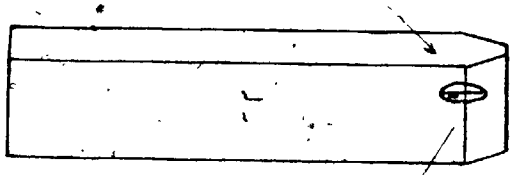
leaf disk

1.
Material embedded on glass microscope slide.

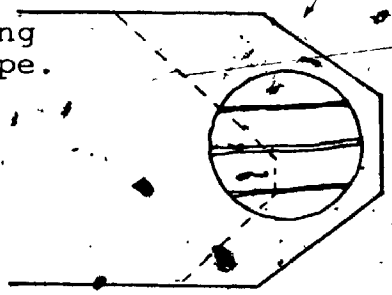


2.
Leaf disks with penetration sites selected under microscope and re-embedded in flat capsule.

conidium and appressorium



3.
Capsule trimmed to penetration site with aid of dissecting microscope.



plastic trimmed to here.

Technique of selection of primary penetration sites for electron microscopy.

Sodium hydroxide pellets were placed at one side of the dish to absorb moisture and carbon dioxide from the air within the dish to minimise contamination due to carbonate precipitation. Each grid was floated face down on the surface of a drop of stain and the dish was then covered. After the desired time had elapsed, the grid was picked up with the fine forceps and washed quickly with 10-15 drops of glass distilled water. The grids were dried and kept on filter paper in a petri dish for examination in the electron microscope.

3.6.7. Electron microscopy

The stained sections were observed in a Philips 200 EM at 60 or 80 KV. Single or double condenser lenses were used. The objective aperture was 40 µm. Photographs were taken either with 30 mm high contrast roll film or 3 1/4 - 4 inch sheet film at different magnifications as required.

3.6.8. Photography and magnification

Exposed film was developed with Kodak D19 developer. Pictures were printed with D72 developer. Different grades of Kodabromide single weight printing paper (F_2-F_5) were used depending on the contrast of the negative. Normally F_3 and F_4 papers were used. The approximate magnification was calculated by using the calibration of the electron microscope and the magnification produced by the enlarger.

CHAPTER 4

OBSERVATIONS AND RESULTS

4.1. Light microscopy

4.1.1. Introduction

Light microscope observations were employed in this study as an adjunct to the ultrastructural investigation. They were especially useful in elucidating the sequence of events occurring during the primary penetration process and the subsequent interactions of the fungus and the barley leaf, which determine whether or not a functional parasitic relationship will be established.

Leaf segments of the barley genotypes were excised at various intervals of time after inoculation with conidia of the mildew, cleared, stained and wet mounts in lactophenol were examined for mildew development. The most effective clearing technique was the methanol-chloroform-lactophenol method. For most purposes a dilute solution of trypan blue in lactophenol was used for staining.

No attempt was made to estimate the germinability of the conidia, because it was impossible to ascertain with this method how many ungerminated spores were washed from the leaf surface during the clearing and staining pro-

cedures. Conidia which had produced appressoria were infrequently dislodged and so counts were expressed as percentages of the number of spores which had produced appressoria.

4.1.2. Compatible Interactions

Compatible interactions, for the first three days after inoculation, were deemed to be those infections in which the appressorium produced an infection peg which had successfully penetrated the epidermal cell of the leaf to form a haustorium and continued in its development to form an elongating secondary hypha (Fig. 5, 6). Such infections occur most frequently on the susceptible genotypes of barley, almost as frequently on the resistant Kwan genotype and rarely on the Algerian variety (Tables 2, 3, 4).

Conidia produce usually two germ tubes, about 7 µm in length and 2 µm in diameter. One of the germ tubes differentiates to form an appressorium. Conidia produce appressoria on all varieties by 10 hr after inoculation. The appressoria normally grow along and within the epidermal grooves between adjacent epidermal cells. The ends of the appressoria are characterized by a short terminal lateral arm, 5-8 µm in length, which was termed the appressorial lobe. Penetration of the epidermal cell was never observed to occur except from such a structure.

The appressoria contain a single nucleus situated just behind the appressorial lobe. Between 10 and 15 hr after

inoculation, a fine peg, which was extruded from the appressorial lobe, penetrates through the epidermal cell wall and the papilla beneath the penetration site. By 15 hr a bulbous pear-shaped haustorium is formed within the lumen of the cell in about 50% of the attempted infections on the non-resistant host, Manchurian (Fig. 1, 2, 3). After 18 hr the haustoria are elongated and are beginning to change their orientation from that of being almost vertical to the external cell wall to a position more or less parallel to it (Fig. 4). After 21 hr, elongating secondary hyphae begin to form as an extension of the main appressorial body and the haustoria are beginning to produce lobes at each of their ends (Fig. 5). The secondary hyphae are elongated after 26 hr to a length of 25 µm or more and branch hyphae are formed originating from the germ tubes which produced the appressoria. The haustoria are enlarged and their lobes well developed (Fig. 6. Diagram 3.1-6 illustrates this sequence of development).

4.1.3. Incompatible interactions

Two different types of incompatible interactions were observed during the first 24 hr after inoculation. The first type was seen on the Manchurian host and on both the resistant hosts. The second type was observed commonly on the Algerian host but only rarely on the Kwan and Manchurian hosts.

a. Prevention of penetration

The mechanism of the first type involved the prevention of the penetration peg from passing through the wall or the host deposit beneath the penetration site. The deposit is sometimes very large (up to 5 µm) but usually appears as a stained swelling beneath the wall about 2 µm thick. Penetration is usually attempted close to a lateral epidermal wall and this is frequently stained locally to the penetration. Haloes around the infection site are sometimes present (Fig. 1, 5, 7, 8).

The appressoria then form a secondary appressorial lobe opposite to the first one or at the other end of the appressorium close to the septum with the germ tube, and attempt infection of the adjacent epidermal cell (Diag. 3.7, 3.8; Fig. 5, 7, 8). If this, too, is unsuccessful a third appressorial lobe is occasionally formed. Secondary appressorial lobes are formed about 20 hr after inoculation and tertiary lobes about 25 hr after inoculation. The host responds in a similar way to secondary and tertiary lobes as it does to primary appressorial lobes.

The incidence of infection from secondary and tertiary appressorial lobes is usually very low, less than 1% of the primary unsuccessful penetrations, and the development of such colonies is much slower compared with the penetrations from primary appressorial lobes. By 48 hr they are only just beginning to form an elongating secondary hypha.

It was noticed that cells overlying the leaf vascular tissues were more commonly associated with this type of unsuccessful penetration than the interveinal epidermal cells. This was true on all the varieties. Epidermal cells overlying the leaf vascular bundles account for about 20% of the leaf surface area whereas only about 6% of the appressoria that formed haustorium and elongating secondary hypha were observed to be established on these cells (Table 5).

b. Epidermal cell staining

It was observed, mainly on the Algerian host but infrequently on the Kwan host, that some of the epidermal cells penetrated by an infection peg from a primary appressorial lobe became completely stained with the trypan blue dye. The staining was most prominent in the cell wall (Fig. 11, 12). This staining reaction was not observed until about 21 hr after inoculation. Appressoria associated with penetrations resulting in the whole-cell staining reaction do not produce secondary or tertiary appressorial lobes. At least 25-35% of such attempted infections result in the formation of a visible haustorium within the cell, which is elongated to about the 18 hr stage of development. No elongating secondary hyphae are formed which suggest that the fungus is rapidly arrested in its development (Diag. 3.9). Whole-cell staining reactions are more commonly associated with the interveinal cells of

the leaf, cells which in a compatible host are more likely to be associated with a successful penetration.

4.1.4. Kinetics of infection

The data of a typical time course experiment on appressorial formation, penetration and production of elongating secondary hyphae are presented in Tables 2, 3, 4. There were wide variations between experiments in the ratio of successful to unsuccessful infections, and the age of the leaf at the time of inoculation was thought to be important in this respect. Low values for successful penetrations, 30% on Manchurian, 30% on Kwan and 0.1% on Algerian were obtained on 14 day old leaves. The higher figures, shown in the tables, were obtained on 7 day old leaves.

There was little difference in the infection rate between the Kwan and Manchurian except at 46 hr after inoculation. There were virtually no whole-cell staining resistant responses on the Manchurian and only about 4% of the appressoria on the Kwan incited this response. About 45% of the attempted infections of both these varieties were unsuccessful. About 50% of the attempted infections on the Algerian incited the whole cell staining response and the other 45% unsuccessful attempts were of the prevention of penetration type. Successful penetrations on Kwan and Manchurian developed similarly and by 48 hr colony development had occurred and secondary

Diagram 3. Early development of Erysiphe graminis on susceptible and resistant barley varieties.

1a, b. Appressorial formation. 10 hr.

2a, b. Formation of penetration hypha. 11 hr.

3a, b. Formation of incipient haustorium. 14 hr.

4. Elongation of haustorium. 18 hr.

5. Formation of elongating secondary hypha and change in position of haustorium. 21 hr.

6. Formation of branch hypha. 26 hr.

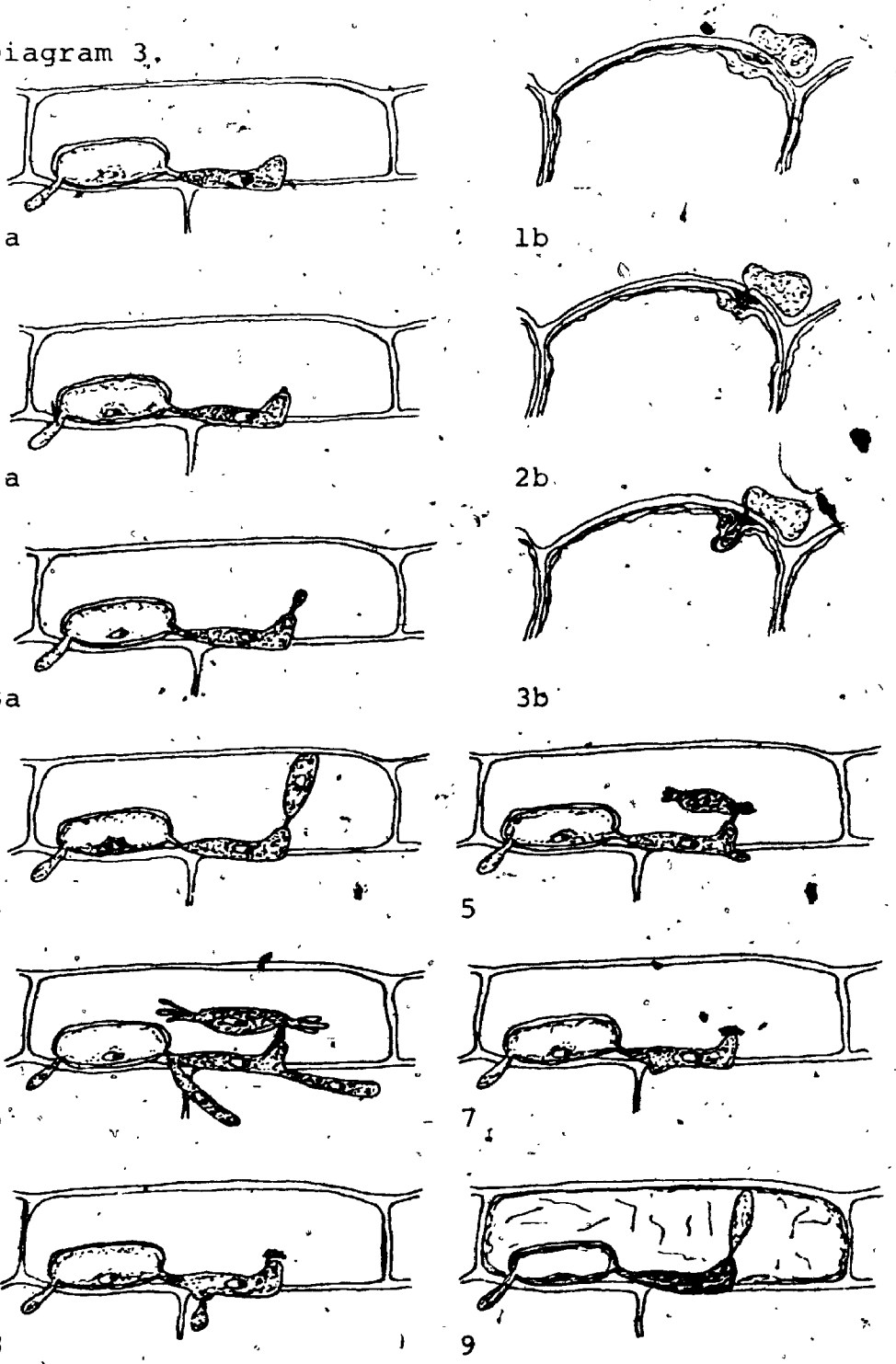
7. Papilla-stopped penetration from primary appressorial lobe and formation of a secondary appressorial lobe. 21 hr.

8. Formation of incipient haustorium from secondary appressorial lobe. 26 hr.

9. Hypersensitive reaction of epidermal cells as observed on the Algerian host. 20 hr.

Figures 1-8 depict stages which occurred frequently on all varieties. All figures surface views looking down on leaf epidermis except 1b, 2b, 3b which are transections through the appressorial lobe and epidermal cell as seen in sectioned material. (See text for full explanation and significance).

Diagram 3.



Early development of Erysiphe graminis on susceptible and resistant barley varieties.

Table 2. Infection of Manchurian variety. Extent of fungal development at various times after inoculation (expressed as percentage of total appressoria and related to the staining response of the infected epidermal cell).

fungal development	time after inoculation			
	15 hr	20 hr	26 hr	46 hr
appressorium only formed				
epidermal cell stained	0.0	0.0	0.2	0.6
epidermal cell unstained	35.9	46.5	42.5	27.2
appressorium and haustorium formed				
epidermal cell stained	0.0	0.0	0.0	0.3
epidermal cell unstained	64.1	53.3	8.0	0.7
appressorium, haustorium and E.S.H. formed				
epidermal cell stained	0.0	0.0	0.0	0.0
epidermal cell unstained	0.0	0.1	49.3	71.2

Table 3. Infection of Kwan variety. Extent of fungal development at various times after inoculation (expressed as percentage of total appressoria and related to the staining response of the infected epidermal cell).

fungal development	time after inoculation			
	15 hr	20 hr	26 hr	46 hr
appressorium only formed				
epidermal cell stained	0.8	2.1	3.6	3.8
epidermal cell unstained	38.5	42.4	42.6	38.2
appressorium and haustorium formed				
epidermal cell stained	0.0	0.2	0.0	0.6
epidermal cell unstained	60.6	51.5	8.4	0.8
appressorium, haustorium and E.S.H. formed				
epidermal cell stained	0.0	0.0	0.0	0.0
epidermal cell unstained	0.0	3.8	45.3	56.5

Table 4. Infection of Algerian variety. Extent of fungal development at various times after inoculation (expressed as percentage of total appressoria and related to the staining response of the infected epidermal cell).

fungal development	time after inoculation			
	15 hr	20 hr	26 hr	46 hr
appressorium only formed				
epidermal cell stained	0.0	3.4	25.2	31.7
epidermal cell unstained	65.7	55.6	55.5	44.9
appressorium and haustorium formed				
epidermal cell stained	0.0	2.2	7.3	16.0
epidermal cell unstained	34.3	38.7	2.7	2.5
appressorium, haustorium and E.S.H. formed				
epidermal cell stained	0.0	0.0	0.0	0.0
epidermal cell unstained	0.0	0.0	9.3	4.9

Table 5. Relationship between successful infection and type of epidermal cell on each of the barley varieties.

type of cell	infection of host variety*					
	Manchurian		Kwan		Algerian	
	26 hr	46 hr	26 hr	46 hr	26 hr	46 hr
Guard cell	0.3	0.3	0.1	0.0	0.0	8.7
subsidiary guard cell	4.4	3.6	4.0	4.3	15.7	13.1
small interveinal cell	48.3	45.8	51.8	43.7	27.6	42.0
long interveinal cell	40.8	34.9	38.0	32.7	49.3	31.9
cells overlying vascular tissue	6.2	15.4	6.1	19.3	7.4	4.3

*Figures are percentages of total number of appressoria with haustoria and E.S.H. at 26 hr and 46 hr after inoculation. Guard and subsidiary cells account for 3-4% of the leaf surface area (Hirata, 1967) and cells overlying vascular tissue for about 20% of the leaf surface area.

haustoria were being formed (Fig. 9). The successful infections on the Algerian host were much slower in comparison and little branching had occurred by this time (Fig. 10).

The resistance of the Kwan gene was effectively expressed only after 72 hr after inoculation, when a necrosis of mesophyll cells started to occur beneath the developing colony. This necrosis continued thereafter resulting in large necrotic flecks on the leaf, plainly visible to the naked eye, by 4 days after inoculation, which resulted in the retardation and restriction of further colony development and few conidia were ultimately produced on this host.

4.2. Electron microscopy - Early interactions

4.2.1. Compatible interactions

a. Formation and structure of the appressorium

The mature conidium contains much glycogen in rosette clusters and it is presumably the metabolism of the glycogen which supplies the spore with energy and material for development, because the conidium, at a later stage, is mainly vacuolated and contains little glycogen. The germ tubes are produced from the elliptical spore on the underside, ie. close to the leaf surface (Fig. 13, 14). The wall of the conidium is composed of two layers, an outer spiny, more electron-dense layer and an inner layer adjacent to the plasma membrane. The cell wall of the germ tubes and the

appressorium is a continuation of the inner wall (Fig. 13, 14, 15). The germ tubes are an extension of the conidium and contain glycogen rosettes and vacuoles which may be extensions of the large central vacuole of the conidium (Fig. 13). The cytoplasm of the spore and its germ tubes also contains mitochondria and ribosomes (Fig. 13, 15). The appressorium is formed from one of the germ tubes. The germ tube swells and elongates, the nucleus of the conidium divides and one of the daughter nuclei moves into the appressorium, which is then separated from the germ tube by the formation of a septum.

The appressorium is a tubular cell, filled with dense cytoplasm and about 25 µm long and 5 µm in diameter (Fig. 14). There was no vacuolation or evidence of the presence of glycogen during the early stages of primary penetration. The nucleus is situated about one third of the length of the appressorium back from the distal end. The nucleus occupies the central part of the cell in cross section (Fig. 17), and is ringed by numerous small vesicles, mitochondria, ER and ribosomal particles. The nucleus is enclosed by the nuclear membrane, and includes an electron-dense nucleolus and a less dense granular matrix in which electron-dense chromatinic regions are dispersed.

Usually, the main appressorial body grows within and along the groove formed by the junction of two adjacent epidermal cells beneath which is their common lateral wall

(Fig. 17, 18). The terminal part of the appressorium is characterized by a short limb or lobe which is produced on one side, commonly at right angles to the main appressorial body. A section through the main body of the appressorium, just behind the lobe, shows mitochondria, small vesicles and lipid bodies enveloping the central portion of the cell (Fig. 18). This central region is noteworthy for the presence of dense polyribosomal structures enclosed by discontinuous membranes. This region is probably important for protein synthesis.

The function of the appressorial lobe is the penetration of the host cell wall (Fig. 19, 20, 21, 24, etc.). An infection pore (Fig. 19, 20) forms in the central part of this appendage where this is in contact with the leaf surface. The formation of this pore is the first indication that an infection peg is about to be formed. The infection pore is surrounded by cytoplasm which contains ribosomes, ER and small vesicles. Mitochondria are present in the appressorial lobe but not in the immediate locality of the pore and occur mainly in the region which is contiguous with the appressorial body (Fig. 19, 21).

The main appressorial body contains extensive strands of ER from the ends of which small vesicles appear to be formed (Fig. 17, 19). Since a conventional golgi apparatus does not occur in Erysiphe graminis, nor in many other fungi, it is likely that the ER is responsible for the formation of small vesicles.

b. Penetration

In order for the fungus to establish itself as a living colony on the host leaf surface, it must penetrate through the epidermal cell wall and the underlying host material. The barriers it must breach consist of firstly, the cuticle, the thin external layer of the cell wall, and secondly, the cellulosic fibres of the cell wall. This is achieved by the penetration hypha, which is extruded by the fungus through the pore of the appressorial lobe. The appressorium is apparently firmly attached to the leaf surface by a mucilaginous secretion of the fungus. This material does not appear very prominently in electron micrographs but can be seen as strand-like material on the outer surface of the appressorium (Fig. 21, 29, 33). Infrequently, the appressorium is not firmly adhered to the leaf surface with the consequence that fungal cytoplasm flows out through the appressorial pore onto the leaf surface and infection is abortive. Usually, however, a peg is formed which penetrates through the cuticle and epidermal cell wall. Fig. 21, 22, 23, 24, 25, 42, 43 illustrate this, where the peg is in or just through the wall. The fungal plasma membrane at the edge of the pore is not so close to the cell wall as in most other regions of the appressorium, and, between this membrane and the wall, an electron-dense material is often present which is confluent with the penetration zone (Fig. 20, 27) and electron-lucent bodies are sometimes present (Fig. 24). The peg

hole shows little distortion and is bounded by an intensely electron-dense zone which is confined to the cell wall and does not affect the cuticle. Figures 23 and 24 show this very clearly; the cell wall is apparently eroded away by the action of the peg beneath the cuticle. In some sections the fibrillar structure of the cell wall appears to be altered (Fig. 25, 29). The wall is slightly swollen around the peg and the microfibrils of the wall are separated.

The differential staining reaction of the wall in the peg region appears minimal in compatible interactions and to be localized in the region of direct contact with the penetration peg. However, in some cases an alteration in the wall staining is present beyond the immediate zone of penetration varying in extent from 0.3 µm to 2 µm from the penetration site (Fig. 22, 29).

The epidermal wall is about 0.5 µm thick and the cuticular layer about 0.05 µm thick. The diameter of the peg is about 0.5 µm where it passes through the cell wall. The peg has a blunt tip and in most sections appears to be lacking a cell wall (Fig. 23, 24). If a cell wall is present (pegs which have passed through the wall and into the host deposition do have a thin wall), it must be exceedingly thin when it penetrates the host wall. The peg does not penetrate at right angles to the wall but at an angle of incidence of about 30-40 degrees from the vertical axis.

Once the peg is through the wall it must then pass through the papilla, the deposit of host material beneath the penetration site. In successful penetrations it is likely that this deposit was penetrated very quickly because almost all the material sectioned of compatible hosts was either of a peg in or just through the wall or the penetration hypha was in the cell lumen and forming a young haustorium.

The penetration hypha which has successfully penetrated through the wall and the papilla invaginates the host plasma membrane and the tonoplast and throughout its development remains ensheathed by these membranes and the host cytoplasm which is situated between them. The tip of the penetration peg, having passed through the papilla, enlarges to form a bulbous or pear-shaped structure which is the incipient haustorium (Fig. 30, 31). The cytoplasmic contents of the penetration hyphae are extremely dense and thus it is difficult to identify the cell organelles and cytoplasmic components inside the hyphae. Ribosomes and small vesicles are the first cytoplasmic constituents to move into the peg and haustorium, followed by mitochondria. Thick strands of membranes pass through the penetration peg into the developing haustorium (Fig. 31, 35). Nuclear movement through the penetration peg was not observed, though it is possible that the electron-dense material attached to the strands of membranes in Figure 31 is nuclear material.

A septum is laid down in the neck of the haustorium (Fig. 33), which then grows in size, forms lobes and continues to develop for two or three days before attaining its mature size.

c. Host response

The response of the host to attempted infection is already well developed by the time the appressorial lobe has formed the penetration pore. Beneath the cell wall; underneath the appressorial pore, an amorphous, electron-dense deposit forms between the plasma membrane and the cell wall. Between the plasma membrane and the tonoplast, a greatly increased concentration of host organelles accumulates surrounding the papilla.

The initial papilla material consists of an amorphous deposit of varied electron-density but, generally, of greatest density directly beneath the point of penetration and of least density at its periphery close to the plasma membrane. Sometimes, it appears to have a globose structure (Fig. 25). Fragments of membranes are frequently dispersed throughout the deposit (Fig. 20, 22, 25, 27, 29). This suggested that the deposit is probably formed by the passage of vesicles through the plasma membrane (Fig. 29) or by a pinocytic action of the membrane (Fig. 27) resulting in vesicles moving to the penetration site and aggregating below the wall there. Often a line of membranes ensheaths this region (Fig. 20, 27, 29, 43-46). The amount

of cytoplasm around the infection site increases during the course of penetration but is extremely variable from one infection site to another and does not appear to be necessarily correlated with the size of the papilla (cf. Fig. 21, 29, 42 which are all representative sections where the peg has just passed through the wall but the amount of papilla material and the amount of host cytoplasm present vary considerably).

The cytoplasm that extends around the papilla contains many mitochondria, vesicles of various size and numerous golgi bodies. With glutaraldehyde fixation it can be seen that much rough ER is associated with the initial cytoplasmic response (Fig. 20, 27). This is not so well preserved with Franke's fixation but can be observed in some sections (Fig. 29). One or two plastids are usually found in the cytoplasmic aggregation. They contain a crystalline prolamellar-like body, membranous strands and osmiophilic globuli (Fig. 26, 34). Many golgi bodies occur, sometimes as many as ten in one section, and are probably responsible for the production of the numerous vesicles in the penetration region.

Normal, uninfected epidermal cells contain little cytoplasm, the tonoplast lying adjacent to the plasma membrane except where a mitochondrion is present (Fig. 39-41). Usually the cytoplasm between these two membranes is about 0.05 μm to 0.2 μm thick. By the time the first deposit of papilla material is observed the layer of cytoplasm, ad-

jacent to the deposit, has increased in thickness to 1 µm or more and continues to increase during the penetration process up to a thickness of 10 µm. The papilla material changes also from a homogeneous or globose deposit to a double-layered deposit of varying thickness by the time the infection hypha penetrates through it. The layer adjacent to the infection site usually is more homogeneous and more electron-dense than the outer deposit. These two regions of the papilla are separated in compatible reactions by an extremely electron-dense channel into which the plasma membrane appears to be infolded (Fig. 30, 32, 33).

d. Undifferentiated germ tubes

It has been stated that only one of the germ tubes produced by the conidium differentiates to form an appressorium. Undifferentiated germ tubes do not form penetration hyphae and thus no infections are established from them. They frequently contain a membranous lamellar body, glycogen, and vacuoles. The cell wall which is in contact with the epidermal cell wall is often swollen and vesicular (Fig. 36). The cell wall beneath the germ tube tip has an increased electron-density and a granular appearance. A papilla is formed though not to the same extent as beneath a penetration site, nor is the host cytoplasm in this region increased as much as in the usual penetration site (Fig. 36, 37, 38).

4.2.2. Incompatible interactions

a. Preamble

Resistance and susceptibility of plants to their pathogens are not absolute phenomena. As was demonstrated in the light microscopy section of the results, inability to effect penetration and the establishment of a functional haustorium occur on susceptible and on resistant hosts. The Kwan variety (Mlk/mla), especially on leaves older than seven days, is little, if at all, more effective than the susceptible (mlk/mla) in preventing the fungus from establishing a primary infection. The Algerian variety (mlk/Mla) is effective. The difficulty in determining whether or not a penetrating peg would be prevented from establishing an infection rendered it necessary to look at material generally at later times than with susceptible material. Most of the material therefore was examined 15 hr after inoculation or later, by which time a functional haustorium should have been well developed if the fungus was successful in its penetration attempt.

b. Kwan (Mlk/mla)

Light microscope studies showed the frequency of unsuccessful infections during the first 24 hr after inoculation to range from 50% to 70% of the spores which formed appressoria on the leaf. Material was examined in the electron microscope in an endeavour to understand the basis for this exclusion.

Conidia germinated and developed appressoria similarly on both the Kwan and Algerian hosts as they did on the Manchurian host. Figure 42, a longitudinal section through the appressorial lobe, shows the typical features as described for appressorial structures on susceptible barley. Penetration of the Kwan host cell wall (Fig. 43-46) occurs at the same hour (eleventh) as on the susceptible. These sections complement penetration studies on the compatible host. In this instance, it appears that penetration of the cell wall resulted in a hole in the wall larger than the width of the peg. This excessively large hole is sealed by large amounts of fungal material. Again, an alteration of the cell wall beneath the cuticle has occurred, in this case on both sides of the peg. The papilla is not well developed, despite the presence of much host cytoplasm, including the nucleus, and the penetration peg is quite close to the plasma membrane of the epidermal cell.

The inability of the peg to penetrate through the papilla or its prevention from so doing was observed in about 50% of the material examined in the electron microscope. Usually, the papilla is large and extends about 5 µm into the cell from beneath the penetration site (Fig. 47, 52-53, 54-56) but this is not always the case (Fig. 48-51). The papilla is double-layered as described in the compatible interactions and the peg is usually stopped inside the inner electron-denser part of the papilla (Fig. 48, 53, 56).

The earliest indication of occlusion of the peg was at 14 hr after inoculation (Fig. 47). In this case the papilla is very large and vacuolated regions occur within the peg. At later stages of occlusion the papilla is surrounded by a dense compact region of cytoplasm, containing mitochondria, plastids, golgi bodies, vesicles and ER (Fig. 52-56).

Frequently, intense staining on the host cell wall for a considerable distance from the penetration site occurs at later times (Fig. 49, 50, 54). Most of the material examined showed that the penetration hypha penetrates deeply into the papilla but this is not always so (Fig. 48). No toxic effects or pathological changes were observed in the fungus because of occlusion, at this time. In all cases the cytoplasm of the fungal appressorium appears normal and no necrotic reactions of the epidermal cell cytoplasm occur.

c. Algerian (mlk/Mla)

The Algerian gene for resistance has been shown to be effective in preventing E. graminis from establishing a functional relationship with its host during the early stages of infection (see light microscopy results). Development of the fungus on this variety is similar to that on the Manchurian and the Kwan hosts during the penetration stages and the formation of a young haustorium (Fig. 57-61).

The appressoria have a similar orientation and

structural organization of those on Manchurian and Kwan hosts, i.e. the peg and pore region are vesicular, contain many ribosomes and mitochondria are situated similarly as in the other varieties. Alteration of the host cell wall (Fig. 59) is comparable to that of the susceptible host (Fig. 21) and the Kwan host (Fig. 50). It can be observed that the wall below the cuticle (Fig. 59) is eroded and that an extensive alteration of the electron-density of the cell wall, a halo type of reaction, is present. The host response, both in the deposition of the double-layered papilla and the concomitant aggregation of host cytoplasm around the papilla is very pronounced (Fig. 57, 58). This series of sections show a peg, either blocked by the papilla or in the process of passing through it. The extremely electron-dense areas within the peg adjacent to the plasma membrane are unusual (Fig. 59). A similar staining reaction occurs in the oblique section through the neck region of the haustorium (Fig. 63).

Pegs which have penetrated through the host barriers to form a haustorium are also apparently quite normal (Fig. 60). The young haustorium, containing numerous small vesicles, mitochondria and polyribosomes (Fig. 61) and surrounded by the host plasma membrane and the tonoplast is comparable to that observed in the susceptible host (Fig. 35).

By 21 hr after inoculation, about the time when the whole cell staining reaction was first observed in the

light microscope, many necrotic reactions were observed. Only one series of sections through a penetration site showed an established haustorium and healthy fungal and host cytoplasm. Interestingly enough, this penetration involves a guard cell, a type of epidermal cell which is rarely penetrated but which appears to be more capable of supporting a compatible type of infection than other types of epidermal cells on the Algerian host (see Table 5). Fig. 62 shows a transection through the appressorial body, similar in many respects to that of Fig. 18 on the Manchurian host, with extensive, centrally located membranes and polyribosomal clusters. The cell wall of the guard cell is normally much thicker than that of other epidermal cells (Fig. 63, 66). The path of the peg through the cell wall as shown in these sections, was four times as long as those through epidermal walls of other cells. Little mechanical distortion of the wall has occurred. The host papilla again consists of an electron-dense region (Fig. 64) close to the cell wall and surrounding the peg. The electron-lucent part of the papilla is homogeneous. The formation of small vesicles by golgi bodies occurs (Fig. 63) and mitochondria are present in the infection site (Fig. 65).

An unusual feature, observed only on the Algerian host, and only at later times, i.e. after 15 hr, is the alteration of the cell wall in the peg area (Fig. 63, 64). This electron-dense line demarks an area inside which the appearance of the cell wall is distinctly changed. The

laminar and striated structure of the cell wall is not visible in this region. In some cases (Fig. 76) it is continuous with the electron-dense papilla region and extends a considerable distance away from the penetration site, but in others (Fig. 67, 69, 70) is restricted to the cell wall close to the penetration site. Sometimes it is only present on one side of the peg (Fig. 67, 70).

The necrosis, or hypersensitive response, of the epidermal cell and the necrosis of the fungal cytoplasm were observed at 21 hr. This is manifested by a disorganization of the epidermal cell cytoplasm (Fig. 67, 69, 70, 71, 72, 73, 74, 75). The plasma membrane and the tonoplast are broken and the membranes appear thick and are very electron-dense (Fig. 67, 72) and later are completely destroyed (Fig. 69, 73-75). Similar changes occur to mitochondria and other cell organelles. In some cases, the aggregation and necrosis of the cell contents is so intense as to render the recognition of cell organelles and constituents almost impossible (Fig. 67, 75). Frequently the epidermal cell is collapsed (Fig. 67, 71, 73).

Not all penetrations initiate a necrotic reaction in the epidermal cell, nor does it appear that the formation of a haustorium is necessary. Necrotic reactions occur where the peg seems to be stopped in the papilla region (Fig. 69, 70). The structure of the haustorium and its size (Fig. 72) suggest that the necrotic reactions arrest the development of the fungus at about the 18 hr stage of

elles. Various types of disorganization of chloroplasts occur. Sometimes spaces are formed close to the grana as if the lamellae are spreading apart (Fig. 84, 86, 88, 97). In other cases there is distortion and coalescence of the granal membranes (Fig. 88, 92). Sometimes the lamellae become vesiculated and filled with matrix material (Fig. 92) or completely break down with disintegration of the chloroplast membrane and loss of the matrix to leave only the lamellar membranes and plastoglobuli (Fig. 86).

The chloroplast membranes are frequently abnormal (Fig. 84, 89, 92), either thickened or distorted, and sometimes are broken (Fig. 86, 88, 89). A significant swelling in the size of plastoglobuli occurs in HR cells compared to chloroplasts in the susceptible host (Fig. 84, 88, 89, 92, 95).

Mitochondria appear to be less affected by the HR than do chloroplasts. Only when the HR is pronounced does a loss of matrix and an abnormal appearance of the cristae and the enveloping double unit membrane develop (Fig. 92, 95, 97).

At later stages in the HR the cytoplasm is highly vesiculated (Fig. 89, 91, 92) and eventually the cells collapse (Fig. 87, 91, 93, 98). The cell contents become homogeneous and electron-dense (Fig. 87, 98).

Fig. 93 through 96 show necrotic cells from an early HR when only two or three cells beneath the developing mildew colony had necrosed. These cells are shown in Fig. 93. Electron-dense material is present adjacent to

development.

The necrosis is usually confined to the penetrated cell but some adjoining cells show pathological changes. These involve the deposition of material between the plasma membrane and the cell wall (Fig. 67, 74). These wall deposits consist of a material similar to that of the papilla and similar to that observed in the wall deposits of the hypersensitive mesophyll responses in the Kwan host referred to later.

Necrotic reactions of the host are associated with similar necrosis of the fungus. These reactions involve the shrinkage of the cytoplasmic contents of the appressorium away from the cell wall, the appearance of electron-dense amorphous bodies in places where this shrinkage occurs, loss of cytoplasmic detail and the breakdown of organelles (Fig. 68, 69, 70, 71, 73-75). Changes in the appearance of the fungal cell wall occur and collapse of the main appressorial body is sometimes observed (Fig. 69, 71, 73). The plasma membrane and the cell wall region of the peg appear in some cases to show an electron-dense region similar to that described earlier (cf. Fig. 70, 71 with 59, 63). The pore region is frequently extremely electron-dense (Fig. 69, 71). The neck region of the haustorium appears particularly susceptible to the necrotic events taking place. The membranes of this region are broken down (Fig. 71) whereas in the main haustorial body (Fig. 72) they are still intact though much thickened.

Not all the interactions that prevent infection on the Algerian host involve necrosis. Some penetrations appear to be stopped by the papilla as described in the previous section and the host cytoplasm and that of the fungus is healthy. In the Algerian host, however, a densely stained region is present in the host cell wall. The papilla is double-layered though the secondary deposit is not so well developed as was commonly seen on the Kwan host and the peg is stopped in the primary deposit (Fig. 76, 77).

4.3. Electron microscopy - Later interactions

4.3.1. Introduction

On the Manchurian and Kwan hosts, the subsequent development of successful early interactions (i.e. those which had produced an elongating secondary hypha) was similar until 3 to 4 days after inoculation. On the Algerian host the few ESH infections that did occur were severely retarded in development (cf. Fig. 9 and 10).

A delayed hypersensitive response (HR) is initiated in the Kwan host and can first be observed 72-96 hr after inoculation. This HR is at first confined to the leaf mesophyll cells beneath the developing fungal colony. These cells become brown in colour and collapse in necrotic clumps.

There is no regularity in the position of cells that first become necrotic. Sometimes they are underneath the

centre of the developing colony and, in other instances, they are at the fringes of it; and cells adjacent to the penetrated epidermal cells are often apparently healthy whereas mesophyll cells, adjacent to the uninfected epidermis on the other side of the leaf, are necrotic.

At first, there is considerable variation in the time of onset of HR from one infection to another, and in the number of cells that are necrotic at any given time. By the seventh and eighth days almost all the cells beneath a colony are necrotic, forming a large brown fleck about 2-3 mm in diameter. Sporulation of the fungus, which is considerable at these times on the susceptible host, also occurs on the Kwan host but is significantly reduced and only a few conidiophores develop.

A comparison of the ultrastructural morphology of epidermal and mesophyll cells of the Manchurian and the Kwan hosts and the associated mildew parasite was made.

4.3.2. Mesophyll cells

a. Manchurian

The mesophyll of the Manchurian barley leaf consists of more or less spherical cells of uniform size. The diameter of these cells is 20-30 µm. There is no palisade layer in barley leaves and thus the mesophyll tissue extends from the upper to lower epidermis. Fig. 78 is a low magnification electron micrograph and shows the general features of a mesophyll cell.

The mesophyll cells are interconnected by common cell walls, which contain middle lamellae perforated by plasmodesmata (Fig. 79, 80). Many intercellular spaces occur between cells. A plasma membrane is situated close to the wall and is attached to the membrane which passes through the plasmodesmata (Fig. 80).

The cells are lined by a thin layer of cytoplasm sandwiched between the plasma membrane and the tonoplast. The cytoplasm contains ribosomes, long strands of ER, golgi bodies and vesicles (Fig. 80, 81). Also enclosed within the cell membranes are the cytoplasmic organelles including chloroplasts, microbodies, mitochondria and the cell nucleus. The central part of the cell is occupied by the cell vacuole (Fig. 78), which is large and sometimes contains a large oil droplet.

The chloroplasts of mesophyll cells from infected leaves are characteristically lens-shaped, 3-4 μm in length and 2-3 μm in width (Fig. 78, 81, 82). They contain numerous lamellae, stacked frequently into granal layers, osmiophilic globuli (plastoglobuli) and a matrix or stroma, all enclosed within a double unit membrane. Occasionally, invaginations of the inner unit membrane occur and small vesicles originating from these invaginations are present in the stroma (Fig. 82). Large starch granules are normally scattered within spaces between lamellae (Fig. 81, 82).

Microbodies are frequently associated with the chloroplasts (Fig. 82). They are enclosed by a single unit mem-

brane and are about 1 μm in diameter. Mitochondria are about 0.5 μm in width and of a variable length (up to 1.0 μm).

b. Kwan-hypersensitive response

Within the period of 3 to 5 days after inoculation mesophyll cells of the Kwan host show varied degrees of disorganization. It appears that the first pathological changes to take place in the hypersensitive-reacting cells are depositions of osmiophilic material on, or associated with, the plasma membrane and the tonoplast. Figure 83 shows an otherwise normal mesophyll cell which has obvious deposits on the tonoplast and some close to the plasma membrane. These depositions are particularly prominent in material fixed by Franke's method (Fig. 84, 85) where the deposition is on the plasma membrane, but are also present in material fixed with glutaraldehyde followed by osmium. With the latter fixation, the depositions are usually situated between the plasma membrane and the cell wall (Fig. 83, 87). Large deposits are present in more disorganized cells. They are situated between the plasma membrane and the cell wall and often have an appearance similar to that of the secondary deposit of the papilla observed in the initial penetration studies (cf. Fig. 32 and 85, also Fig. 87, 90, 95). Sometimes the deposits are electron-dense and globular in appearance (Fig. 86, 96).

Chloroplasts are the most affected of the cell organ-

elles. Various types of disorganization of chloroplasts occur. Sometimes spaces are formed close to the grana as if the lamellae are spreading apart (Fig. 84, 86, 88, 97). In other cases there is distortion and coalescence of the granal membranes (Fig. 88, 92). Sometimes the lamellae become vesiculated and filled with matrix material (Fig. 92) or completely break down with disintegration of the chloroplast membrane and loss of the matrix to leave only the lamellar membranes and plastoglobuli (Fig. 86).

The chloroplast membranes are frequently abnormal (Fig. 84, 89, 92), either thickened or distorted, and sometimes are broken (Fig. 86, 88, 89). A significant swelling in the size of plastoglobuli occurs in HR cells compared to chloroplasts in the susceptible host (Fig. 84, 88, 89, 92, 95).

Mitochondria appear to be less affected by the HR than do chloroplasts. Only when the HR is pronounced does a loss of matrix and an abnormal appearance of the cristae and the enveloping double unit membrane develop (Fig. 92, 95, 97).

At later stages in the HR the cytoplasm is highly vesiculated (Fig. 89, 91, 92) and eventually the cells collapse (Fig. 87, 91, 93, 98). The cell contents become homogeneous and electron-dense (Fig. 87, 98).

Fig. 93 through 96 show necrotic cells from an early HR when only two or three cells beneath the developing mildew colony had necrosed. These cells are shown in Fig. 93. Electron-dense material is present adjacent to

the cell wall, within it and scattered throughout the necrotic cytoplasm (Fig. 94). Adjacent cells form a wall deposition next to the necrotic cells and the cytoplasm is vesicular and contains strands of ER (Fig. 95, 96).

4.3.3. Epidermal cells, haustoria and hyphae

A. Manchurian

a. Infected epidermal cell

The host cytoplasm of infected epidermal cells is reduced from the early intense concentration observed during the initial penetration stages. The collar surrounding the haustorial neck is more homogeneous than at earlier times and electron-lucent (Fig. 99, 103), but the double-layered structure of the papilla is maintained (Fig. 100, 103). The amount of cytoplasm present in the infected cell varies from cell to cell but the usual cytoplasmic components are present (cf. Fig. 100, 103).

b. Haustoria

Fungal haustoria are well developed after 4 days and are extensively lobed. They are surrounded by a sheath enclosed within an electron-dense extra-haustorial membrane (Fig. 99, 100, 101, 102, 103). Vesicles are present in the sheath matrix (Fig. 101, 102, 103) and invaginations of the extra-haustorial membrane occur indicating that these vesicles are formed from the membrane (Fig. 101). The sheath is sometimes only a thin band of material and the extra-

haustorial membrane closely follows the contours of the haustorium (Fig. 99, 102). In other instances it is expanded (Fig. 100, 101). The haustoria and lobes contain long mitochondria, many small vesicles (sometimes arranged in a reticular network as in Fig. 102), large lipid-like bodies and ER (Fig. 99, 100, 101, 102). Vacuolar structures of various sizes containing heterogeneous material are present (Fig. 102, 103). These structures are similar to lysosomes, but morphological criteria are not sufficient to permit identification of them as such. The haustorial neck normally contains dense cytoplasm (Fig. 103) but frequently the appressorium broke away during the fixation and embedding procedures permitting escape of cytoplasm from the neck (Fig. 99, 100).

c. Hyphae

Four days after inoculation the external hyphae of the mildew on susceptible barley leaves are filled with cytoplasm containing vacuoles and lipid bodies. Many lysosome-like structures, similar to those in the haustoria, are present containing various inclusions (Fig. 104). Strands of ER and numerous mitochondria are situated at the periphery of the cell and ribosomes are scattered throughout the cytoplasm (Fig. 104, 105).

B. Kwan

a. Epidermal cell and haustoria

Epidermal cells are affected by the HR at a later time (i.e. 5 days after inoculation) than are mesophyll cells. As in the mesophyll cell, the first indication of epidermal cell necrosis is the deposition of osmiophilic deposits on the plasma membrane adjacent to the inner wall of the cell (Fig. 107, 108). When the HR is more advanced, the deposits become more pronounced. Sometimes these deposits are electron-lucent (Fig. 109) and in other cases large globose electron-dense deposits are formed (Fig. 117).

The HR causes the cell to collapse (Fig. 111, 112), the destruction of the cell membranes and the aggregation and disintegration of the cytoplasm and cell organelles (Fig. 109, 111, 114). The cell walls contain electron-dense regions which are spherular and sometimes annular in shape (Fig. 112, 113, 114). Frequently present in the cells are structures comprised of convoluted whorls of membranes (Fig. 115, 116).

At the time the first wall depositions are formed, the haustoria are still healthy. However, unlike haustoria on the Manchurian host, glycogen deposits are present close to the haustorial plasma membrane (Fig. 107, 108). No intermediate stages of necrosis were observed. Either the haustoria appear healthy and the cytoplasm is well preserved (Fig. 106, 107, 108, 110) or the haustoria have collapsed and the cell organization is destroyed (Fig. 109,

111, 112, 114, 117). Usually the extrahaustorial membrane is broken and the sheath matrix is more electron-dense and granular (Fig. 114, 117). Sometimes, it appears vesicular (Fig. 109). Frequently electron-dense material is present in the haustoria (Fig. 109, 111, 114). Eventually, the haustoria are shrunken, granular and no organization of cell structure remains (Fig. 117).

b. Hyphae

External hyphae of the mildew on the Kwan host were more frequently dislodged from the leaf surface during fixation and embedding, especially after epidermal cells had become necrotic, than those on the Manchurian host. Hyphae are shrunken and the cytoplasm is granular and contains no ER, or recognizable ribosomes. Large vacuoles without a surrounding membrane are formed (Fig. 118) and these vacuoles appear to be products autolysing cell components (Fig. 120). Mitochondria appear swollen and contain abnormal cristae (Fig. 118). Electron-dense material (Fig. 119, 121) is present in hyphae, sometimes aggregated around membrane bound structures (Fig. 121) which may be necrotic mitochondria. Vesiculation close to the plasma membrane is sometimes evident (Fig. 121).

CHAPTER 5.

DISCUSSIONS

The purpose of this study was to elucidate and clarify some of the morphological changes which take place during the parasitism of barley by Erysiphe graminis. Particular emphasis was laid on the early interactions between host and parasite and on later interactions which were considered relevant in determining resistance of the host to the parasite.

The discussion will generally follow the sequence of the results obtained by electron microscopy, complemented where necessary, with evidence obtained by light microscopy. At the onset perhaps it should be pointed out that some parts of the discussion may consider in detail aspects which have already been briefly touched upon. The necessity for this lies in the fact that some structures observed at one stage in development of the host-parasite interaction may be analogous, if not homologous, to structures observed at a later time. An example is the formation of the papilla during the primary penetration process and the formation of wall deposits in mesophyll and epidermal cells during the hypersensitive response of the Kwan variety four and five days later.

Light microscope studies, particularly of the early development of the mildew on the resistant and susceptible barley, were an invaluable complement to electron microscope studies. Because only a minute amount of material can be studied in the electron microscope, incorrect and misleading interpretations may easily be made unless these observations can be related to the broader observations using the light microscope. A crucial part of this work was the development of a technique whereby primary penetration sites could be readily located and sectioned for electron microscopy. Stavely and Hanson (1966) published a method employing the use of a conventional microtome. This involved the cutting of thick sections of plastic-embedded leaf tissues, selecting the sections which contained the peg region and then re-embedding for thin sectioning, a laborious procedure at best. The technique developed by me (McKeen and Rimmer, 1973) was found to be relatively quick and simple and to be effective in locating infection sites of even smaller spores than those of E. graminis, eg. Botrytis cinerea (McKeen, 1974).

This study is the first to examine serial sections of early stages of primary infection of E. graminis on naturally growing susceptible and resistant barley leaves although Edwards and Allen published a paper on the primary infection of barley by E. graminis in 1970. However they examined material 48-72 hr after inoculation whereas, as many studies have shown, primary infection occurs 10-20 hr

after inoculation. It seems likely that they were observing material containing well-established haustoria, penetrations associated with secondary haustorial formation, and, possibly, papilla-blocked penetration hyphae. This is supported by the fact that their electron micrographs showed appressorial lobes which were vacuolated and contained glycogen-like particles neither of which were observed in appressorial lobes during cell wall penetration in the present study. I observed that material containing haustoria, if sectioned obliquely, often resulted in sections of infection hyphae (haustorial necks) which gave the appearance of being only part way through the wall (Fig. 27, 28). This emphasizes the importance of using serial sections in this type of study so that the limits of the progress of such structures can be determined.

Stanbridge et al. (1971) studied early stages of infection of detached leaves which had been inoculated after being placed on water agar containing 200 ppm (µg/ml) of benzimidazole. In some preliminary experiments I observed that leaves floating on water containing 100 ppm and 200 ppm benzimidazole were much more resistant than intact leaves. Dekker (1963) and Cole and Fernandes (1970) observed similar effects with kinetin on powdery mildew infections. Some of the results presented in this dissertation, on primary infection of susceptible barley by *E. graminis*, were published earlier (McKeen and Rimmer, 1973).

The ability of pathogenic fungi to invade their hosts is often dependent on the formation of appressoria which are specialized organs differentiated from the germ tube of hyphae. E. graminis forms a long, undifferentiated germ tube when conidia are germinated on glass slides but when they are incubated on the host leaf surface, however, one of the short germ tubes differentiates to form an appressorium 6-8 hr after inoculation. This indicates that the conidium and germ tube respond to external stimulation at an early stage and that the host surface is influential in the differentiation of the appressorium. Prior to penetration the appressorium forms a lobe, usually distally at right angles to the main axis, and it is from such a structure only that penetration is attempted.

The appressorium always has a prominent nucleus and is separated from the germ tube by a septum of the ascomycetous type. The dense cytoplasmic structure of the appressorium is in contrast to the sparse cytoplasmic contents and the vacuolation of the germ tube and conidium. This is not unexpected as it is reasonable to consider that the appressorium would need to be in a more active metabolic state during the infection process than the germ tube.

Glycogen granules, which have been shown to be abundant in dormant conidia (Leong, 1971) are sparsely present in the germinated conidia and germ tubes but are never observed in appressoria at the time of infection. It is likely that during the early stages of germination they

are utilized or converted into simple sugars which are translocated to the appressorium. Akai and Ishida (1967) reported that glycogen-like particles in the conidia of Colletotrichum lagenarium seem to flow into the germ tubes and they thought, consequently, that these particles are utilized during germination for cell wall synthesis. It is considered that active fungal cell wall synthesis and the secretion of materials including material for adhesion and enzymes for cell wall degradation occur in the appressorium where it is in contact with the host cell wall at the infection site. Before penetration this region of the appressorium always contains numerous vesicles and an abundance of ribosomes. A close contact between appressorium and epidermal cell is always observed in material which has not been dislodged during the embedding procedures.

In transmission electron micrographs the cuticular layer appears as a thin smooth layer on the outer surface of the epidermal cell wall and is about one tenth the thickness of the cell wall. Before penetration it is intact and sometimes slightly indented where it is in contact with the pore of the appressorial lobe. During penetration some erosion of the cuticular layer is occasionally observed, sometimes it appears fragmented, but no change occurs in its electron density. However, significant alteration in the electron density of the cellulose layer of the cell wall is observed around the penetration site. This increase

in electron density is most pronounced at the edges of the hole produced by the infection peg and is localized to the wall around the peg more at the time of penetration (11-12 hr) than at later times.. Separation and swelling of the fibrillar structure of the cell wall is not unusual. The peg is blunt and appears to have no cell wall or else it is exceedingly thin while it passes through the wall. These observations suggest that the penetration of the wall involve chemical alteration of the wall, but whether the cuticular layer is degraded enzymatically is uncertain. In addition, larger holes are occasionally formed in the walls and apparently plugged by fungal material. Penetration of guard cell walls also occurs. These are much thicker than other epidermal cell walls but still do not prevent penetration from occurring.

Most investigators of primary penetration (Bracker and Littlefield, 1974; Corner, 1935; Leong, 1971; Stanbridge et al., 1971) have considered cuticular penetration to be the result of mechanical pressure exerted by the penetration peg. Roberts et al. (1960) showed that rose leaves infected with Sphaerotheca pannosa have only a quarter of the cutin characteristics of healthy leaves and Linskens and Haage (1963) demonstrated cutinase activity by Botrytis cinerea, Rhizoctonia solani and other plant pathogenic fungi. Recently Van den Ende and Linskens (1974) have reviewed the evidence for plant pathogenic degradation of cutin. Scanning electron microscope examination of

E. graminis on barley has shown the outer epidermis of the leaf to consist of a layer of waxy protuberances and that extensive degradation of this layer occurs beneath the appressorium and germ tube (Schwinn and Dahmen, 1973). In order to achieve close contact with the cell wall, it seems likely that it is necessary for the fungus to remove these waxy protuberances of the outer epidermis. These structures are not observed in transmission electron micrographs and are presumably removed by solvents during preparation. If these waxy layers are degraded by the fungus there is little reason to suppose that the fungus would be incapable of degradation of the cutin layer.

The alteration of the cell wall around the zone of penetration observed in electron micrographs has been associated with the halo that appears round penetration sites in stained light microscope preparations. Akai et al. (1968) reported that the haloes observed on the epidermal cell wall are the results of the degradation of the cell wall constituents. McKeen and Bhattacharya (1969) studied histochemically the halo around the infection site. They showed that cellulose is lacking in the halo and that after differential extraction of polysaccharides the Periodic acid-Schiff (PAS)-negative halo was always surrounded by a PAS-positive band even after pectin, hemicellulose and other non-cellulosic polysaccharides have been removed.

Kunoh (1972) found that the halo does not stain with Sudan III indicating an alteration in the cuticular layer of the cell wall. He confirmed McKeen and Bhattacharya's findings that the halo is PAS-negative and also that pectin and cellulose reactions (Ruthenium red, Iron reaction and Zinc-chlor-iodide tests) are negative. However he did show that reduced compounds and aldehyde groups are present and that pentose and uronic acids are present, results that would be expected if cell wall degradation has taken place. There is some difficulty, however, in interpreting these data with respect to penetration. I observed haloes not only surrounding the actual penetration site beneath the appressorial lobe but also, and often even more intensely stained, beneath the undifferentiated germ tubes. In this study a penetration of the cell wall from an undifferentiated germ tube was never observed to occur.

Stanbridge et al. (1971) also reported that they never observed penetration from undifferentiated germ tubes. They suggested that the fact that the appressorium contains a nucleus may be an important factor in this regard.

It has been mentioned that cell wall alteration is localized to the cell wall adjacent to the peg. Only after the peg has penetrated through the cell wall is the alteration of the wall extended beyond this region, up to 2 µm radius from the penetration hyphae. Assuming that cell wall degrading enzymes are produced this could indicate that diffusion of enzymes into the cell wall from the

penetration hypha, results in continued alteration of the wall after penetration has occurred. Alteration of the cell wall and the formation of papillae are observed beneath undifferentiated germ tubes. These are more extensive and electron-dense than around penetration sites. If haloes do indicate enzymatic alteration of the cell wall and the evidence suggests this to be the case, then the question arises as to why the fungus should secrete enzymes in places where no penetration attempt will follow. This would seem to be wasteful of the energy resources of the fungus which one would logically consider should be conserved for rapid penetration and haustorial formation.

Before addressing this problem further it is important to discuss the host response to infection because it is not to be expected that the one will not affect the other. Evidence has been presented that stimulation of the host occurs before the appressorium begins to penetrate the host cell wall. This stimulation results in an aggregation of host cytoplasm beneath the infection site and undifferentiated germ tubes. This host aggregate responds to the threatened infection attempt at both these locations by forming a papilla.

The aggregation of host cytoplasm consists of rough ER, mitochondria, golgi bodies, numerous small vesicles and plastids. The plastids are similar to etioplasts (Gunning, 1965a, 1965b) and consist of a prolamellar structure and lipid globules. The papilla itself is at first a

small protuberance of the cell wall of electron-dense material beneath the appressorial lobe and germ tubes. It is deposited outside the host cell plasma membrane. The papilla is an amorphous structure and has been shown to contain polysaccharides but no cellulose or lignin (Kunoh, 1972). Callose has been reported to be present in the papilla (Edwards and Allen, 1970).

The presence of numerous golgi bodies and small vesicles in the host aggregate suggests that they are probably responsible for the formation of the papilla. Evidence that small vesicles are incorporated into the papilla has been presented. The papilla contains membranes interspersed amongst the amorphous material. Similar observations were presented by Griffith (1971) for lignituber formation in Verticillium-infected roots of tomato and pea seedlings. He suggested that the deposition of material on to the penetrated cell wall results from metabolic activity within the living protoplast and is accompanied by vesicular secretion through the host plasma membrane. Chou (1970) also considered the sheath formation surrounding the hypha of Peronospora parasitica results from a process of fusion and incorporation of vesicles derived from the host protoplast.

The involvement of vesicles in the process of papilla formation is consistent with the well documented evidence for the passage and incorporation of cytoplasmic materials across the plasma membrane into the developing cell wall

(Cronshaw, 1965, 1967; Pickett-Heaps and Northcote, 1966). Northcote and Pickett-Heaps (1966) provided evidence that the golgi apparatus is important in the secretion of polysaccharides in plant cells.

Successful penetration of the papilla occurs quite rapidly compared to the penetration of the cell wall, because the peg is frequently found within or just through the cell wall but only infrequently observed within the papilla. Only at later times, when infection sites without haustoria present were selected, are pegs frequently observed to be stopped within the papilla. Papillae in these instances are usually large and have a secondary or tertiary deposit surrounding the primary papilla. These secondary deposits are usually less electron-dense and often stellate in appearance. The rapidity and extent of papilla formation seems to be important in the prevention of infection of the host. Light microscope data showed that prevention of infection by the papilla is non-specific, i.e. it occurs with about the same frequency on the susceptible and resistant varieties.

When it successfully blocks penetration, the papilla does not bring about immediate death of the fungus. This is shown by the fact that appressoria which have their initial penetration attempt blocked by the papilla form a secondary appressorial lobe and reattempt infection from this structure. The frequency of infections, however, from these secondary attempts is usually low. Probably this is

because the fungus has less inoculum potential (sensu Garrett, 1956) after its first unsuccessful attempt, and/or that the host has mobilized sufficient reserves to contain quickly this penetration attempt.

Lin and Edwards (1974) found that the age of the leaf is a factor in non-specific resistance due to the prevention of haustorial formation. The type of epidermal cell is also important in this regard. Hirata (1967) showed that subsidiary cells of stomata are more susceptible than other epidermal cells. In this study, it was observed that the long cells overlying the leaf vascular tissues are the most resistant cells.

The function of the papilla in powdery mildew infection of barley is thought to be a host defense mechanism for blocking penetration and haustorial formation. Similar host deposits have been observed in a variety of plant diseases and have sometimes been referred to as callosities (Young, 1926). Fellows (1928) described them as they occur in wheat plants infected by Gaumannomyces graminis as lignitubers because they contain lignin but no callose. Because deposits similar to these formed in response to infection can be produced by penetrating cells mechanically with fine needles, Wood (1967) suggested that they are a generalized wound response. However the fact that papilla formation in barley is initiated before fungal penetration of the cell wall has commenced suggests that they may be chemically stimulated and that they result from cytoplasmic

activity. Moreover, because they have been observed to contain successfully the invading fungus in up to 50% of penetration attempts, it is concluded that they play an important role in cellular resistance of barley to E. graminis.

In support of this, Bushnell and Bergquist (1975) observed the development of the papilla and concluded that most of its growth occurs within 15-20 min and that, in coleoptiles, it develops concurrently with the haustorium. On leaves, however, the papilla response seems to be under a more decisive on-off control as papillae are seen only when haustoria do not form, and papilla deposition is not seen around necks after haustoria are produced. It is difficult to relate these observations to the evidence of electron micrographs of penetrations where papillae are obviously present when the haustoria have been formed. They concluded that the papilla is apparently an important component of generalized host resistance to powdery mildew. More work on the formation of the papilla and its role in the establishment of host parasite relations seems to be desirable to clarify these contradictions.

If papillae are an important defense mechanism of the host against invasion then it is possible to speculate that the fungal-induced cell wall alterations beneath the germ tubes, which was mentioned earlier, may aid the penetration of the epidermis by the infection peg by dividing the host cell's capabilities of response between various locations.

If the host response is concentrated solely on the appressorial infection site the papilla may be formed too quickly for successful penetration to occur frequently. It should be possible to test this hypothesis experimentally and further work comparing the histochemistry of haloes beneath germ tubes and the appressoria would be valuable.

When the tip of the peg is 3-5 μm beyond the papilla, within the lumen of the epidermal cell; it enlarges to form an incipient haustorium. The haustorium at this time contains thick strands of membranes, many small vesicles and ER. McKeen (1972) suggested that similar strands within the hyphae and conidiophores are responsible for nuclear movement in E. graminis. Their conspicuous presence in young haustoria at about the time that nuclear movement from the appressorium to the haustorium takes place suggests that they may well play a similar role at this stage of development.

The development of the mildew on susceptible and resistant varieties of barley up to the formation of a young haustorium was similar on all varieties studied. The Manchurian and Kwan hosts show a similar development up to 70 hr after inoculation and no evidence was obtained of any expression of resistant mechanisms before this time due to the presence of the Kwan gene. The Algerian variety, however, was observed to prevent further development of the fungus in both light and electron microscopy studies by a hypersensitive response occurring about 20 hr after inocula-

tion.

In the light microscope studies, it was observed that some of the cells which had been penetrated become permeable to stain in contrast to uninfected cells. When haustoria can be seen in these cells they have developed to about the 18 hr stage, i.e. they are elongated but have not formed lobes. Usually 40-50% of the appressoria formed are associated with these stained epidermal cells. No secondary appressorial lobes occur on appressoria associated with these cells. Less than 5% of appressoria continue in their development to form an elongating secondary hypha. Several other workers observed less than 5% successful primary infections on the Algerian variety (Ellingboe, 1972; Masri and Ellingboe, 1966; McKeen and Bhattacharya, 1969; Stanbridge et al. 1971). Only Kunoh (1972) and Stanbridge et al. (1971) suggested that two distinct resistant mechanisms are in operation, blockage by the papilla, and hypersensitivity.

Electron microscopy revealed that most of the material at 20-21 hr after inoculation is necrotic. This hypersensitive type of response involves the simultaneous necrosis of both host cell and the fungus.

Electron micrographs of the appressoria show them to be collapsed, with the plasma membrane destroyed or broken, cytoplasm is often shrunken and electron-dense material is situated between the shrunken cytoplasm and the fungal cell wall. Similar destructive changes occur in the epidermal

cell - broken membranes, coagulated cytoplasm and collapse of the cell. The altered permeability of these cells to stains is probably due to the fact that membranes have lost their integrity. Frequently, cells adjacent to the necrotic epidermal cell form wall deposits similar to those which occurred in hypersensitive cells on the Kwan host. These will be fully discussed later.

Ellingboe and his coworkers (Ellingboe, 1972; Masri and Ellingboe, 1966; McCoy and Ellingboe, 1966) exhaustively studied the development of appressoria and hyphae but they did not report observing papillae, haustoria or cytoplasm inside epidermal cells and therefore did not relate incompatibility to hypersensitivity or other events.

Haywood and Ellingboe (1974) reported observing a similar staining response of the epidermal cell to infection of wheat by E. graminis but, again, did not relate this to hypersensitivity. Bushnell and Bergquist (1975) reported that a hypersensitive response of coleoptile epidermal cells of the Algerian host occurred only after haustorial formation.

The light microscope data for infection of the Algerian host is anomalous. About 60% of attempted infections have only formed appressoria at 20 hr (40% have successfully formed a haustorium). Only a few percent of appressoria are associated with stained epidermal cells. At 26 hr, however, 80% of the attempted infections are 'appressoria only' (Table 4). 10% have appressoria and

haustoria and 10% have progressed to form a hypha as well. Apparently 20% of the population have regressed. The only logical way this can be explained is that those haustoria have disintegrated. This is precisely the percentage increase in appressoria associated with stained cells. Electron micrographs show material which does not contain haustoria but the cellular disorganization is so great that it is impossible to tell whether or not a haustorium has ever been present. Consequently, it is considered that hypersensitive responses only occur in epidermal cells which have been penetrated by the fungus infection hyphae to form haustoria.

Another conspicuous difference between the Algerian and the other varieties is the formation of electron-dense lines in the cell walls close to the penetration peg. These lines are not formed during the actual penetration of the wall but become apparent about 20 hr after inoculation. They are not confined to necrotic cells but also occur in cells containing haustoria and in papilla-blocked infection sites. Within the cell wall region demarked by this electron-dense line, definite alteration of the cell wall fibrillar structure is apparent which may indicate the limits of the influence of the peg on the cell wall. That this cell wall staining only occurs in the Algerian variety indicates that the cell wall may be different in some way from that of other varieties. It may be related to the resistant gene, or, at least to some closely linked gene,

as these varieties are near-isogenic. More work is necessary to investigate this possibility.

Resistance to powdery mildew by the barley variety containing the Kwan gene is expressed about 3 days after inoculation. This also involves a hypersensitive collapse and necrosis but is initiated within the mesophyll tissue underlying an infection site. This is an unusual occurrence because, unlike necrotic reactions which have been studied in other host parasite combinations, the mesophyll cells are not penetrated by the fungus, but are the first cells to collapse and only after this response do the penetrated epidermal cells become necrotic. White and Baker (1954) and Hirata (1967) observed that delayed hypersensitivity involved the collapse of mesophyll cells. This study confirms these observations in the light microscope and extends them to show the fine structural changes which are associated with this response.

The first indications of resistance in the Kwan host are the browning and necrosis of two or three mesophyll cells beneath a developing colony. The first changes in cell structure which are observed in electron micrographs are the deposition of osmiophilic material on the plasma membrane and the tonoplast, and the formation of small electron-dense inclusions between the plasma membrane and the cell wall. Osmiophilic deposits on the cell membranes have been shown to occur naturally in some plant cells and are thought to consist of lipoidal material, but little is

known of their function (Ledbetter and Porter, 1970). In this study they occur only in cells of the Kwan host associated with hypersensitive collapse. Comparable membrane deposits have only been reported to occur in hypersensitive cells of one other host-parasite combination (Heath, 1972). It is of interest that Heath only observed these droplets to occur on cell membranes of the intermediate resistant cowpea host (Purple Hull Pinkeye) and not on immune varieties. The Kwan host is similar to this host in that it, too, shows an intermediate type of resistant response.

The inclusions between the plasma membrane and the cell wall are greatly enlarged in cells which are more disorganized. In some cells they are composed of an electron-lucent material, similar to that of the papilla, and in others consist of an electron-dense, globose matrix. These depositions are most frequently present on cell walls adjacent to necrotic cells but are also often present in the necrotic cells.

Similar deposits of amorphous material on the walls of uninfected cells where they are in contact with necrotic cells have been reported in plants showing a hypersensitive reaction to fungal infection (Heath and Heath, 1971; Heath, 1972; Mercer et al., 1974; Skipp et al., 1974) and in virus-infected plant cells (Hiruki and Tu, 1972; Ross and Israel, 1970; Spencer and Kimmins, 1971). These deposits usually contain membranes and are electron-lucent (Heath

and Heath, 1971; Heath, 1972; Hiruki and Tu, 1972; Mercer et al., 1974; Ross and Israel, 1970). Callose has been detected in material of this appearance (Heath, 1971; Heath and Heath, 1971; Hiruki and Tu, 1972). Granular, electron-dense material of unknown composition has been reported in association with electron-lucent material (Heath, 1972; Mercer et al., 1974; Skipp et al., 1974) or by itself (Heath, 1972; Hiruki and Tu, 1972). Wall deposits have been observed in cereals in fine structural studies (Gill, 1974; Skipp et al., 1974) and also by light microscopy (Allen, 1923; Leath and Rowell, 1969). Mercer et al. (1974) considered these deposits, which they called reaction material, to be largely composed of callose at first, which then probably became impregnated with electron-dense material. The electron-dense material, they thought, most probably consisted of oxidized polyphenols. Heslop-Harrison (1966) has shown that the temporary callose of walls in pollen grains of Lilium sp. is much less permeable to certain compounds than is cellulose. Mercer suggested that the production of these wall deposits may be an attempt to prevent the passage of potentially toxic substances from adjacent necrotic cells. Tu and Hiruki (1971) considered that deposition of material on the cell wall, if formed quickly enough, could be effective in preventing the passage of virus from one cell to another through plasmodesmata. In support of this hypothesis they observed that wall-thickened cells ringed necrotic cells

in local lesions of potato virus M infections of kidney beans.

Some confusion exists in the literature with respect to both the terminology of wall thickenings and their relationship to the structures known as lomasomes. Marchant and Robards (1968) have briefly reviewed the formation, function and fate of lomasomes or paramural bodies. Lomasome-like vesiculate bodies occasionally occur in reacting cells (Fig. 97) but wall deposits are almost always associated with ER and small vesicles, as are the papillae formed beneath appressoria. However, Ross and Israel (1970) frequently observed vesiculate, membranous bodies appressed to cell walls which closely resembled 'plasmalemmasomes' (yet another term which has been used for these structures). They considered that these structures may reflect the movement of the structural wall materials concomitant to heat or virus-induced collapse of the cells. Comparable structures to those observed by Ross and Israel are found in oat roots treated with victorin (Hanchey et al., 1968). They termed these structures 'lomasome-like wall lesions' and thought of their presence as a manifestation of cell wall degradation brought on by the pathotoxin. Erhlich et al. (1968) considered lomasome-like structures more likely to be formed as a host response rather than a degenerative effect of the pathogen. Ross and Israel supported this contention and believed the presence of wall lesions to be a transi-

tory effect that preceded actual cell collapse. This interpretation is most likely the case in the hypersensitive response of the Kwan host. The fact that mesophyll cells are not in contact with the pathogen suggests that some diffusible agent incites this cellular response. The formation of wall deposits may be an attempt to prevent further passage of this agent or toxic substances formed in cells already responding to such an influence. It is probable that callose is present in these wall deposits particularly those which have an electron-lucent matrix. Because these wall deposits seem to be a general phenomenon of pathological tissues, it seems clear that more work is required to enable us to understand the role and composition of these structures and the cellular events related to their formation and function.

Chloroplasts are the cellular organelles most quickly and variably affected by the hypersensitive response. Four types of chloroplast disorganization were observed. These involved the spreading or separation of granal layers, distortion and coalescence of granal layers, the vesiculation of granal lamellae incorporating matrix material, and the disintegration of the chloroplast membrane and loss of the matrix to leave only a skeletal lamellar structure and plastoglobuli.

Plastoglobuli increase in size in chloroplasts of hypersensitive cells from a mean diameter of 0.15 μm to greater than 0.25 μm. Similar swelling of the plasto-

globuli was observed by Heath (1974) during infection of cowpea leaves by Uromyces phaseoli. Plastoglobuli did not appear to be more numerous in hypersensitive cells. Lichtenthaler (1966) reported that plastoglobuli may either enlarge or increase in numbers during the ageing of chloroplasts.

These observations are similar to those of Goodman and Plurad (1971) on chloroplasts in hypersensitive cells responding to bacterial infiltration. Heath (1974) studied the ultrastructural changes of chloroplasts occurring in rust-infected and in ethylene-treated cowpea leaves. She observed what appeared to be prolamellar structures within chloroplasts of both infected and ethylene-treated tissues and considered that infected tissues may produce ethylene in vivo resulting in the observed changes. Prolamellar bodies were not observed in chloroplasts of hypersensitive reacting tissues in this study and, as Hislop and Stahmann (1971) have shown ethylene levels to be reduced in the Kwan host at this time compared to the Manchurian, it seems unlikely that ethylene is important in the necrosis of those cells. However, Camp and Whittingham (1975) in a study comparing chloroplast ultrastructure in mesophyll cells of susceptible barley with uninfected tissue observed lamellar degeneration in chlorotic tissues that often resembled aberrant prolamellar bodies. Chloroplasts in the green islands surrounding the chlorotic areas were

persistent though fewer in number than in the controls. Chlorosis of tissue on susceptible barley does not occur until about the time of sporulation, after the stages of infection examined in this study. These changes may more closely resemble those of natural senescence than does the hypersensitive reaction. Similar to the findings of this study was the general observation that the most drastic alterations in organelle fine structure occurred in chloroplasts whereas other organelles were virtually unaffected.

Other cellular organelles such as the nucleus, mitochondria and microbodies are eventually disintegrated but only when cellular disorganization has progressed beyond the formation of wall deposits and membrane breakage has occurred. The cellular membranes are broken at about the same time that chloroplast changes are observed. Cells then collapse probably due to loss of osmotic turgor of the cells when membranes lose their integrity. This is not an unexpected observation as one of the well known physiological effects of hypersensitivity is electrolytic leakage from affected cells (Goodman, 1968; Wheeler and Hanchey, 1968). Whether this leakage commences before membranes are broken is difficult to detect. It may be that membranes are significant components of the cellular response and the fact that these membranes are the first to contact any toxic substances diffusing from adjacent cells or from the fungus may explain why the first indications of hypersensitivity were osmiophilic depositions on membranes.

The final stages of necrosis are characterized by the complete collapse of the cell sandwiching an electron-dense mass of unrecognizable cell contents between the cell walls. Darkly staining inclusions in the cell wall occur, sometimes ring-shaped. These are especially prominent in the interconnecting walls of epidermal and mesophyll cells. These are usually associated with the middle lamella of the cell wall and occur most frequently in this portion of the cell wall where the cell walls separate.

Epidermal cells, including cells containing haustoria, are affected one or two days later than the underlying mesophyll cells. They collapse rapidly, because intermediate stages in necrosis, which are observed frequently in mesophyll tissue, are rarely observed. The initial responses are again osmiophilic depositions on the plasma membrane and the formation of wall deposits. This is followed by collapse and aggregation of cytoplasm into electron-dense masses. Frequently, membranous whorls occur within these cells possibly due to the coiling of broken sheets of membranes. Skipp et al. (1974) observed similar membranous whorls in cells adjacent to necrotic cells. Fowke and Setterfield (1969) have suggested that these may be fixation artefacts but their absence in non-necrotic tissues makes it more likely that their formation is an effect of cell necrosis. These are not observed in mesophyll cells and this may be due to the much greater amount of cytoplasm in these cells which could prevent

this coiling.

The necrosis of the epidermal cells which contain haustoria result in the necrosis of the fungus. Electron-dense regions occur in these haustoria, less pronounced but nonetheless similar to those observed in appressoria on the Algerian host. Frequently the external hypha of the fungus was dislodged during the embedding procedures after epidermal cell collapse had occurred. This made it difficult to determine the effect of haustorial necrosis in the external hyphae but those which are intact are necrotic also. This is in contrast to the situation which has been found in rust infections where only the haustorium mother cell is affected by hypersensitive collapse of mesophyll cells containing haustoria (Allen, 1923; Heath, 1972; Skipp and Samborski, 1974; Skipp et al., 1974). It appears that in rust infections the presence of an intervening septum prevents the spread of fungal necrosis from a dead haustorium mother cell to the rest of the infection hypha. Why the septum in the haustorial neck of E. graminis should not similarly prevent necrosis spreading into the hypha is obscure. The necrosis of fungal hyphae involves the dissolution of membranes and the occurrence of electron-dense deposits where membranes had been. Cytoplasmic detail is lost and sometimes vacuoles are formed which appear to autolyse cellular components such as mitochondria.

The hypersensitive response of plant cells to invasion by plant pathogens is the single most important defense

mechanism of plants. In the case of powdery mildew infection of barley it results in haustorial death in association with epidermal cell necrosis. This is the case both on the Algerian host within the first 24 hr after inoculation and on the Kwan host 5 days after inoculation. Though the cause of haustorial death is unknown, as Skipp et al. (1974) have pointed out it is to be expected that the haustorium would meet very unfavourable conditions after the death of the host cell. The breakdown of cellular structures inevitably leads to decompartmentalization of enzymes and many of the changes which occur in the necrotic cells are suggestive of the action of hydrolytic enzymes. It has been reported that acid phosphatase, ribonuclease, esterase, protease and B-glucosidase disperse within plant cells when they become necrotic (Mace, 1973; Pitt and Coombes, 1968, 1969). Such enzymes may directly affect the fungus or be involved in the production of toxic compounds (Knott and Kumar, 1972). The nature of the triggering mechanism of hypersensitive responses is unknown but almost certainly involves genetic control and the exchange of information. Recently Keen (1975) isolated a specific elicitor of the anti-fungal phytoalexin hydroxyphaseollin, which appears to be the basis for resistance of certain soybean cultivars to incompatible races of Phytophthora megasperma var. sojae. He found that race 1 and race 2 cultures of the fungus produced metabolites which, in cell-free bioassays on soybean plants, elicit higher levels of hydroxyphaseollin in the

monogenic disease-resistant cultivar Harosoy 63 than in the near-isogenic susceptible cultivar Harosoy. A similar kind of elicitation of hypersensitive necrosis may be involved in the specificity of obligate biotrophs though as yet no evidence in support of this hypothesis has been obtained. Phytoalexins themselves may be involved in the specificity of hosts to obligate biotrophs and some evidence for this has been obtained for the barley-powdery mildew system (Oku et al., 1973). The challenge for the future in this field lies in the resolution of these problems.

PHOTOMICROGRAPHS

LIGHT MICROGRAPHS

PLATE 1

Manchurian. Successive stages of infection and haustorial development in susceptible barley.

Figure 1. Conidium, germ tube and appressorium. Penetration of the host cell has not been effected. Note the formation of the papilla (arrow) beneath the appressorial lobe. 11 hr. Trypan blue x1900.

Figure 2. Similar to Fig. 1 but a peg has been formed from the appressorial lobe. There is no papilla formed but an extensive host reaction (HR) is present around the penetration site. 11 hr. Trypan blue x1000.

Figure 3. A young haustorium. Note the expanded sheath around the haustorium. 15 hr. Trypan blue x1275.

Figure 4. A later stage (18 hr) in the development of a compatible interaction. The haustorium is elongated and parallel to the leaf surface. 18 hr. Trypan blue x1000.

Figure 5. The haustorium is lobate and an elongating secondary hypha has been formed. The upper spore made an unsuccessful penetration attempt with its primary appressorial lobe but penetrated successfully at its second attempt (SAL). 25 hr. Trypan blue x600.

Figure 6. A branch hypha has been formed at the germ tube and appressorial septum. The ESH has continued to elongate. 30 hr. Trypan blue x600.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4
5
6

PLATE 2

Kwan and Algerian. Development of the fungus on the resistant hosts.

Figure 7. Papilla-stopped infection attempts on the Kwan variety. The appressorium has attempted infection from two appressorial lobes. Note the unhealthy appearance of the appressorium. Kwan 25 hr. Trypan blue x1700.

Figure 8. Papilla-stopped infection attempts on the Algerian variety. Unsuccessful penetrations from primary and secondary appressorial lobes. Algerian 25 hr. Trypan blue x700.

Figure 9. Normal development of a colony on the Kwan variety. Note the long lobes of the haustorium and good hyphal development. Kwan 42 hr. Trypan blue x880.

Figure 10. Resistant reaction of the Algerian gene. This spore has produced a well-lobed haustorium but no colony development has occurred. Compare with Fig. 9. Both are secondary appressorial lobe infections. Algerian 42 hr. Trypan blue x880.

Figure 11. An unsuccessful infection in the resistant Algerian host. Observe the haustorium at the upper left corner of the cell. Note the whole cell has taken up the trypan blue stain. Walls especially are stained (arrow). Algerian 26 hr. Trypan blue x350.

Figure 12. Similar to Fig. 11. A haustorium has been formed but the development has stopped at about the 18 hr stage (Fig. 4). The whole epidermal cell is stained. Algerian 26 hr. Trypan blue x400.

ELECTRON MICROGRAPHS

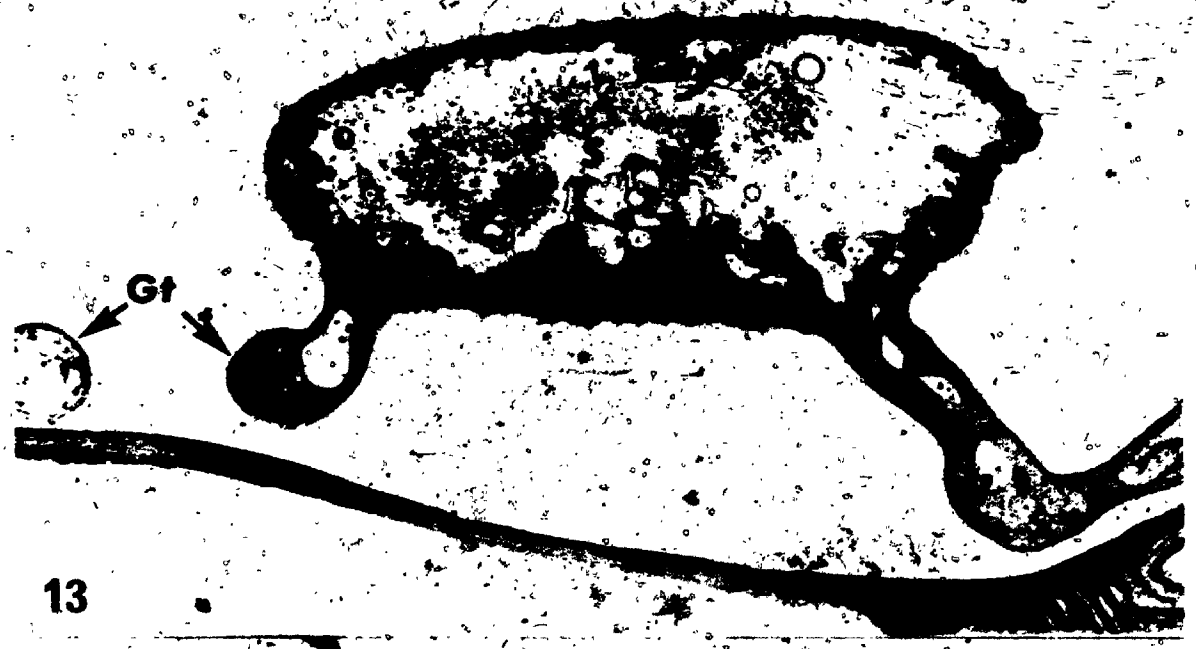
PLATE 3

Conidium, Germ tubes and Appressorium

Manchurian 11 hr. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 13. Longitudinal section through a conidium (S) which has germinated to form two germ tubes (Gt). The germ tube on the right has formed an appressorium. Observe the double layered, spiny wall of the conidium, its large vacuole and the numerous glycogen particles scattered among the cytoplasm and in the vacuole. x5,500.

Figure 14. A serial section of the same spore as in Fig. 13. This shows the germ tube (Gt) and appressorium (A). Note the difference between the germ tube cytoplasm, which is simply a continuation of the spore, and that of the appressorium. The appressorium has no vacuole, nor glycogen but contains many mitochondria and dense cytoplasm. x7,000.



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14

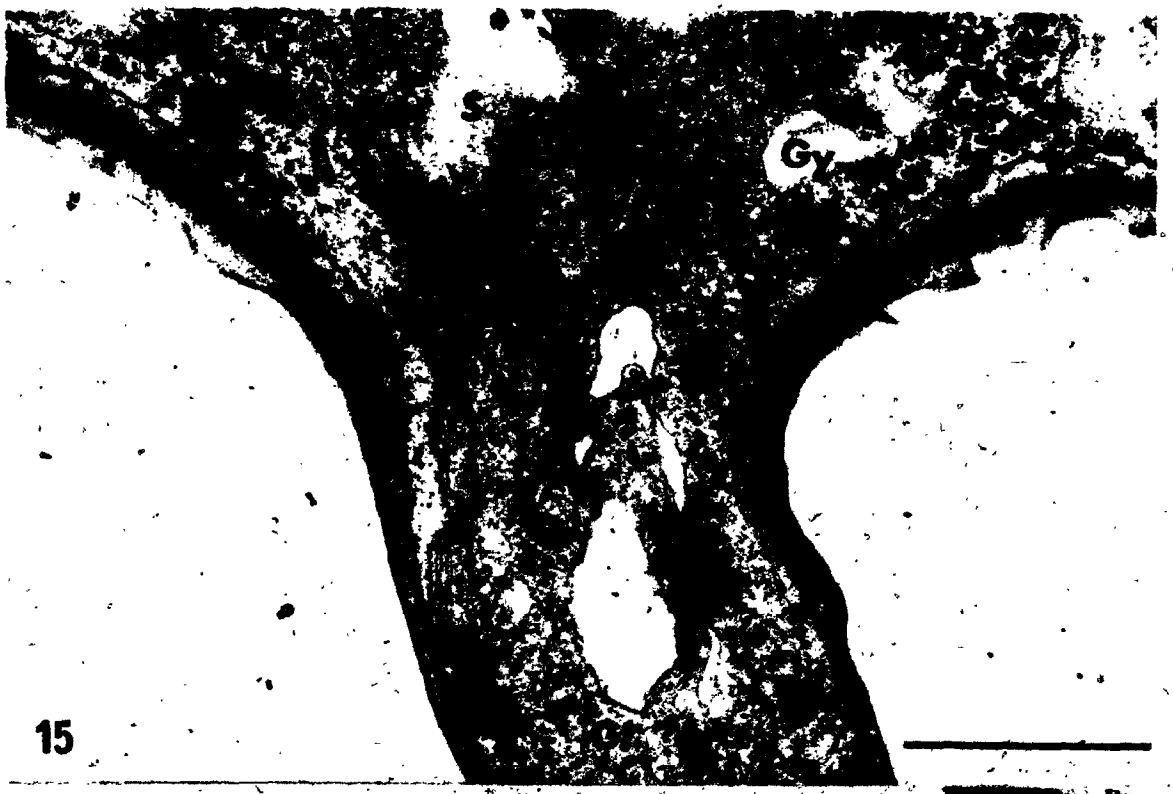
PLATE 4

Enlarged views of parts of the conidium, germ tube and appressorium

Manchurian 11 hr. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 15. High magnification of part of Fig. 13, showing the region where the germ tube joins the conidium. Note the wall of the germ tube is a continuation of the inner layer of the double-layered wall (SW) of the conidium. Note also the rosettes of electron-dense glycogen particles (Gy). x37,875.

Figure 16. High magnification of part of Fig. 14, showing the tip of the appressorium. Observe the small mitochondria (M) often lying close to the plasma membrane. Note the large numbers of ribosomes (R) and ER. x27,500.



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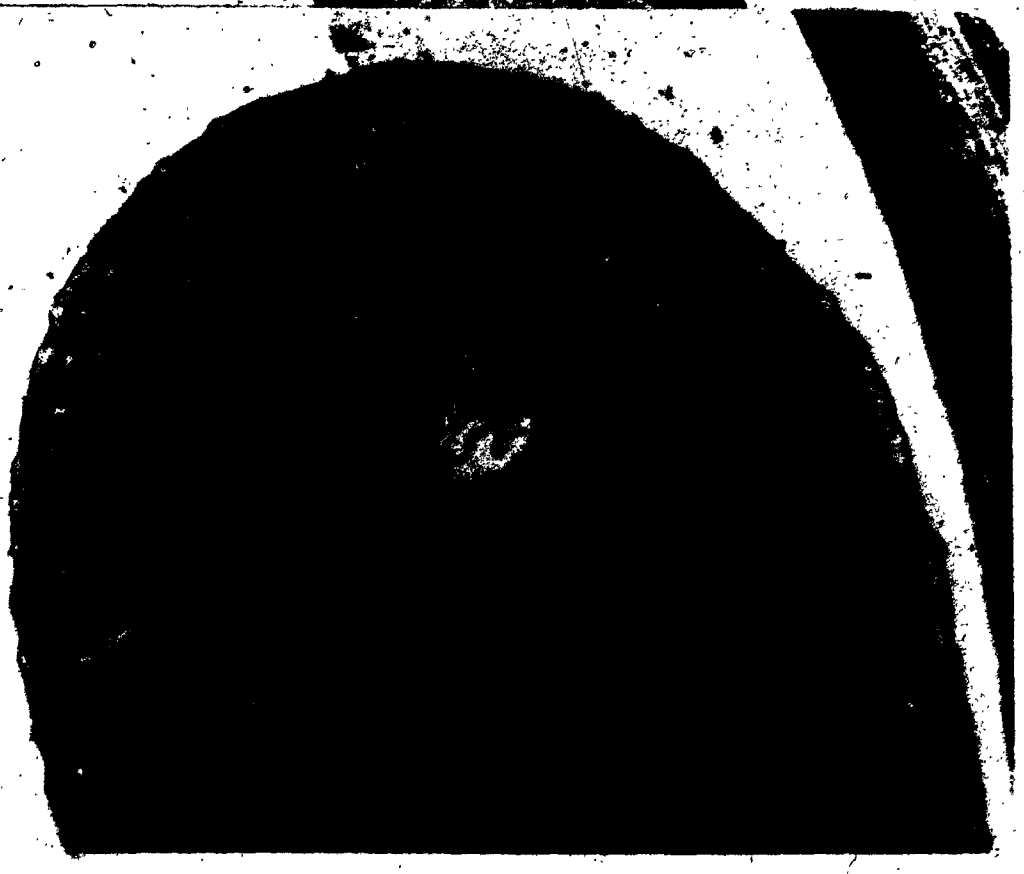

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PLATE 5

Appressorium

Manchurian 14 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 17. A transection of the appressorium through the nuclear region, about one third back from the tip. The appressorium is lying in the groove between adjacent epidermal cells. Again observe the mitochondria close to the plasma membrane, the extensive strands of ER and the numerous small vesicles (SV). Observe that small vesicles appear to be formed from the ER by pinching off from the ends of such strands (arrow). Note the nucleus (N) surrounded by the nuclear membrane and containing a darkly staining nucleolus (Nu). x30,800.

17

PLATE 6

Appressorium

Manchurian 14 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 18. A transection through the same appressorium as in Fig. 17, closer to the tip of the appressorium. Observe the central part of the cell (unlabelled arrows) which contains groups of polyribosomes (PR) but no mitochondria or small vesicles. This region is ringed by strands of ER. Note strands of mucilaginous material (Fam) between the appressorium and the epidermal cell. x29,300.


18

PLATE 7

The appressorial lobe (prepenetration)

Figure 19. A transection through an appressorium (A) where the appressorial lobe (AL) is formed. Note again the numerous strands of ER in the appressorium and the small vesicles formed from them. Note the breakdown of the wall in the appressorial lobe, the pore (Po), through which the penetration hypha develops. Note the numerous small vesicles and ribosomes in this region and the absence of mitochondria which are mainly present in the main body of the appressorium. Notice that even before penetration has begun a papilla has already been formed (Dl) in the epidermal cell beneath the pore. Keystone 14 hr. x22,900. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 20. A transection of the appressorial lobe through the pore. Note electron-dense regions between the plasma membrane and the fungal cell wall in the vicinity of the pore. The cuticle (Cu) which can be seen as a thin layer on the surface of the epidermal cell wall, is not visible beneath the pore and the cell wall (EW) is slightly indented. Note the well developed papilla and the rough ER (RER) close to the plasma membrane surrounding the papilla. Manchurian 11 hr. x39,000. Glutaraldehyde followed by osmium. Uranium and lead stained.


19
D1
Cu
D1
Ve
pm
20
RER

PLATE 8

Appressorial lobe, penetration peg and papilla.

Keystone 14 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 21. A transection of an appressorium through the appressorial lobe (AL). Note the peg in the epidermal cell wall (EW), the well-formed papilla (Dl) and the small amount of host cytoplasm surrounding it. Observe that the mitochondria are aggregated in the main body of the appressorium. x18,000.

Figure 22. Higher magnification of the peg region of Fig. 21. Note the alteration in the staining of the epidermal cell wall (EW) around the peg (arrows). Membranes are present in the papilla. Note the numerous small vesicles (SV) and ribosomes (R) in the appressorial lobe close to the peg. x39,000.

Figure 23. High magnification of the peg (P). Observe the extremely electron-dense staining of the wall in contact with the peg (arrows). The tip of the peg is hard to define because of the oblique sectioning of the plasma membrane in this region. x101,500.

21

PLATE 9

Penetration pegs, papillae and organelles of host cytoplasm

Keystone 14 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 24. High magnification of a peg in the epidermal cell wall. Observe the intense electron-density of the epidermal wall (EW) around the peg (P) (arrow). The wall beneath the cuticle (Cu) is eroded on the left side of the peg. The fungal plasma membrane of the peg expands just beyond the cuticle to lie almost adjacent to the limits of the hole in the wall. Note the electron-lucent bodies between the plasma membrane and the cell wall of the fungus on the left of the peg and the electron-dense material on the other side of the peg. x83,250.

Figure 25. Similar stage of penetration as Fig. 24. Note again the expansion in width of the peg (P) beneath the cuticle, the angle of penetration and the globose structure of the papilla (Pl). x42,500.

Figure 26. High magnification of organelles in the host cytoplasm around the papilla. Included are a plastid (Pd), lipid body (L) and mitochondria (M). Note the rough ER close to the lipid body. x54,000.

24

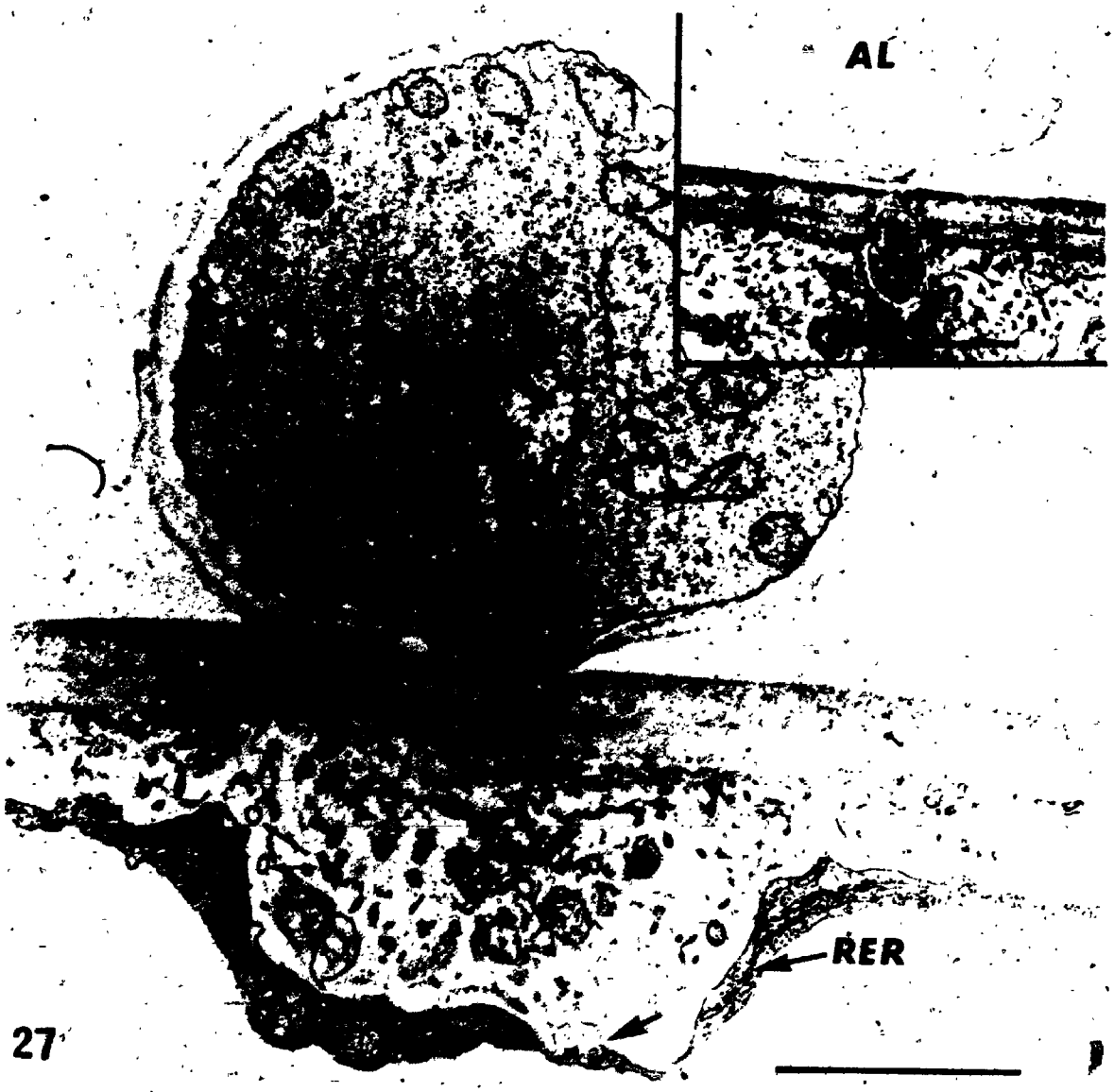
PLATE 10

Appressorial lobe, penetration peg and papilla

Manchurian 17 hr. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 27. A transection through an appressorial lobe of a successful penetration with a haustorium formed (not shown). Notice that mitochondria (M) are now in the pore region. Observe that the papilla contains many membranes and vesicles (V) which may be pinched off by pinocytosis from the plasma membrane (arrow). The papilla is surrounded by long strands of rough ER and mitochondria. x38,400.

Figure 28. The same infection site as above but showing the neck of the haustorium (P) as it passes through the wall and papilla. Note that by sectioning from this angle, i.e. longitudinal to the appressorium, it is not possible to get a continuous picture of the penetration hypha (P) and the appressorial lobe (AL) because of the angle of penetration of the fungus through the epidermal wall. x23,400.



27

PLATE 11

Penetration site

Manchurian 14 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 29. An oblique section through the penetration region and the papilla. Note the halo (Ha) around the peg in the epidermal cell wall. The microfibrils of the wall are slightly separated and the wall around the peg is swollen. The cuticle (Cu) is unaffected. The peg itself does not appear healthy (cf. Fig. 47). Observe the double-layered papilla composed of an electron-dense region (D1) close to the wall and the penetration peg and the heterogeneous outer layer (D2). Notice that many membranes are interspersed in the matrix of the papilla (white arrows) and the vesicle which appears to be in the process of passing through or fusing with the plasma membrane (black triangle). Note the thin strand of fungal adhesive material (Fam) close to the cuticle and attached to the appressorial lobe. x46,500.

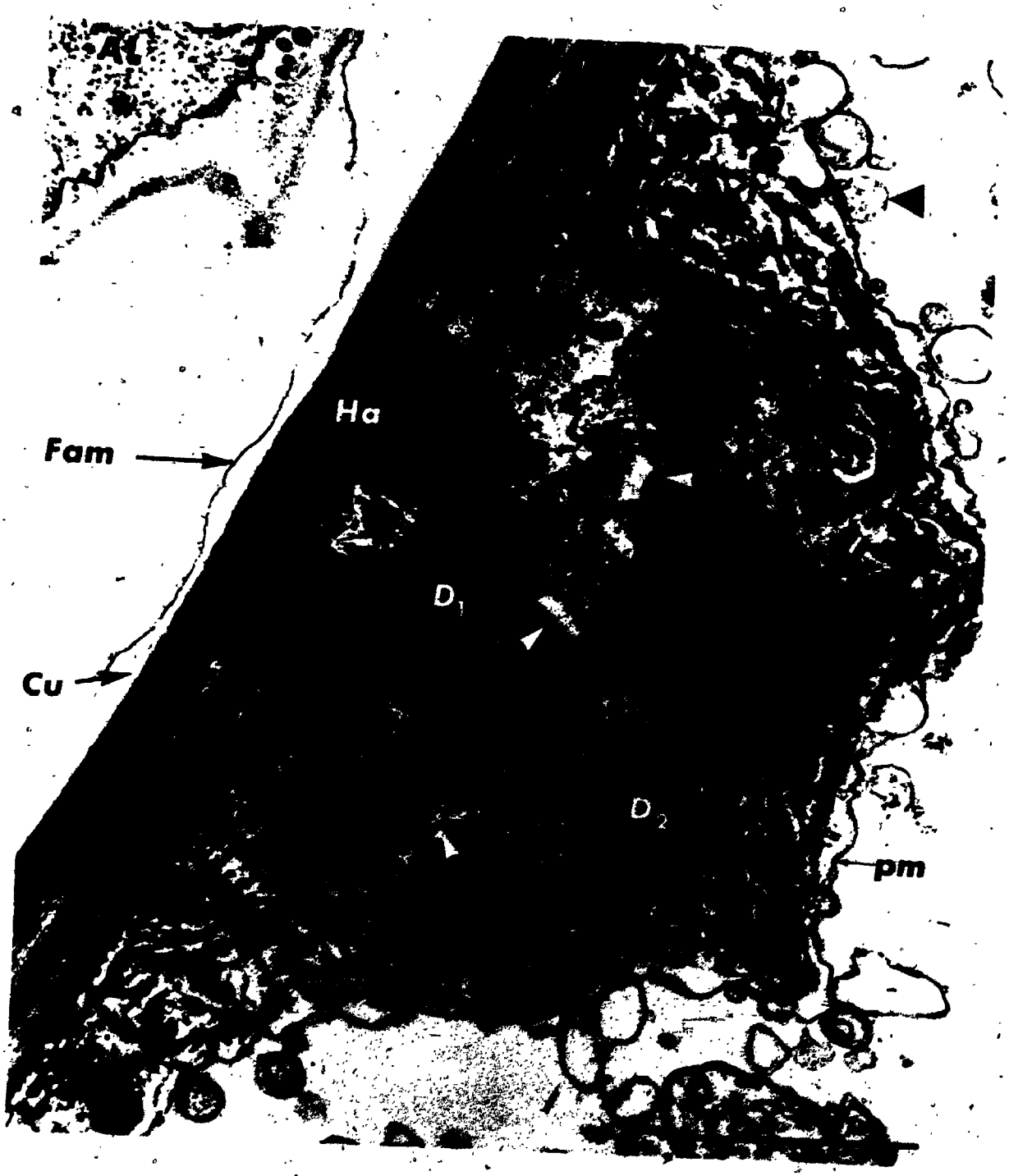

Ha
Fam
D1
Cu
D2
pm

PLATE 12

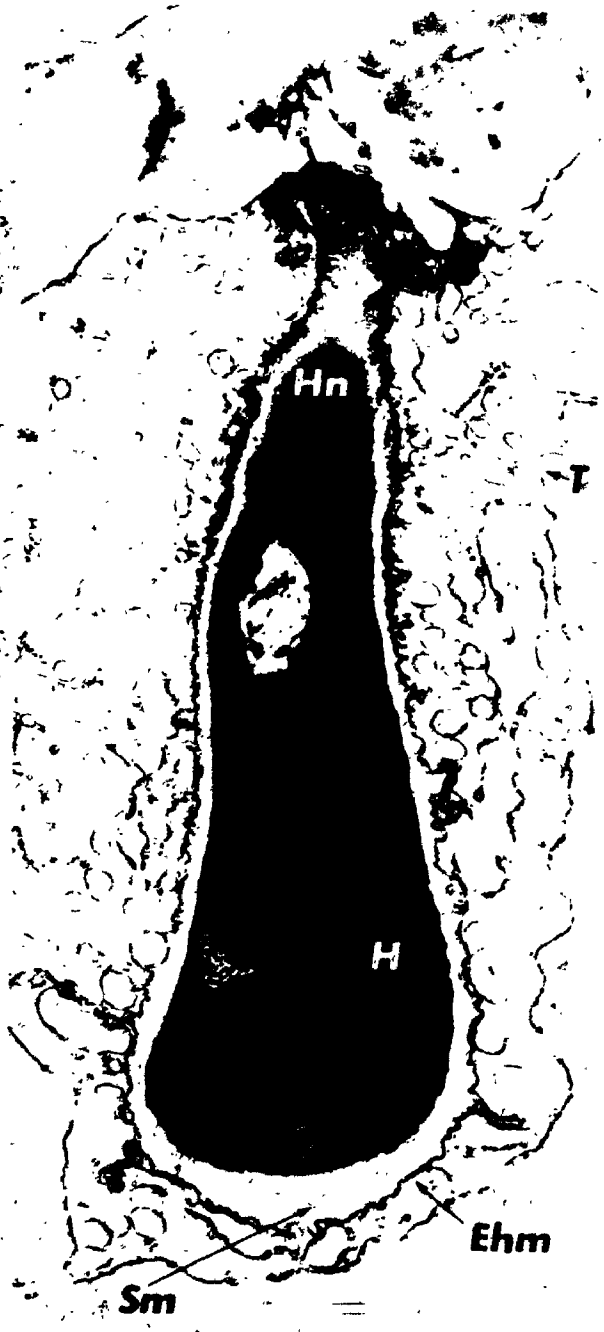
An incipient haustorium

Keystone 14 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 30. A section through an incipient haustorium, penetration hypha and appressorium. Note the extensive, vesicular cytoplasm surrounding the haustorium. The fungal cytoplasm is extremely dense within the haustorium (H) and the identification of the organelles within it is difficult. x20,000.

Figure 31. Serial section of the haustorium shown in Fig. 30. The haustorium contains thick, strand-like components. Notice the convoluted form of the extrahaustorial membrane (Ehm) (the invaginated host plasma membrane) and the thin sheath (Sm) which surrounds the haustorium. x31,000.

30



31

PLATE 13

Young haustoria

Figure 32. A section through a haustorium, haustorial neck and appressorium. The haustorium (H) is filled with dense groundplasm, vesicles and ER. Mitochondria are present in the neck (Hn). A double-layered papilla is prominent (D_1, D_2) and the host cytoplasm surrounding the penetration area contains golgi apparatus, mitochondria and vesicles. Keystone 15 hr. x21,900. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 33. A section through a haustorium, haustorial neck and appressorium. The haustorium (H) has been separated from the appressorium (AL) by a septum (Se). Notice the deep invagination of the papilla (D) alongside the neck of the haustorium (Hn). Manchurian 15 hr. x18,300. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

32

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PLATE 14

Haustorial neck and a haustorium

Figure 34. A section through the neck of a haustorium, surrounded by a papilla and host cytoplasm. Notice the separation of the neck of the haustorium (Hm) from the papilla (D_2) by an electron-dense zone, similar to the electron-dense invagination as seen in Fig, 33. Note the vesicles in the papilla and the presence of golgi apparatus (Go), a plastid (Pd) and mitochondrion (M) in the host cytoplasm. Manchurian 24 hr. x48,300. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 35. A section through a young haustorium, showing the surrounding extrahaustorial membrane (Ehm) and tonoplast (T). Notice that the sheath, the region between the haustorial plasma membrane (Hpm) and the extrahaustorial membrane is very thin. The haustorium is filled with dense cytoplasm, consisting of ribosomes, ER and vesicles, and mitochondria. Note the thick strands of membranes (white arrow) in the central region of the haustorium. Keystone 15 hr. x32,800. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

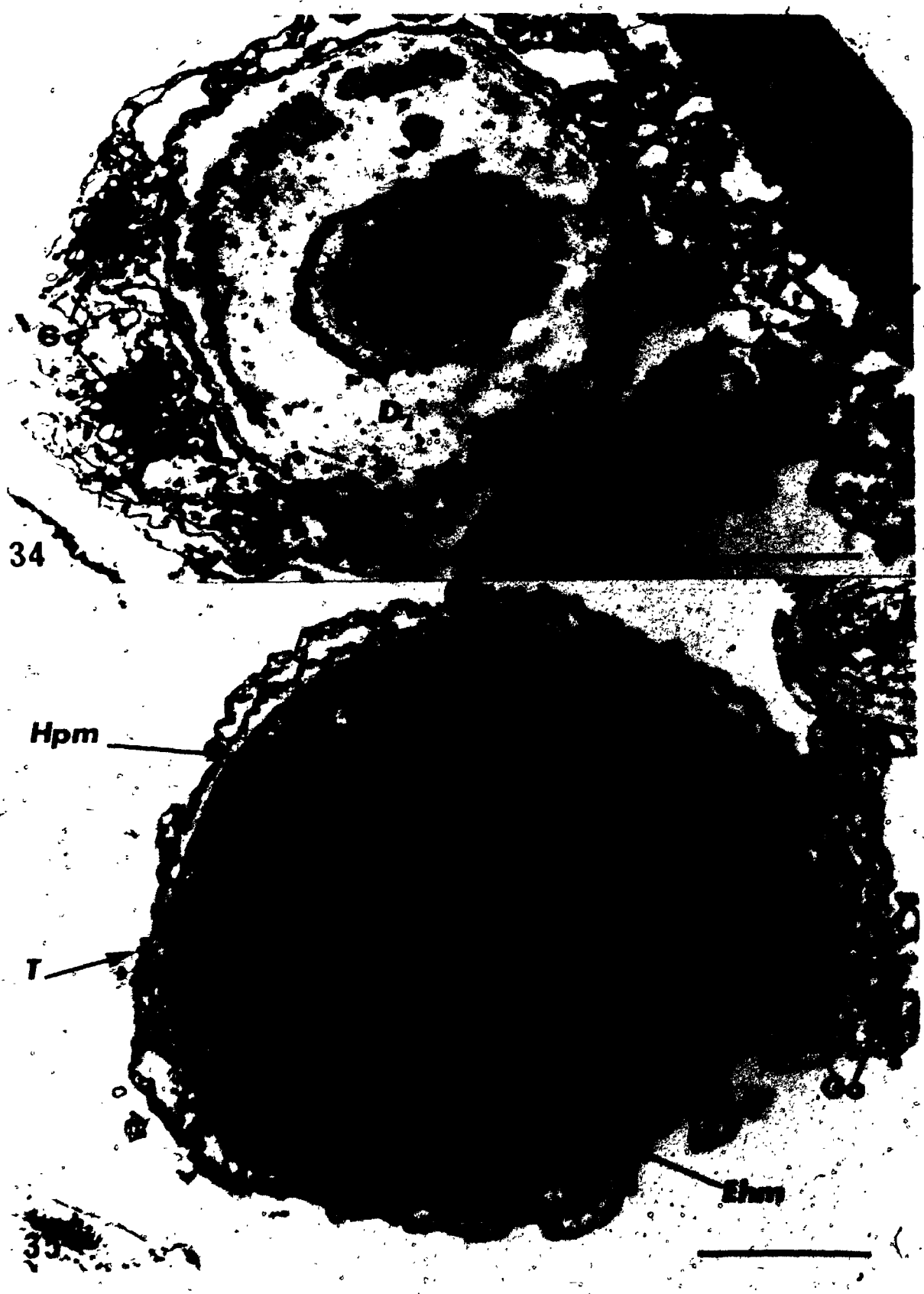

Ec
D1
34
Hpm
T
Ehm
33

PLATE 15

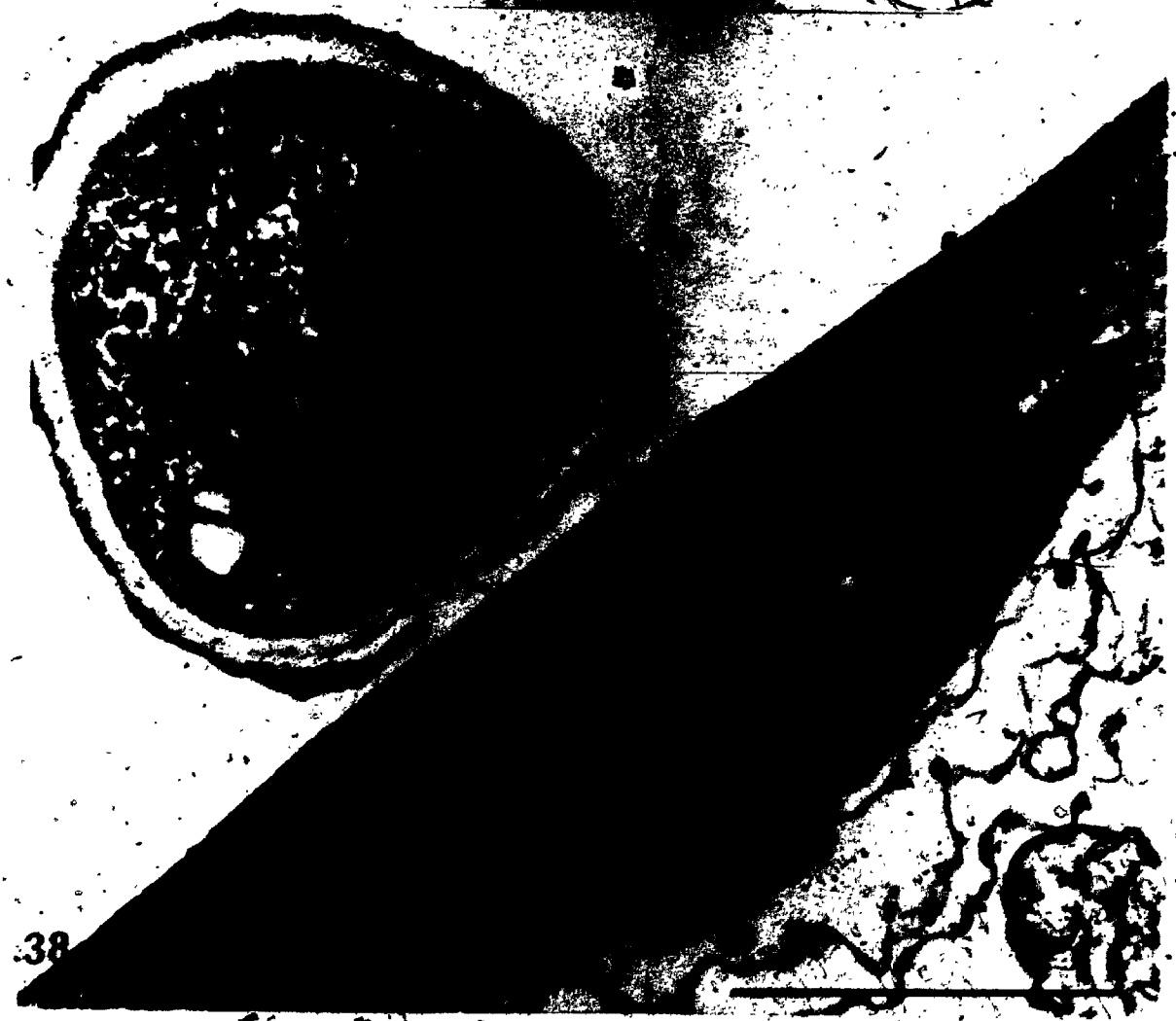
Undifferentiated germ tubes

Figure 36. Section through an undifferentiated germ tube. The epidermal cell wall beneath the germ tube shows an alteration in electron density (Ha), and a small papilla is present. Notice the vesicular body (vb) in the cell wall of the fungus. These structures are commonly seen (quite close to the tip) on the underside of germ tubes. Manchurian 11 hr. x24,850. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 37. Section through an undifferentiated germ tube. Note the large vacuole (V) in the germ tube and the presence of glycogen. Again, a halo in the epidermal cell wall and a small papilla are present. Manchurian 14 hr. x20,150. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 38. Section through an undifferentiated germ tube. Note the glycogen (Gy) present in the germ tube. A halo and papilla are present. Notice the rough ER beneath the papilla. Kwan 14 hr. x56,800. Glutaraldehyde followed by osmium. Uranium and lead stained.

36



38

PLATE 16

Normal uninfected epidermal cells

Kwan 7 day plant. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 39. Normal epidermal cell from uninfected barley. Notice the exceedingly thin layer of cytoplasm, except where the nucleus (N) is situated. Most of the cell is vacuolar (V). x4,875.

Figure 40. Junction of two epidermal cells, from uninfected leaf. Note the darker staining middle lamella region (ml) which extends beneath the cuticle (Cu). The plasma membrane (Pm) and the tonoplast are adjacent except for regions where cytoplasmic organelles such as mitochondria are present. x29,900.

Figure 41. Portion of healthy epidermal cell. Note the layer of cytoplasm rarely exceeds 0.5 µm in thickness except where the nucleus (N) is situated. x7,700.


V
39
pm
Cu
T
40
41

PLATE 17

Kwan. Appressorial lobe and penetration peg

Kwan 11 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 42. A section through an appressorial lobe, penetration peg on the resistant host, Kwan. Note that penetration was effected with only little papilla formation (D_1). The appressorial structure is similar to that on susceptible varieties (cf. Fig. 19, 21). The presence of the host nucleus in the area of penetration is fortuitous. Note golgi apparatus (Go), vesicles and rough ER in the cytoplasm around the papilla. x23,300.

42

PLATE 18

Serial sections through a penetration site

Kwan 11 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figures 43, 44, 45, 46.

Serial sections through the same peg as shown in Fig. 42. Note that the hole formed is much wider than is usual and that large amounts of fungal material have been secreted into the hole (Fig. 43, 44). This material has similar density to the fungal cell wall. Fragments of the cuticle (large arrows) are present in this material. Vesicles and membranes (small arrows) are present in the papilla. x45,250.


45
45

PLATE 19

Papilla-stopped penetration peg

Kwan 14 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 47. Section through an appressorium, peg and papilla. The peg is apparently blocked in the large papilla (D_1, D_2). Note the electron-lucent areas (arrow) where the cytoplasm is shrunken away from the tip of the peg. The cytoplasm in the appressorium is normal. x21,200.


D
47

PLATE 20

Serial sections of a papilla-stopped penetration

Kwan 20 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 48. A section through the peg and papilla at a later time, 20 hr, after inoculation. This figure shows the peg as far into the papilla (D_1) as it penetrated. Note the double-layered papilla and the loose aggregation of cytoplasm in the infected area. x19,800.

Figure 49. Serial section of the same infection site shown in Fig. 48. Fungal cytoplasm in the appressorium is healthy. Note the intense halo reaction (Ha) of the epidermal cell wall. x19,800.


P
D

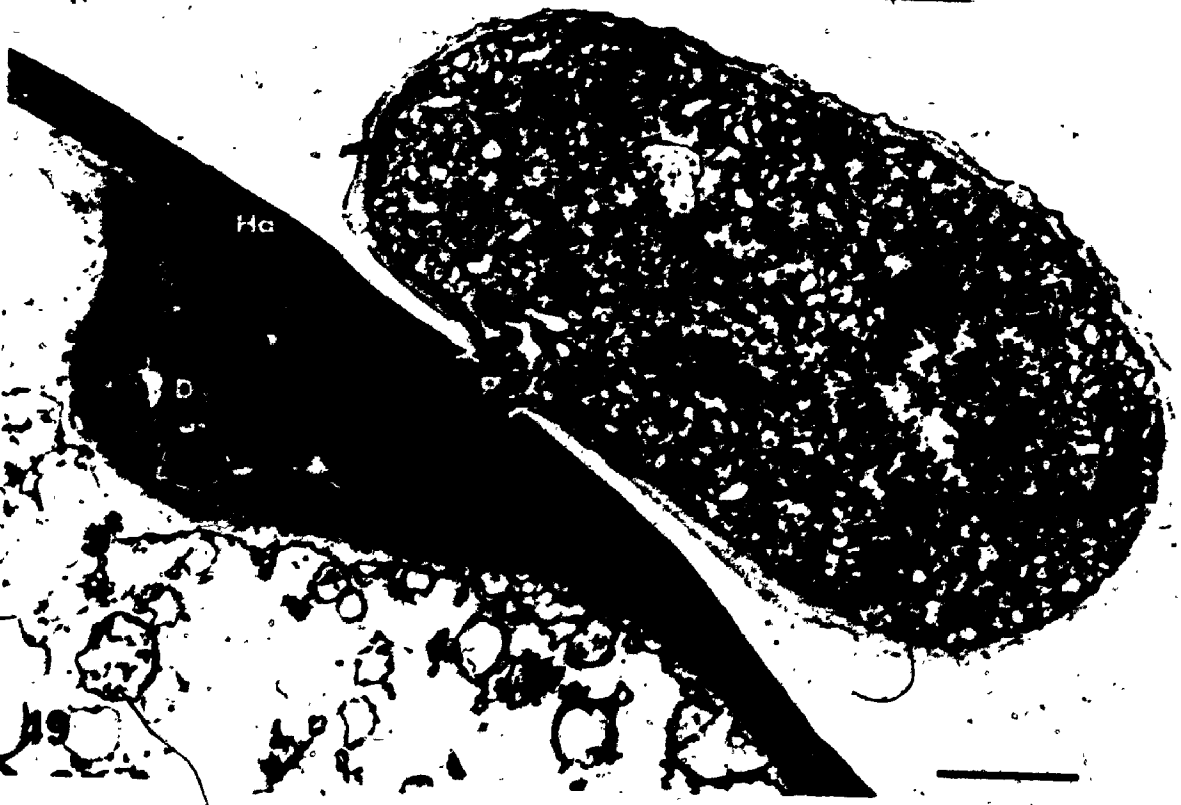

Ha
D

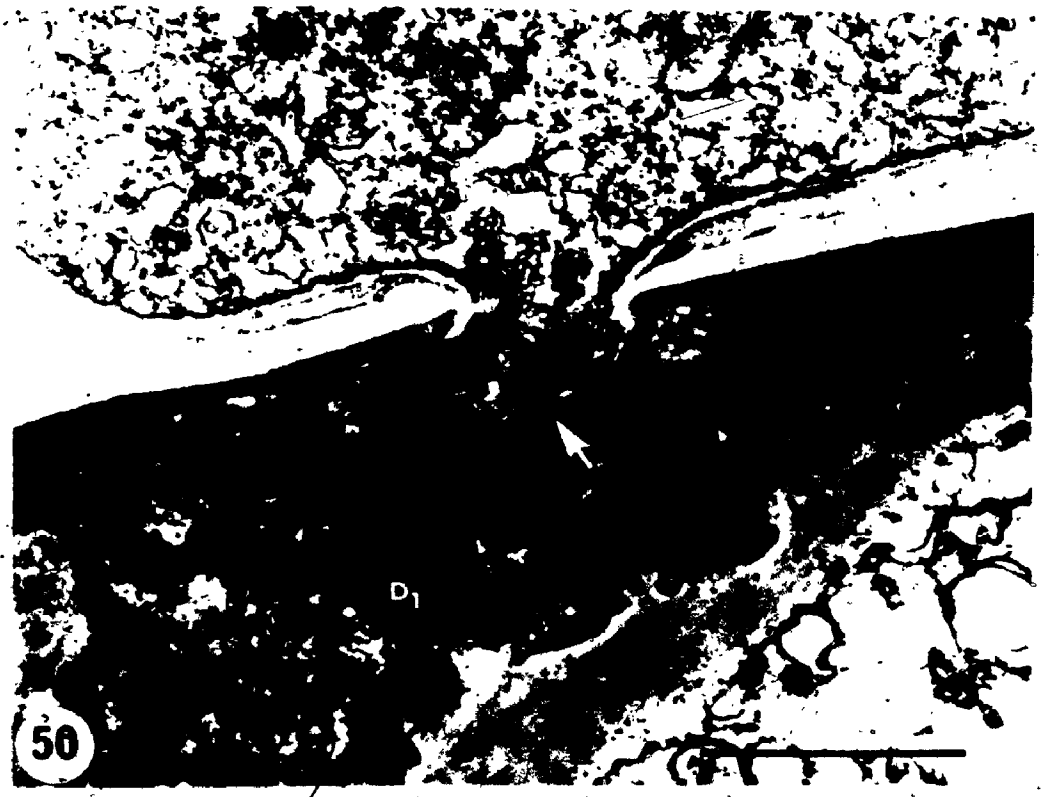
PLATE 21

Same series as Plate 20 (cont'd.)

Kwan 20 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 50. High magnification of Fig. 49. The peg is in the wall. Observe that the wall is split and the microfibrils separated. A spur of cell wall material (large arrow) has apparently been pushed in and turned up. A line of membranes (small arrows) can be seen in the primary deposit of the papilla. Such lines of membranes are commonly present in the papilla. x38,300.

Figure 51. Serial section of the same infection site shown in Fig. 48, 49 just to one side of the peg. Observe the dark stained material between the fungal plasma membrane and the cell wall passing into the peg hole (cf. Fig. 24). x19,800.


D1
50

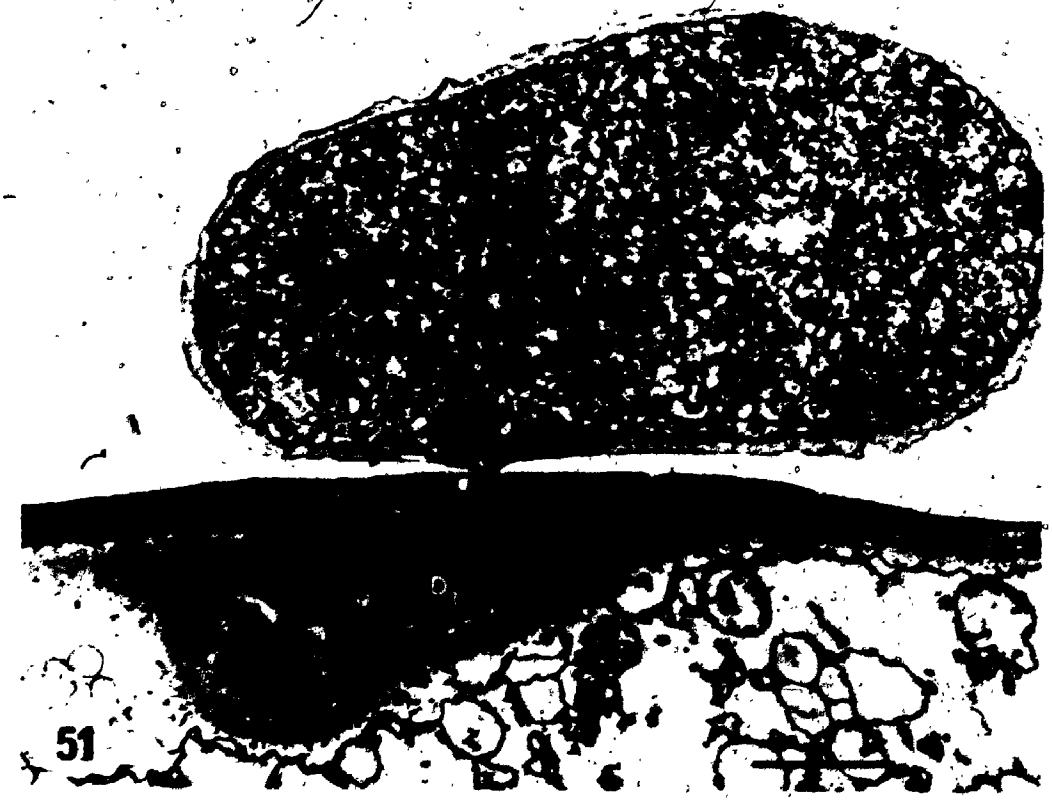

51

PLATE 22

Papilla-stopped penetration

Kwan 20 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 52. An appressorium, peg and papilla. Observe the large stellate papilla (D_1, D_2) and vesicular cytoplasm of the host. The fungal cytoplasm appears normal. x19,800.

Figure 53. Serial section of the same infection site shown in Fig. 52 showing the limit of peg growth (P) in the papilla. The papilla has prevented further growth but the fungal cytoplasm appears normal. x29,000.

52

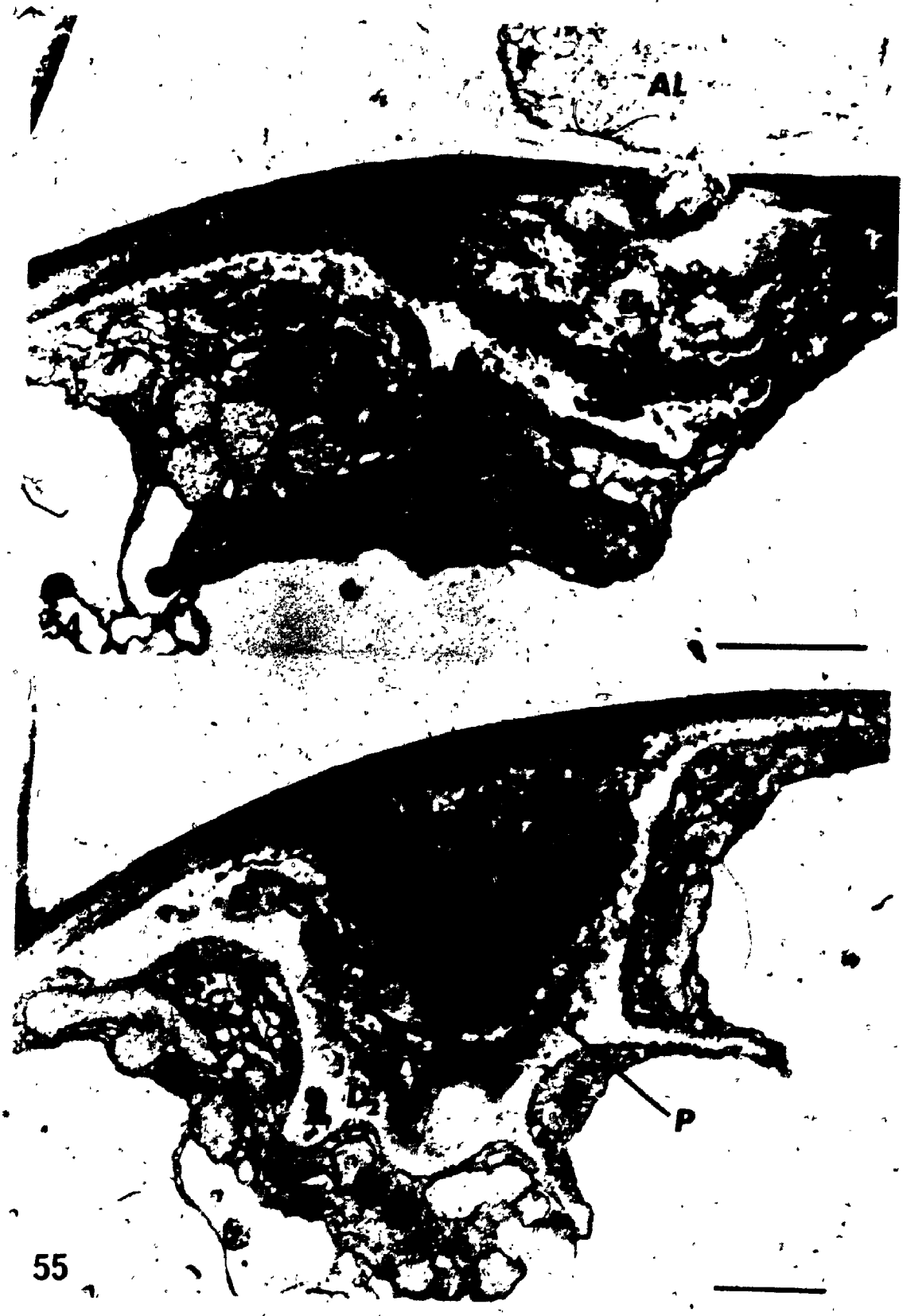
PLATE 23

Papilla-stopped penetration

Kwan 20 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 54. The first of a series showing a peg blocked in the papilla. This shows the healthy appressorial lobe and the peg within the wall. Note the intense halo reaction of the wall and the membrane layers in the papilla (D_1). The host cytoplasm is densely aggregated around the papilla and includes a plastid (Pd), mitochondria, golgi bodies, vesicles and ribosomes. x27,500.

Figure 55. Serial section of the penetration shown in Fig. 54. Note the peg, surrounded by electron-dense material, in the D_1 part of the papilla. Notice that the region between the stellate D_2 papilla and the plasma membrane contains osmiophilic amorphous material. x19,800.



55

PLATE 24

Same series as Plate 23 (cont'd.)

Kwan 20 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 56. A serial section of the penetration shown in Fig. 54, 55 showing the tip of the peg (P) stopped in the papilla. The oblique section through the papilla shows it to be surrounded by host cytoplasm. The peg would be about 5 μm in length to extend this far into the papilla. Note the presence of a second plastid (Pd) and the golgi apparatus (Go). x24,200.

56

PLATE 25

Algerian - Appressorial lobe and penetration peg

Algerian 15 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 57. An appressorium, penetration peg in the Algerian host. Note the similarity to figures of the infection process in the susceptible hosts and the Kwan host. The appressorial structure is comparable to that of the susceptible (Fig. 21) and the Kwan (Fig. 42) varieties. Note the presence of a halo reaction (Ha) in the cell wall. x20,900.

Figure 58. Serial section of the penetration site shown in Fig. 57. Note the large cytoplasmic aggregation around the penetration site, reaching to the lower wall of the epidermal cell. Many golgi bodies (Go), vesicles (Ve), mitochondria and ER are present in the loose cytoplasm surrounding the double layered papilla. x13,000.

Figure 59. Higher magnification of the peg region of Fig. 58. Notice the unusual electron-dense areas of the plasma membrane in the peg (arrows). The fungal cytoplasm in the peg is granular and devoid of organelles. x39,400.

Hd
57
D
Go
58

PLATE 26

Appressorium and haustorium

Algerian 15 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 60. An apparently healthy appressorium, and haustorium on the Algerian host, 15 hr after inoculation. Cf. Fig. 32 and 33. Note the double-layered papilla (D_1, D_2) and the intensely electron-dense region between the haustorial neck (Hn) and the papilla. The cytoplasm is vesicular (Ve) and contains golgi apparatus (Go), mitochondria (M) and a plastid (Pd). x14,000.


D_1
D_2
Pd
60

PLATE 27

Haustorium

Algerian 15 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 61. Haustorium from serial section of the haustorium shown in Fig. 60. Compare with Fig. 35. Note the numerous small vesicles (SV) and ribosomes (R) of the young haustorium (H). Typical of haustoria at this stage is the closeness of the extrahaustorial membrane (Ehm) to the body of the haustorium. x31,800.

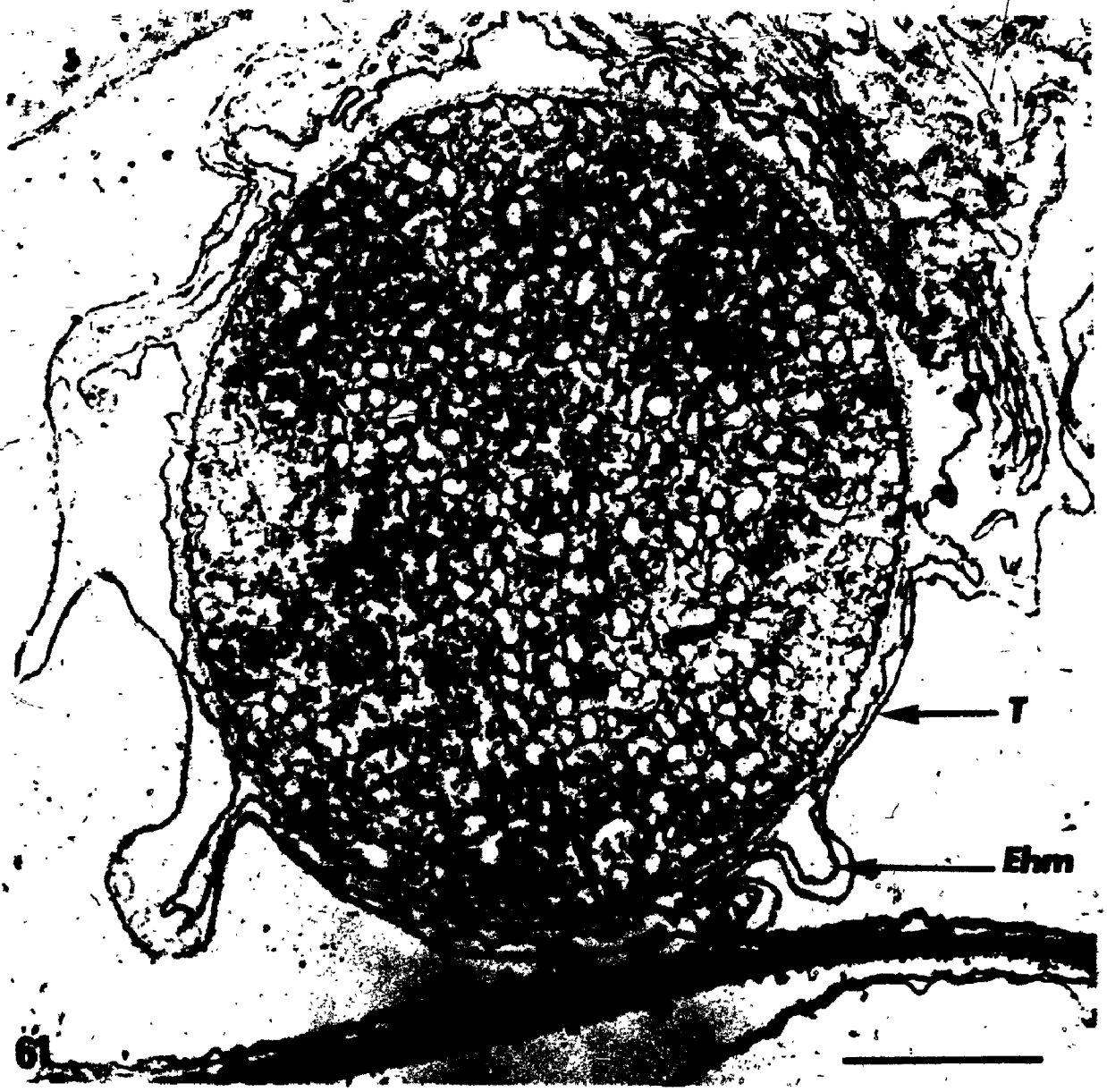

T
Ehm

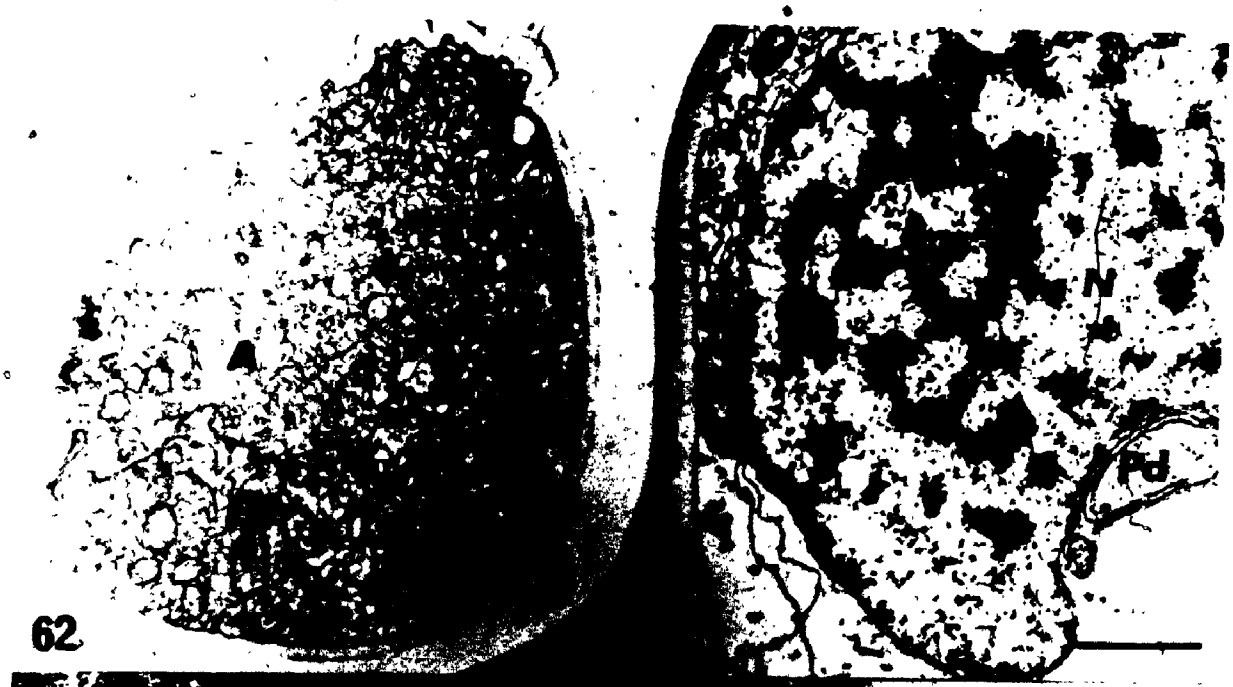
PLATE 28

Serial sections of guard cell infection

Algerian 21 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 62. Transection of an appressorium in the epidermal groove between an epidermal cell and a subsidiary guard cell. The appressorium is compressed but otherwise similar to that in Fig. 18. Note the central region of the cell containing strands of ER and groups of polyribosomes. x18,200.

Figure 63. Serial section of the appressorium shown in Fig. 62, showing the appressorial lobe over the subsidiary cell and the penetration peg in the wall of the guard cell of the stomate. Guard cells are rarely infected by E. graminis. Note the thickened guard cell wall (GCW) and the papilla (D). The unusual feature of penetrations of the Algerian host is the frequent appearance of an electron-dense zone of demarkation in the wall (unlabelled arrow). x22,000.



62

63

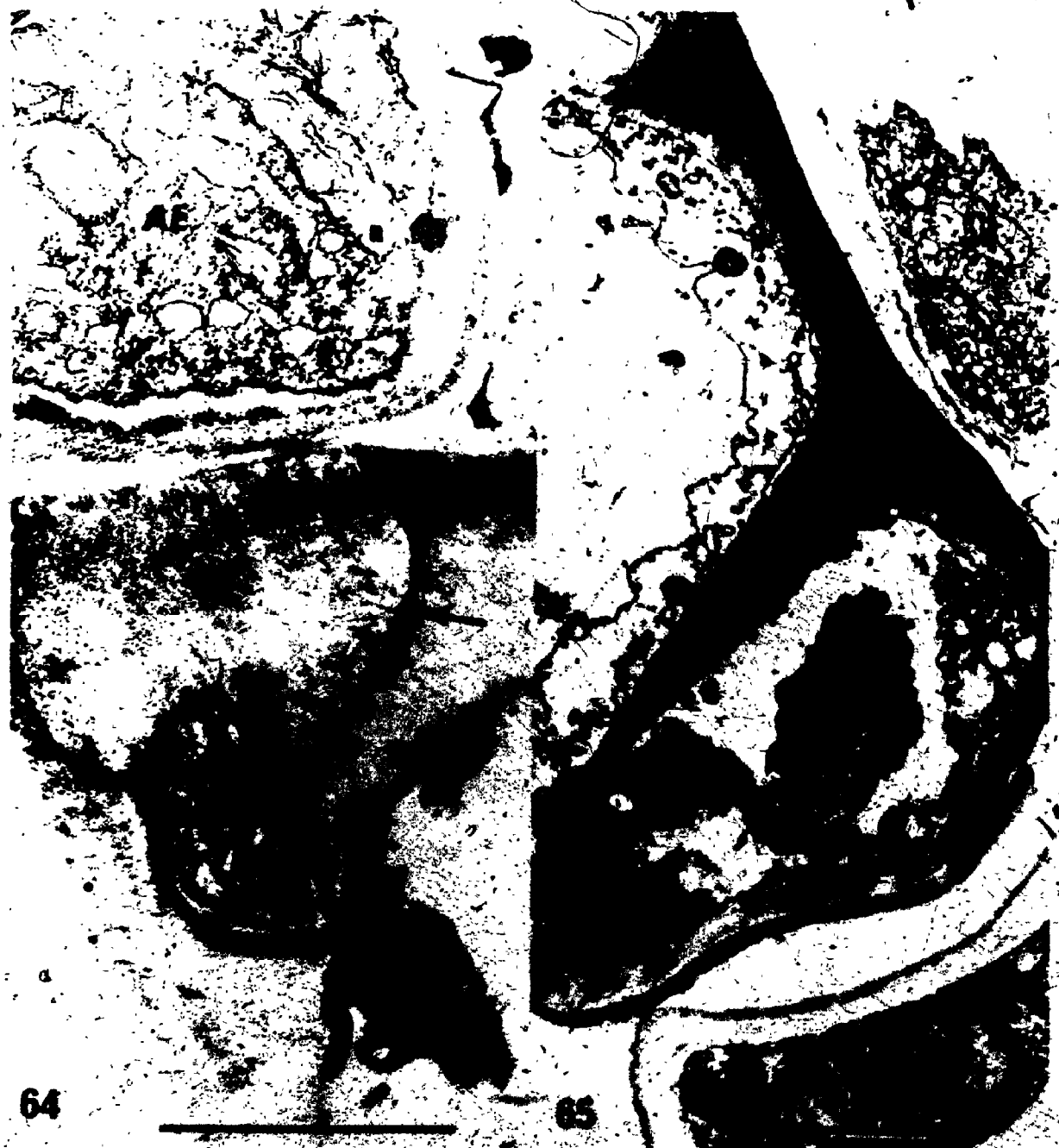
PLATE 29

Same as Plate 28 (cont'd.)

Algerian 21 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 64. High magnification of the peg shown in Fig. 63. The peg is almost through the cell wall here. Alteration of the wall within the electron-dense zone (arrow) can be seen, especially the loss of the electron-dense layer of the wall directly beneath the cuticle. Fig. 63 also shows this well. x48,900.

Figure 65. Serial section beyond the wall alteration apparent in Fig. 64. Note the electron-dense region surrounding the neck of the haustorium (Hn). x16,400.



64 65

3 3

OF/DE

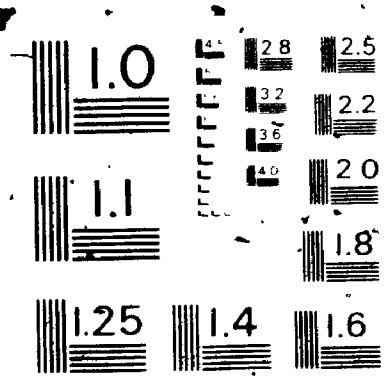


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PLATE 30

Normal uninfected guard cells

Algerian 7 day plant. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 66. Healthy, uninfected guard cells from uninoculated leaf. Note the presence of plastids (Pd) with grana and the otherwise thin layer of cytoplasm and the large vacuole (V). x17,700.

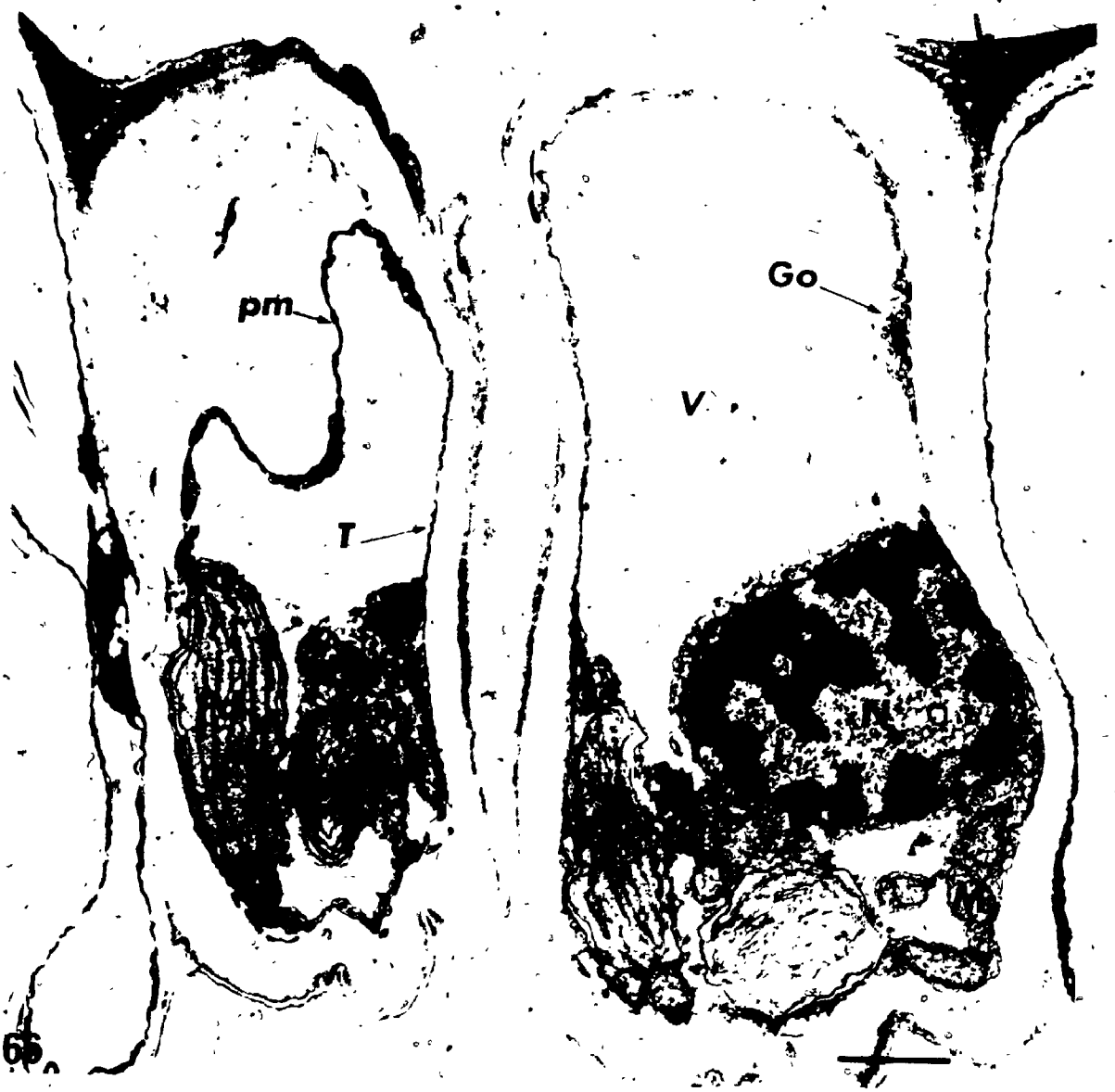

pm
T
Go
V

PLATE 31

Hypersensitive response to infection

Algerian 21 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 67. Section of a hypersensitive epidermal cell in the Algerian host. Notice the epidermal cell has collapsed and the cytoplasm is necrotic. Membranes are thick and coarse. The appressorium has pulled away from the leaf during the EM preparation and pulled with it the peg and the necrotic haustorium or cytoplasm. The penetrated part of the wall shows the darkly staining zone (arrow) which was only observed in the Algerian host. Observe the wall deposits (WL) formed in the adjacent epidermal cell. x22,700.

Figure 68. Transection of the same appressorium as in Fig. 67. Note the breakdown of the fungal plasma membrane and disintegration of cytoplasmic organelles, and the abnormal nucleus (N). x16,600.


A
68
A
WL
67

PLATE 32

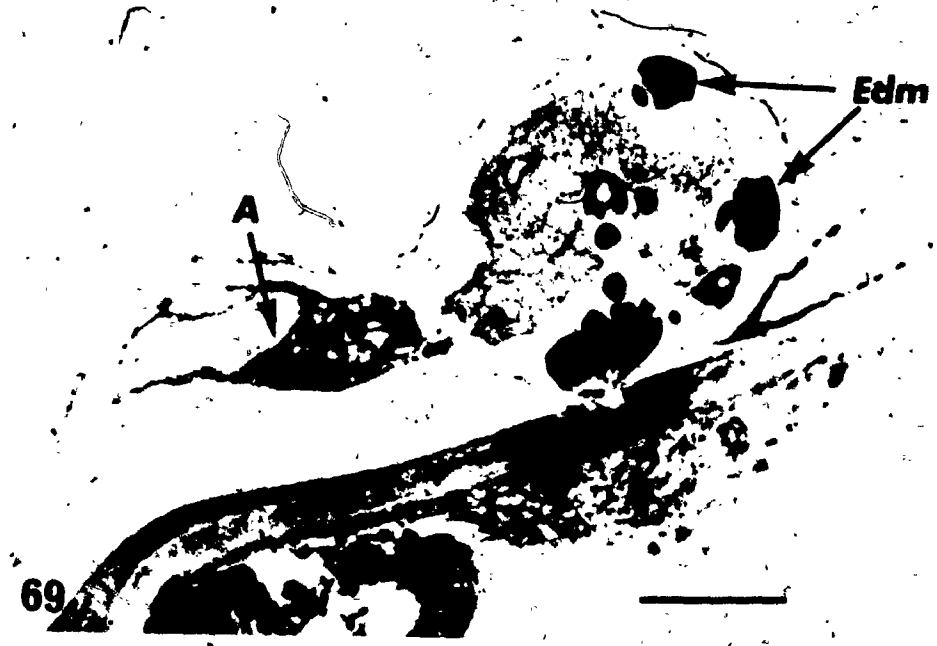
Hypersensitive response showing electron-dense deposits in the appressorium

Algerian 21 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

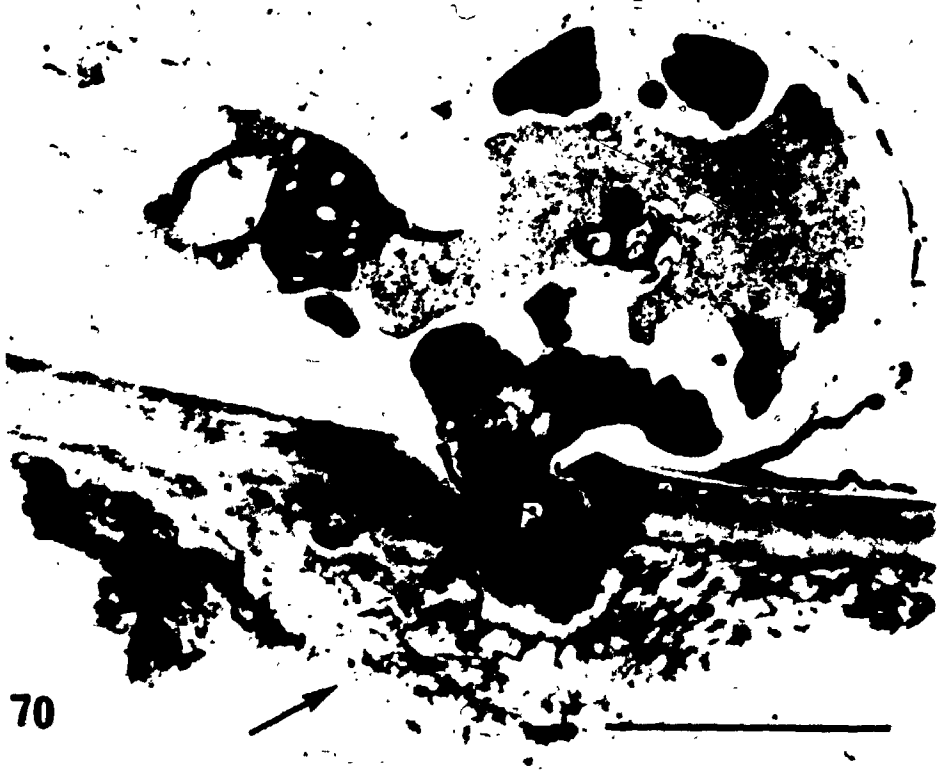
Figures 69, 70. Serial sections of an epidermal cell showing a hypersensitive response to infection in the Algerian variety. Note the electron-dense material (Edm) in the space where the necrotic fungal cytoplasm has shrunk away from the cell wall. The appressorium has collapsed (A). The host cytoplasm is also necrotic and pulled away from the papilla. The host plasma membrane and tonoplast are absent (unlabelled arrow Fig. 70). Note that the peg appears stopped in the papilla. Fig. 70 shows the furthest that the fungus was observed to penetrate into the host. Note, again, the intense halo and the electron-dense line in the cell wall.

Fig. 69 x22,100.

Fig. 70 x43,300.



69



70

PLATE 33

Necrotic appressoria, haustoria and epidermal cell

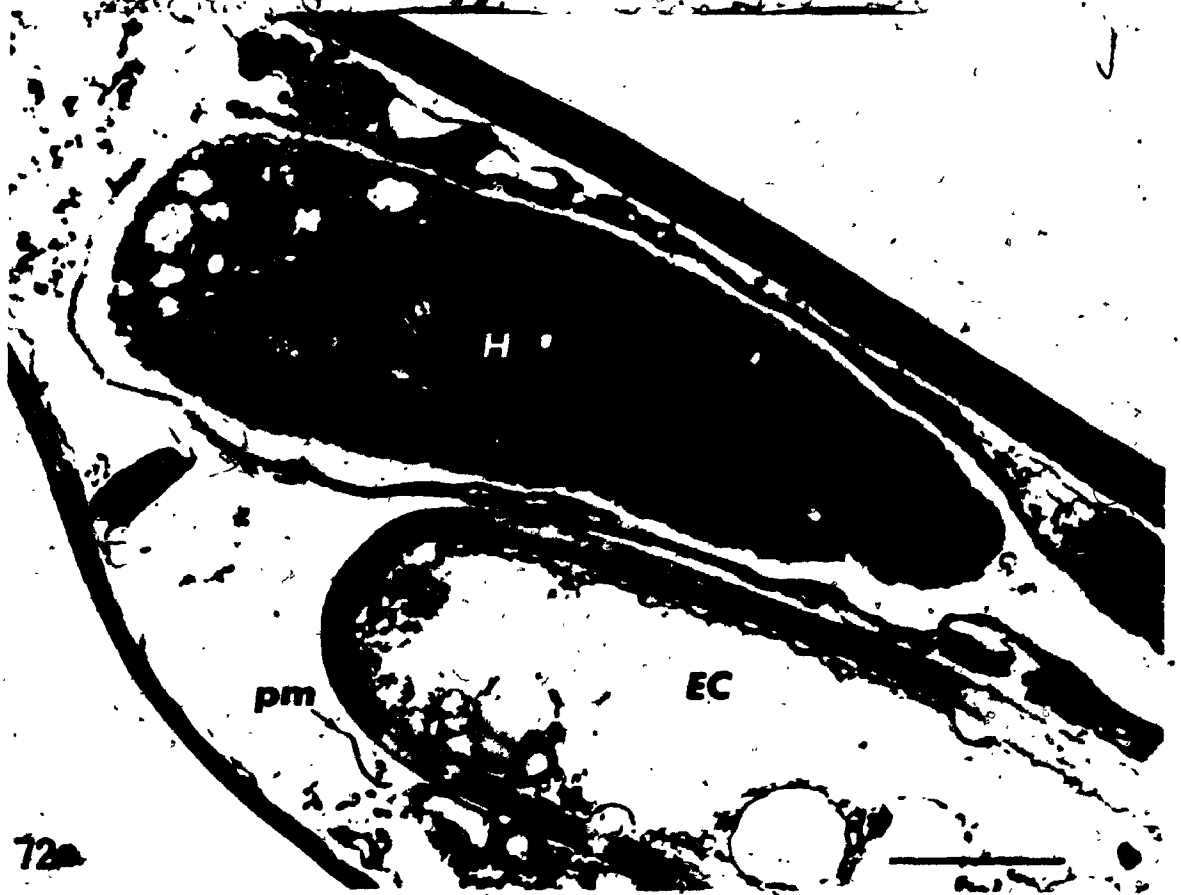
Algerian 21 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figures 71, 72. Appressorium, haustorium neck (Fig. 71) and haustorium (Fig. 72). Notice, again, the electron-dense material (Edm) in the appressorial lobe and the collapsed appressorium. The cytoplasm is aggregated and shrunken from the cell walls. The epidermal cell has collapsed and the cytoplasm is necrotic. Notice the thick electron-dense appearance of the plasma membrane (pm) compared to that of the adjacent healthy epidermal cell (EC). The haustorial cytoplasm is completely necrotic and the extrahaustorial membrane is broken.

Fig. 71 x18,700

Fig. 72 x25,700

71



72

PLATE 34

Serial sections through a hypersensitive epidermal cell, showing the appressorium, penetration hypha and haustorium

Algerian 21 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 73. Note the appressorium (A) is collapsed (arrow) and its cytoplasm coalesced. x18,600.

Figure 74. The epidermal cell is collapsed, the penetration peg (P) broken and the papilla dispersed. Note the absence of the plasma membrane and the tonoplast (arrow). Note the formation of wall deposits (WL) in the adjacent cell. x22,000.

Figure 75. Part of the disorganised haustorium with membranous whorls (cf. Fig. 113), and surrounded by necrotic cytoplasm. x32,600.

75

PLATE 35

Papilla-stopped penetration

Algerian 21 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 76. Section of appressorial lobe and papilla. Note the healthy host cytoplasm and fungal appressorium. The zone of demarkation continuous with the papilla is present in the epidermal cell wall (arrows). Apart from this cell wall staining, this figure is similar to those shown of infections on the Kwan host (Fig. 47-56). x22,500.

Figure 77. Same infection site as shown in Fig. 76. This shows the peg stopped by the papilla. Note the peg (P) has grown along and within the epidermal cell wall rather than into the papilla. This section showed the limit of fungus development. x68,400.

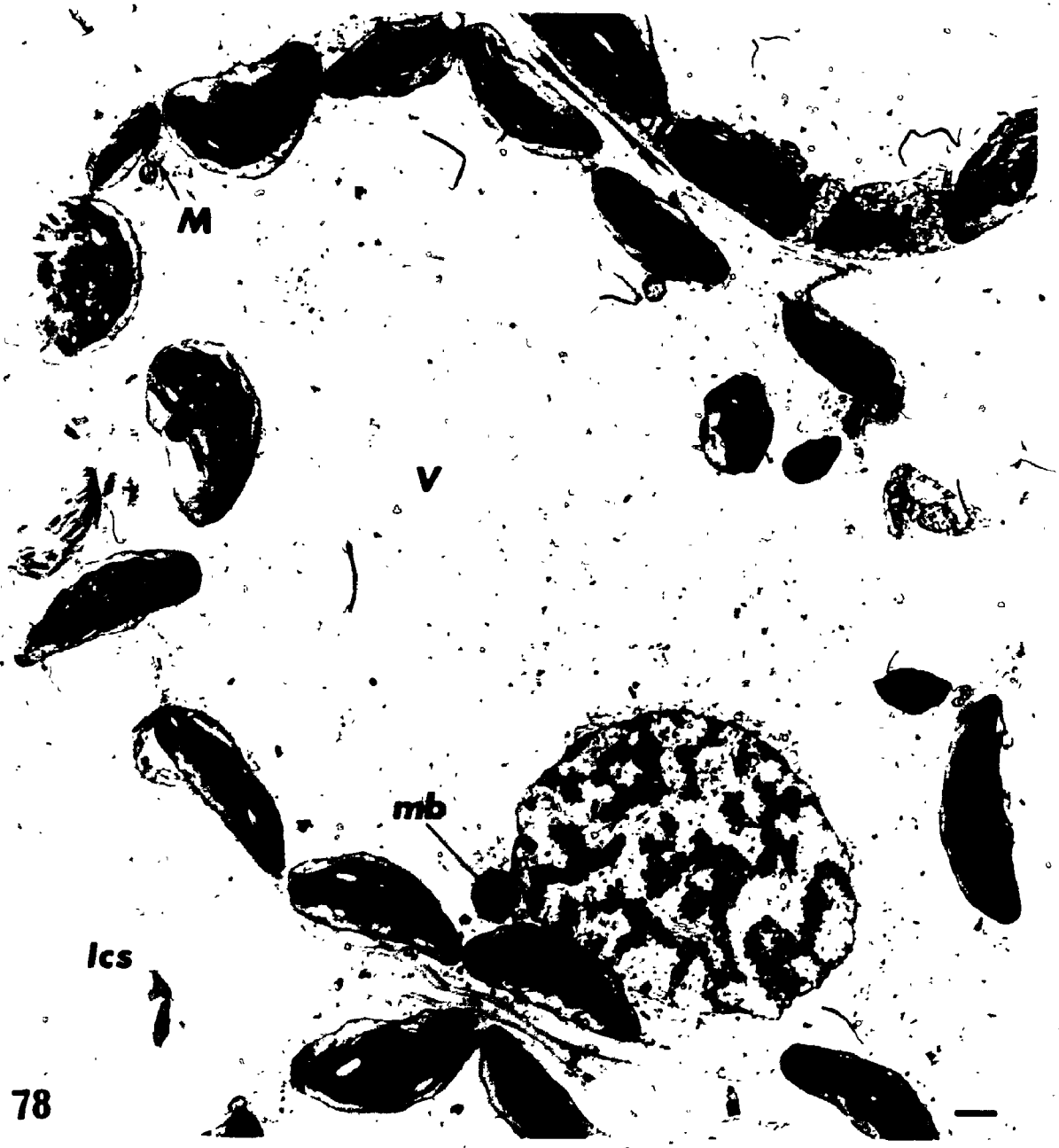

0 5

PLATE 36

Mesophyll cell from infected Manchurian leaf

Manchurian 95 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 78. Section of a mesophyll cell from an infected Manchurian leaf. Note the chloroplasts (C), mitochondria (M), microbodies (mb), and the nucleus (N) are situated close to the cell wall, enclosed within the tonoplast and plasma membrane. There is a large amount of intercellular space (Ics) interspersed between the mesophyll cells. The cytoplasm frequently appears to be loosely aggregated when fixed by Franke's method. x6,650.



78

PLATE 37

Mesophyll cells from infected Manchurian leaf

Manchurian 95 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 79. Section of part of a mesophyll cell from an infected Manchurian leaf, again showing diffuse cytoplasm. Note the plasmodesmata (pa) in the cell wall. Compare Fig. 79 and 80 with 81 and 82 and note the more diffuse appearance of the cytoplasm with this fixation. x12,700.

Figure 80. Section of part of two mesophyll cells from infected Manchurian leaf. Note the mesophyll cell wall with a well defined middle lamella (ml) and plasmodesmata. The cytoplasm contains ER, golgi bodies (Go), microbodies, mitochondria and chloroplasts. Note the starch grains and spaces around starch grains in the chloroplasts. Many plastoglobuli are present, of variable size and distribution and are not especially electron-dense. The chloroplast grana are generally closely stacked. x15,400.


pa
T
pm
79
ER
ml
Go

PLATE 38

Chloroplasts of mesophyll cells in the Manchurian host Manchurian. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 81. Section of part of a mesophyll cell from an infected Manchurian leaf. Glutaraldehyde fixed. Note the compact cytoplasm around the periphery of the cell. Chloroplasts again contain starch grains (sg) and the grana (gr) are closely stacked and electron-dense. Note the small plastoglobuli (pg) present in the chloroplasts. 144 hr. x18,700.

Figure 82. Part of a mesophyll cell from infected Manchurian leaf. Two chloroplasts sandwiching a microbody. The grana are closely stacked and the plastoglobuli are small (0.1 - 0.2 μm). The host cytoplasm is packed close to the plasma membrane. Note the small vesicles pinched off from the chloroplast membrane. 120 hr. x19,800.

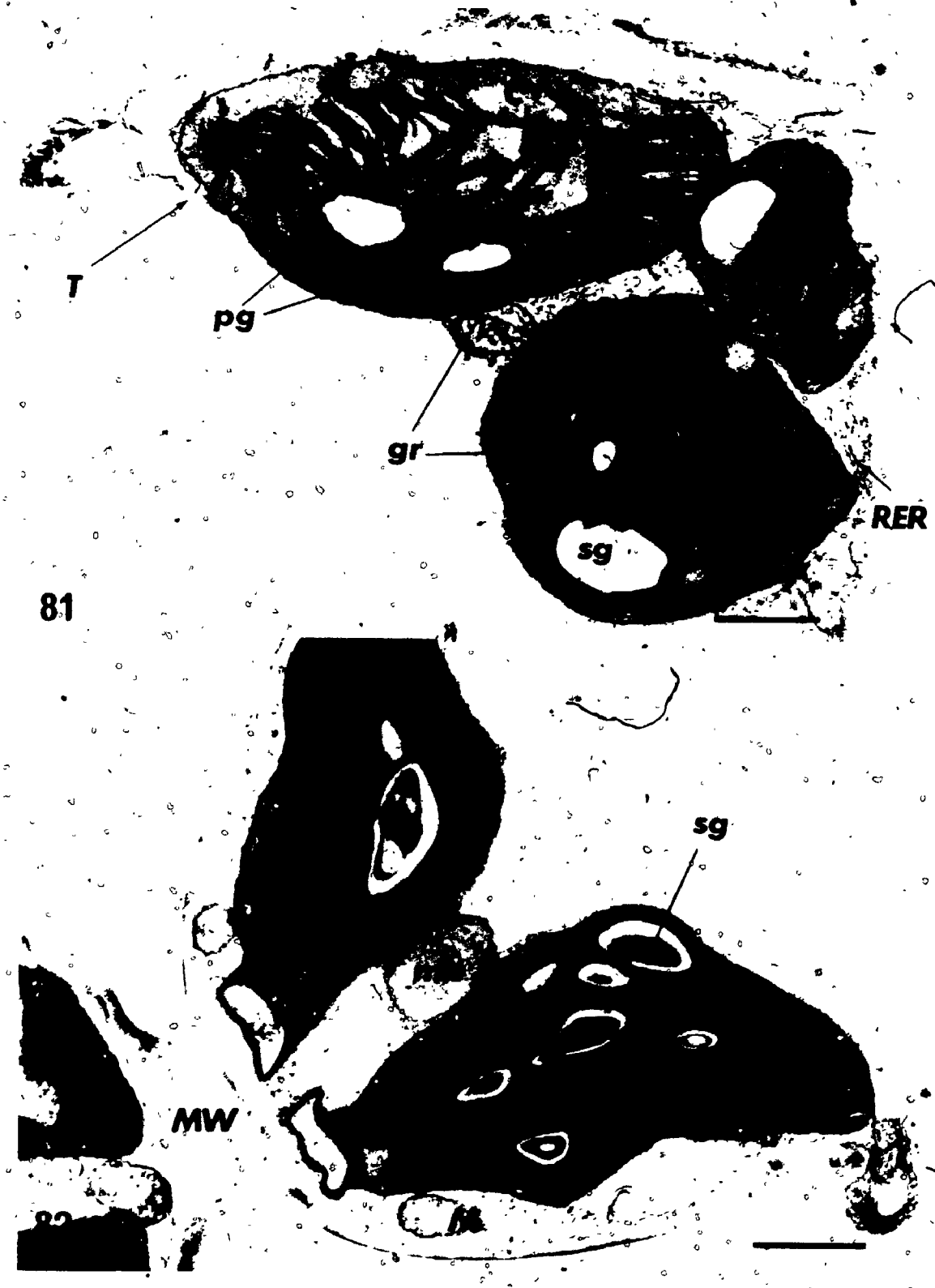

T
pg
gr
RER
sg
81
sg
MW

PLATE 39

Kwan - Mesophyll cell

Kwan 115 hr. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 83. Section of a mesophyll cell of the Kwan host close to an infected epidermal cell. Glutaraldehyde fixed. Similar to Manchurian variety except for the deposits on the tonoplast membrane (unlabelled arrows). The plastoglobuli are intensely osmiophilic and the grana are closely stacked. No starch grains are present. Cytoplasm surrounds the chloroplasts and is close to the plasma membrane. It includes many mitochondria and strands of ER. x20,500.

83

PLATE 40

Effects of HR on mesophyll cells. Initial effects.

Kwan 95 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 84. Oblique section through the mesophyll wall and part of two mesophyll cells of infected Kwan leaves. Initial changes of hypersensitive mesophyll necrosis. Note (black arrows) the electron-dense deposition on the mesophyll plasma membrane. This is the first observed symptom of cell necrosis. Also some disorganization of chloroplasts has begun. This normally involves a swelling or increase in size of plastoglobuli (white arrow). x20,350.

Figure 85. Section of part of mesophyll cells of infected Kwan leaves. Note the prominent deposition (WL) next to the wall, similar to papilla material and the proximity of ER and golgi apparatus. Note the dark staining deposits on the plasma membrane of adjacent cell (large arrow). x29,400.

85

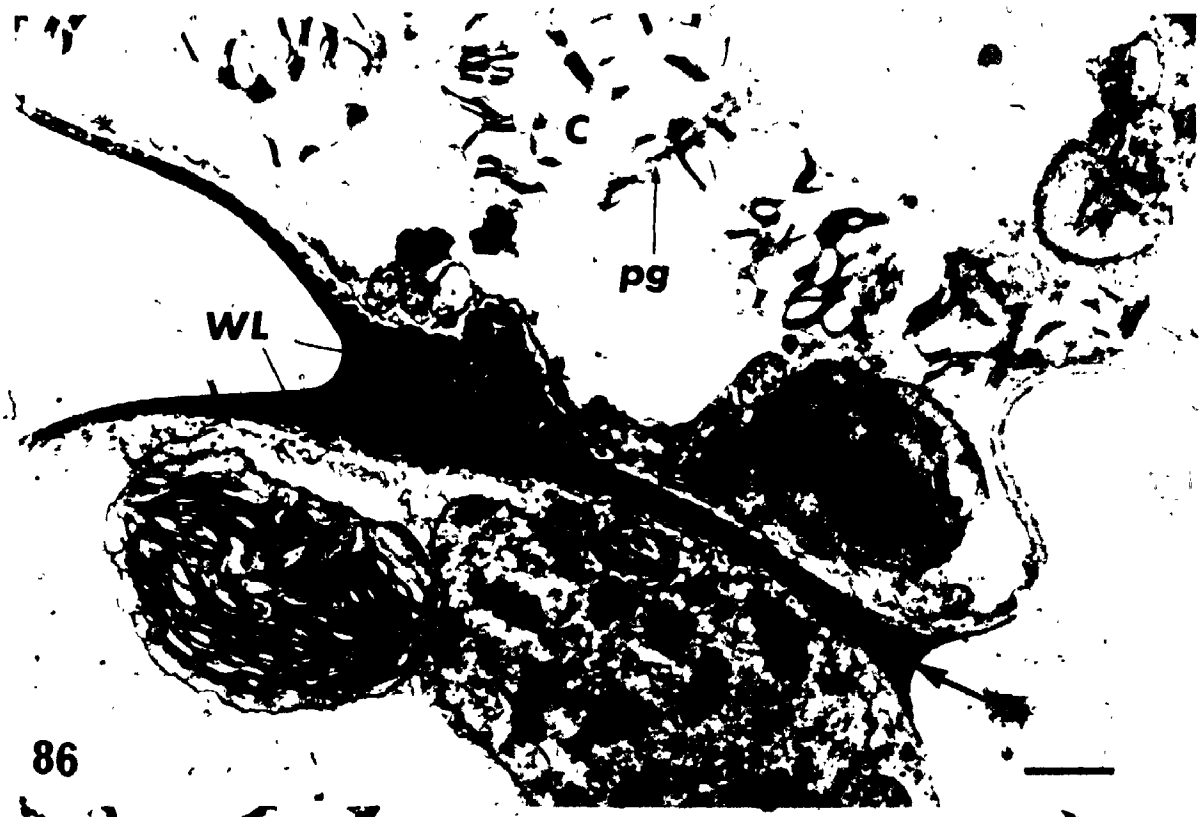
PLATE 41

Effects of HR on mesophyll cells. Chloroplasts and wall deposits

Kwan. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 86. Section through the adjoining cell wall of two mesophyll cells of an infected Kwan leaf. Note the globose electron-dense wall lesions (WL) and alteration in staining of the wall (arrow) cf. Fig. 113. The chloroplasts in the upper cell are broken down leaving membranous fragments and enlarged plastoglobuli (pg). 95 hr. x13,000

Figure 87. Section through three adjoining mesophyll cells of infected Kwan leaf. The cell on the left is completely necrotic (NMC). The adjacent cell has wall deposits (WL) around the whole of the cell and is collapsed (arrows). The cell on the right has wall deposits adjacent to the middle cell but not elsewhere. 120 hr. x13,300



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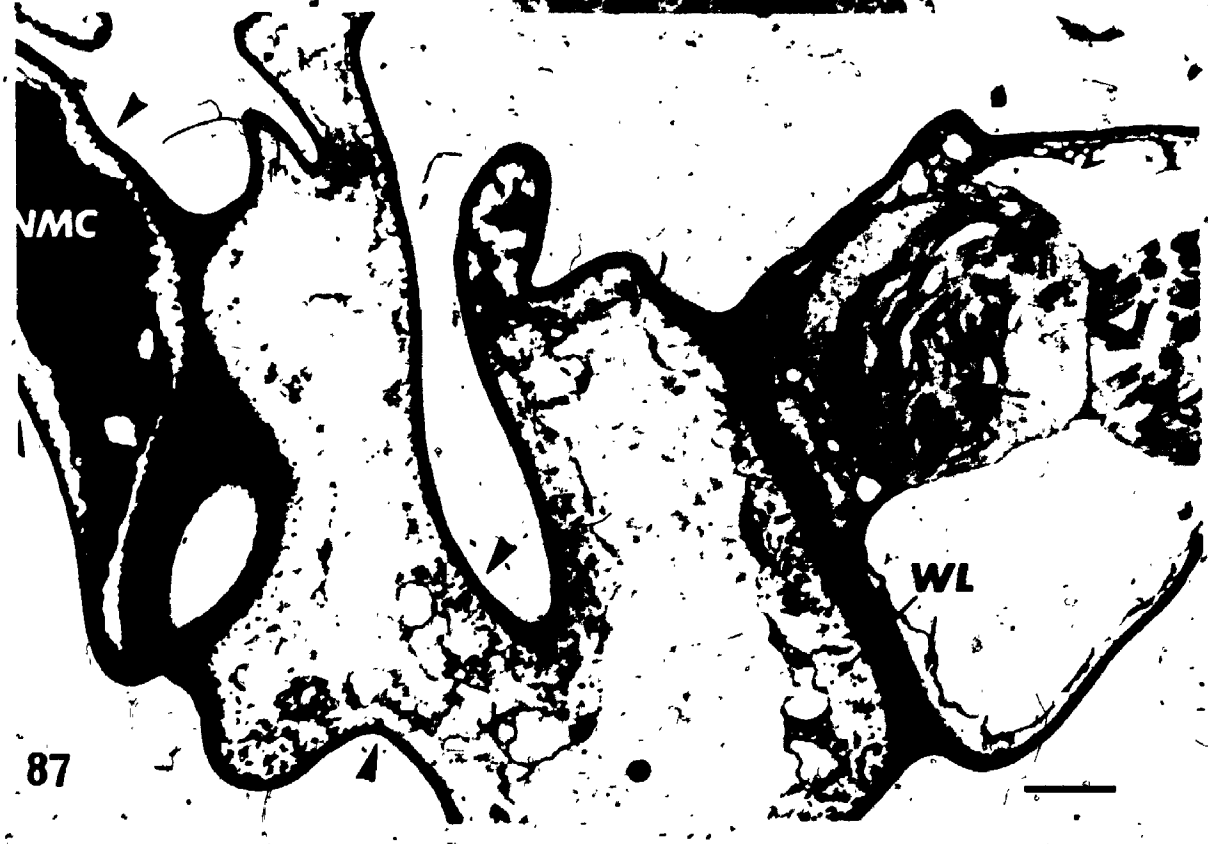


87

PLATE 42

Effects of HR on mesophyll cells. Chloroplasts.

Kwan. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 88. Section of disorganized chloroplasts in mesophyll cells of an infected Kwan leaf. Note the disorganized chloroplasts with separation of grana and large electron-lucent spaces present and that plastoglobuli (pg) are enlarged (up to 0.25 - 0.3 μm). Note the mesophyll plasma membrane is intact but chloroplast membranes are broken. Some grana (gr) have coalesced and have lost all vestige of membrane structure. 95 hr. x28,000

Figure 89. Section through a mesophyll cell close to a necrotic haustorium-containing epidermal cell of an infected Kwan leaf. Note that the plasma membrane (pm) in this cell is broken and the membrane of one chloroplast is completely disintegrated and that of another (Cm) is almost so. Note the swelling of the plastoglobuli, especially pronounced in the chloroplast whose plasma membrane is disintegrated. Grana have also coalesced here. 120 hr. x26,400


pg
88
Cm
pm
89

PLATE 43

Effects of HR on mesophyll cells. Cellular collapse, wall deposits and chloroplasts

Kwan 144 hr. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 90. Section of a necrotic mesophyll cell of an infected Kwan leaf. The plasma membrane is separated from the wall by deposited material (WL). The cytoplasm next to the deposited material (lower cell) contains vesicles, and strands of ER. x18,900.

Figure 91. Section through a collapsing mesophyll cell. The cell is very disorganized. Deposition between the plasma membrane and the cell wall has occurred and the chloroplasts are disorganized. x8,900.

Figure 92. High magnification of chloroplasts shown in Fig. 91. Observe the chloroplast ground plasm is vesicular (arrows) in appearance and that some of the plastoglobuli (pg), which are much enlarged, have a double membrane around them. Granal structure has been destroyed. Note the mitochondrion (M) appears abnormal. x31,300.

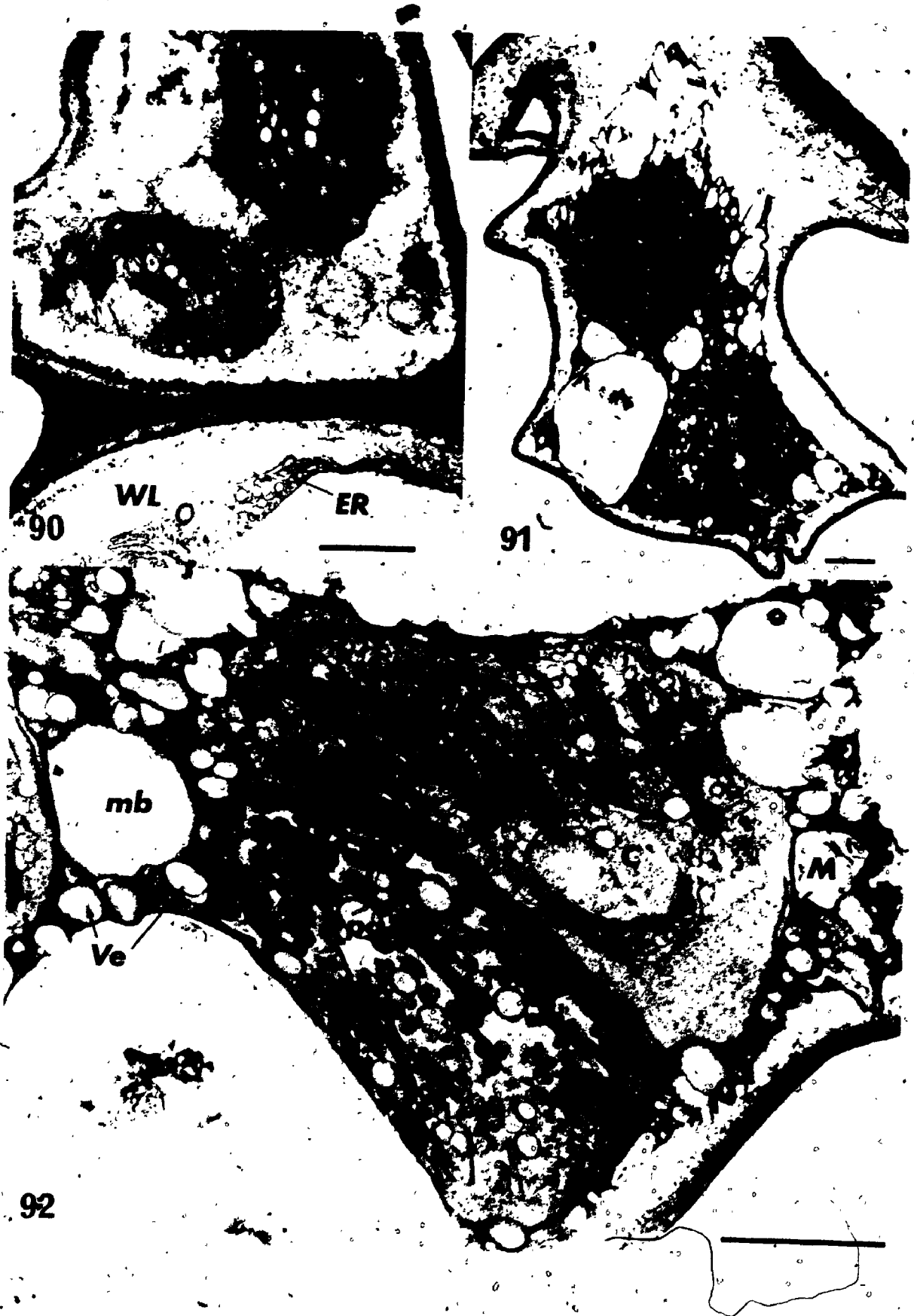

WL
ER
90
91
mb
M
Ve
92

PLATE 44

Effects of necrotic mesophyll on adjacent cells

Fig. 93-96 from an infection site with only four or five necrotic cells. Kwan 90 hr. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 93. Two dead mesophyll cells of an infected Kwan leaf. Note that the cells are completely collapsed in places (arrow). The cytoplasm is destroyed and unrecognizable except for remnants of chloroplast starch granules. This is a section from a 90 hr infected leaf and these cells were among the first four or five cells to become necrotic in this infection court. x6,000

Figure 94. High magnification of part of Fig. 93. Note the alteration of cell wall staining, electron-dense staining of the plasma membrane region and total disintegration of the organelles. x38,000

Figure 95. Effect of necrotic mesophyll on adjacent mesophyll cells. Deposition of material on the common cell wall has occurred but other parts of the cell not adjacent to the necrotic cell appeared healthy. Note that vesicles and ER occur in the cell cytoplasm and the healthy chloroplast. Note the large size of the plastoglobuli. x22,700

Figure 96. Similar to Fig. 95 but the deposit is more electron-dense and globose. Note ER and vesicles. x18,800

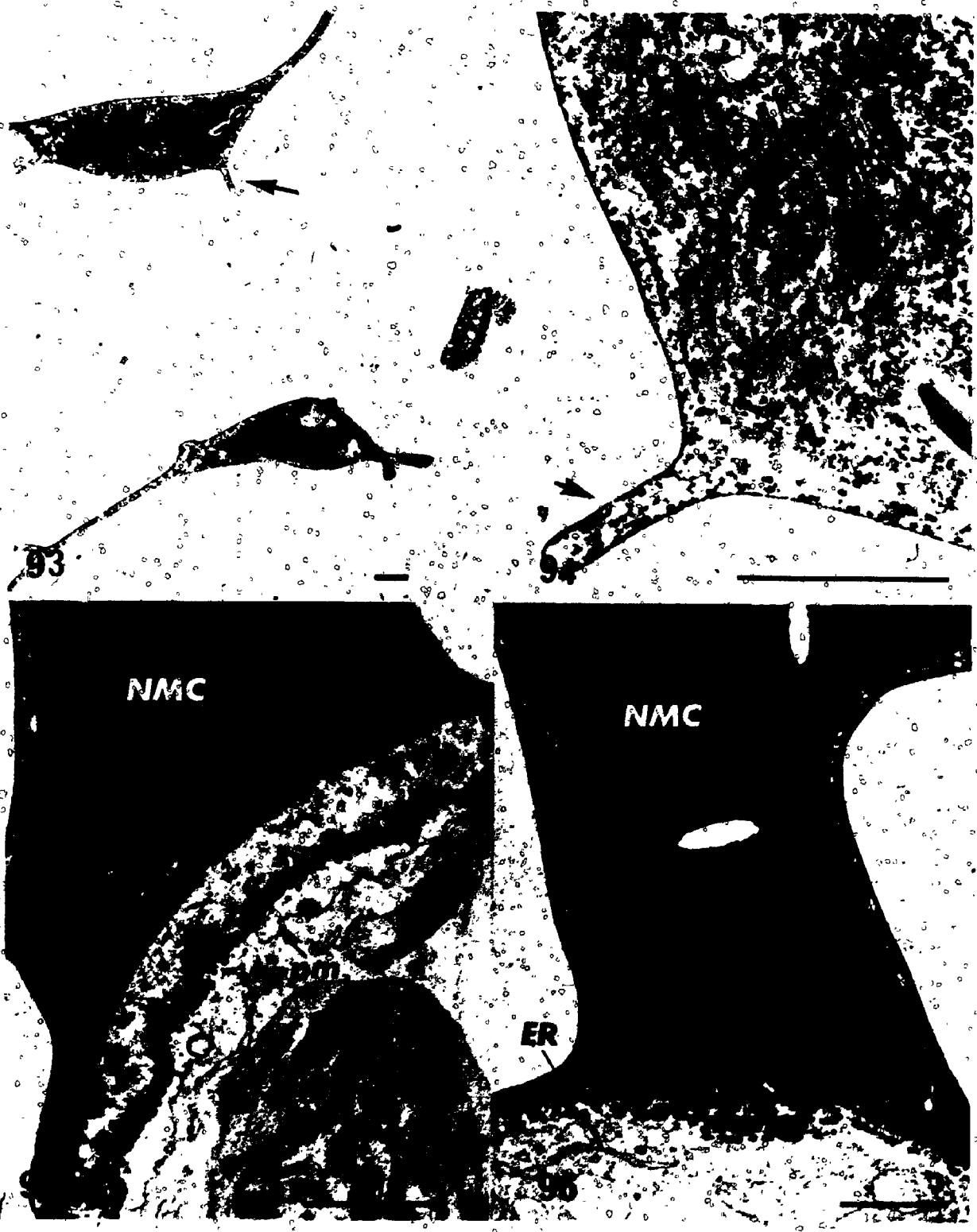

93
NMC
NMC
ER

PLATE 45

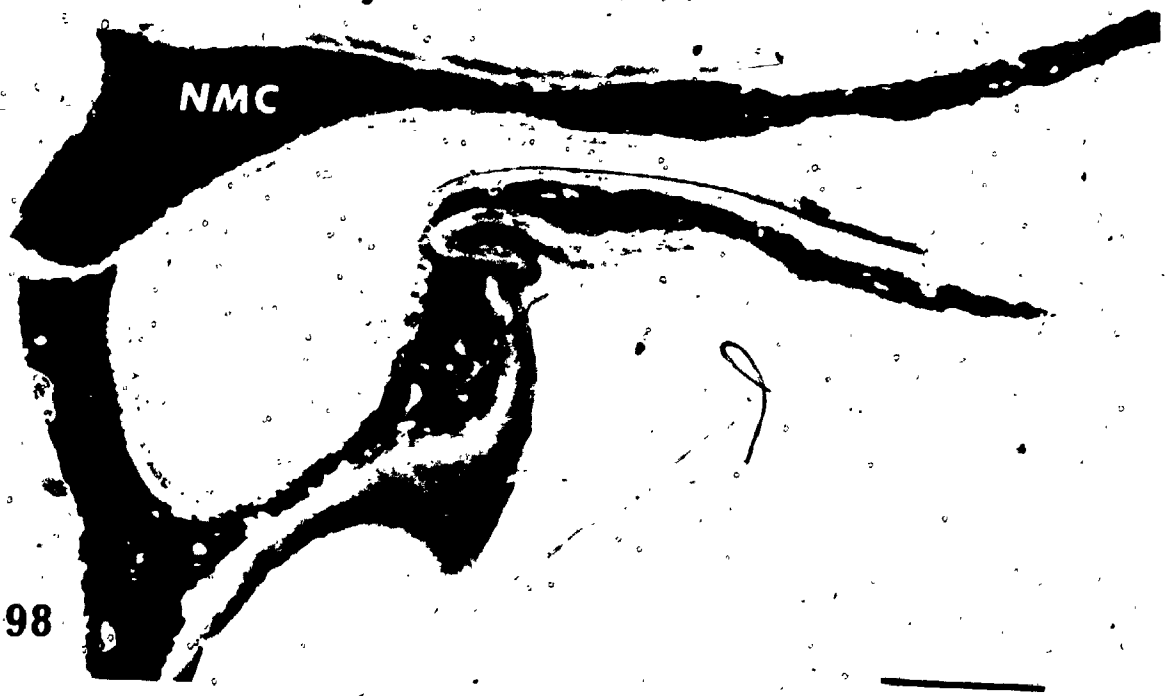
Final stages of necrosis

Kwan 95 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 97. Section of a mesophyll cell adjacent to a collapsed mesophyll cell. Infected Kwan leaf. Note the plasmodesmata between the necrotic cell and the adjacent collapsing mesophyll cell and the membranous aggregation by the wall. x19,800

Figure 98. Totally collapsed and necrotic mesophyll cell. Observe that the cell has collapsed to less than 1 µm across and cytoplasmic contents are electron-dense and unidentifiable. x24,200

97



98

PLATE 46

Manchurian. Haustorium and epidermal cell

Manchurian 95 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 99. Section through a haustorium. Haustorial lobes (Hl) and the penetration site. This figure shows the typical features of an established haustorium. Note that the sheath is contracted close to the haustorium. Note the lack of host cytoplasm. The cytoplasm of the haustorium contains many small vesicles and mitochondria. x20,000

Figure 100. Section through a similar aged haustorium as Fig. 99. Observe the double layered papilla and associated host cytoplasm. The sheath of the haustorium is expanded and the sheath matrix (Sm) is less electron-dense than in Fig. 99. x25,700

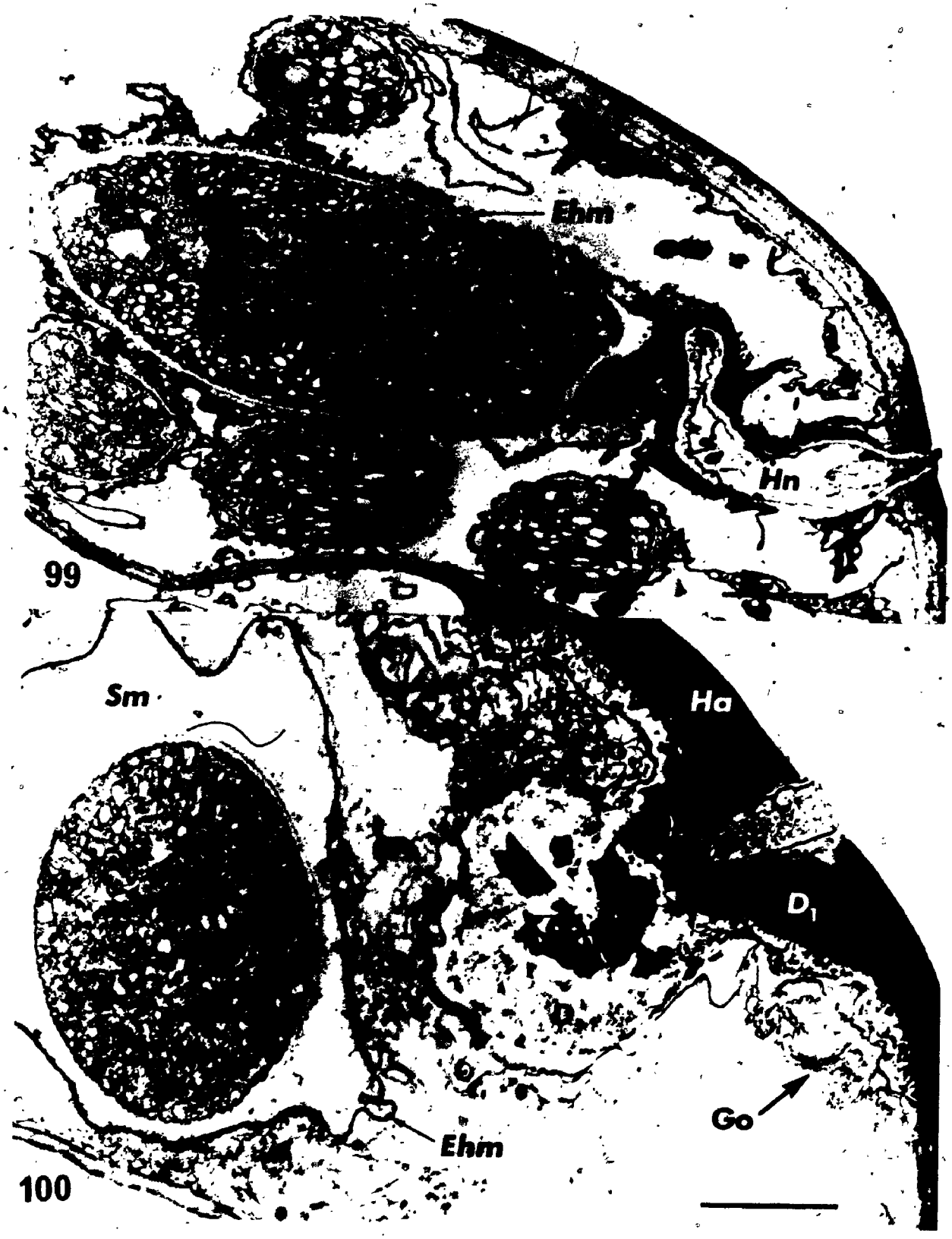

Ehm
Hn
99
Sm
Ha
D1
Go
Ehm
100

PLATE 47

Haustorium and epidermal cell

Manchurian. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 101. Transection through the main body of a haustorium. Again the sheath is expanded. Note the invaginations of the extrahaustorial membrane (arrow) and the darker staining of the sheath matrix close to regions of dense host cytoplasm. 95 hr. x20,900

Figure 102. Longitudinal section through the main body of an older haustorium. Note that vacuolar regions (V) are formed. These contain electron-dense amorphous material. Again note the vesicles and the invagination of the extrahaustorial membrane in the sheath matrix (arrow). 115 hr. x20,500

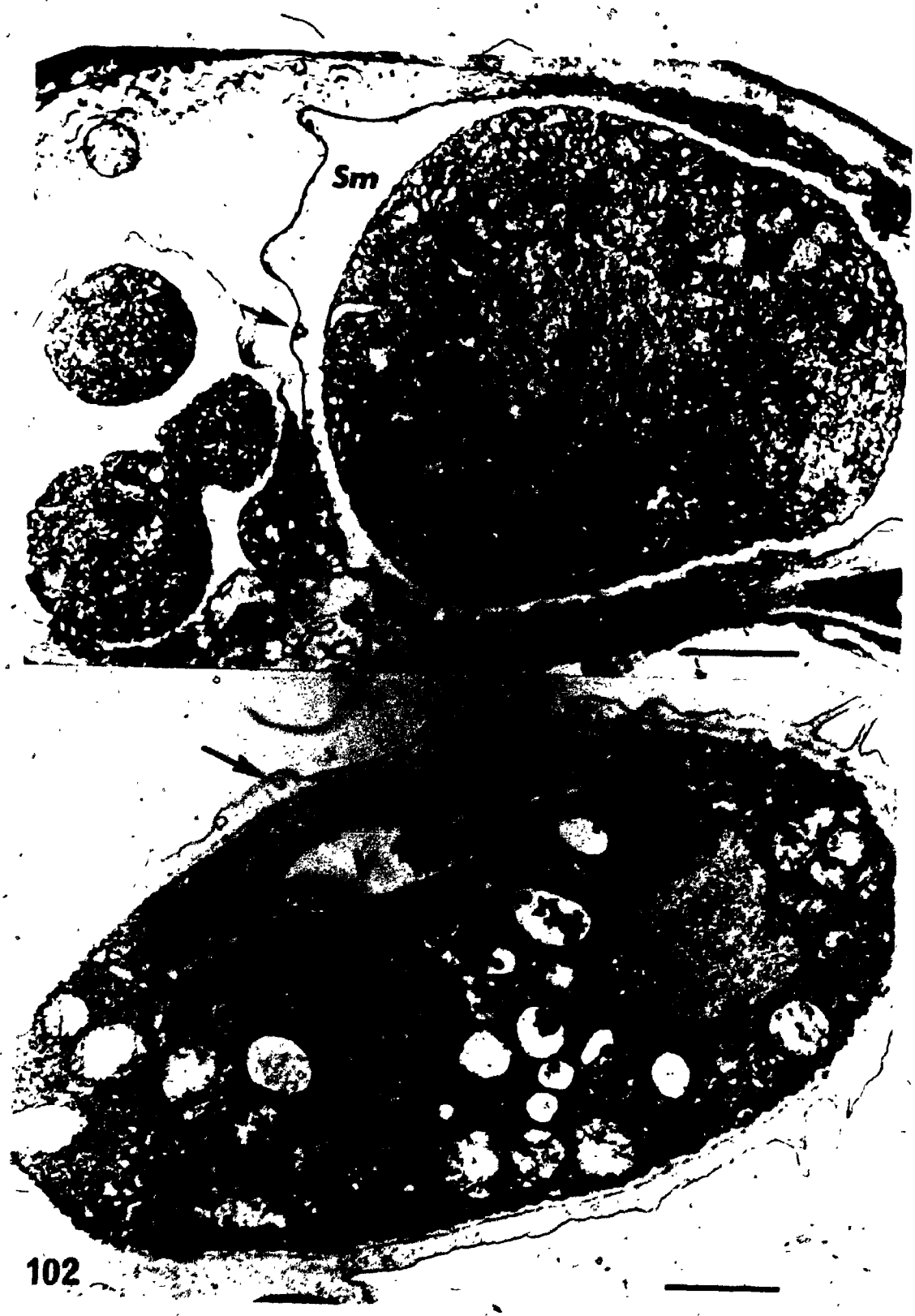

Sm
102

PLATE 48

Appressorium, haustorium and epidermal cell

Manchurian 115 hr. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 103. Transection through an appressorium, hypha, penetration hypha and haustorium. Note again the vacuolar contents of the haustorium, and the presence of lipid-like bodies (L). Note the dense, healthy cytoplasm of the appressorium and the adjacent fungal hypha (Hy). x12,750

103

PLATE 49

Fungal hypha

Manchurian 120 hr. Glutaraldehyde followed by osmium., Uranium and lead stained.

Figure 104. Transection of a healthy fungal hypha. Note the formation of electron-dense bodies in the vacuoles (arrows) and the dense cytoplasm, filled with strands of ER and ribosomes. Note the small mitochondria (M) ringed close to the fungal plasma membrane. x25,300

104

PLATE 50

Fungal hypha

Manchurian 120 hr. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 105. Transection of a healthy hypha. It is similar to Fig. 104 but passes through the nuclear region. Note the enlarged vacuole (V) containing electron-dense material (L) and the dense ribosome-filled cytoplasm. x25,300

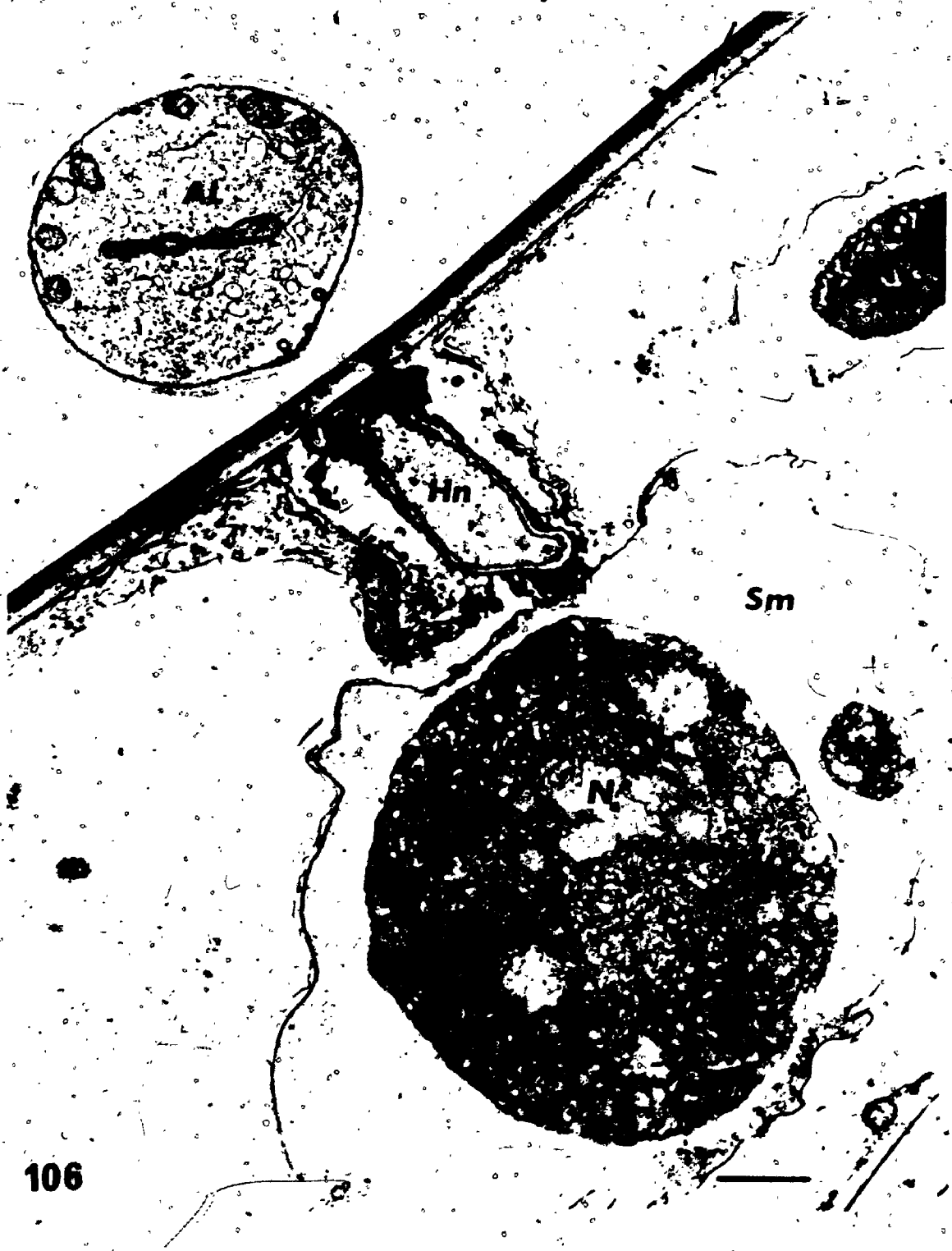
105

PLATE 51

Kwan - Haustorium and epidermal cell

Kwan 95 hr. Glutaraldehyde/osmium followed by osmium. Uranium and lead stained.

Figure 106. Section through a penetration site on the resistant Kwan host. The haustorium at this time appears quite healthy and similar to those on the Manchurian host at this time, cf. Fig. 100, 101. Note the expanded sheath (Sm) surrounding the haustorium and the small vesicles (SV) and lipid bodies (L) within the haustorium. Little host cytoplasm is present. x16,200



106

PLATE 52

Initial effects of HR on haustoria and epidermal cells

Kwan 115 hr. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 107. Section through a haustorium at a later stage. Note the indications on the cell wall (arrows) of a hypersensitive reaction. The haustorium contains vacuoles (V) similar to those of similar aged haustoria on the susceptible host (Fig. 102, 103). The dark regions close to the plasma membrane are probably regions of glycogen formation (Gy). Note the invaginations of the extrahaustorial membrane (Ehm) similar to those in haustoria in the Manchurian host (Fig. 101, 102). x15,400

Figure 108. Section through a haustorium. Note the network of small vesicles (SV), the long mitochondria, glycogen rosettes (Gy), and lipid bodies (L) in the haustorium. Both the haustoria in Fig. 107 and 108 have formed sheaths with a dense matrix. The cell wall here too has the beginnings of electron-dense depositions (arrow). x15,400


Ehm
Cy
Cy

PLATE 53

Effects of HR on haustoria and epidermal cells

Kwan 120 hr. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 109. Section of a collapsed haustorium in an epidermal cell. Note the wall deposits (WL) on the inner wall of the epidermal cell and the clumped cytoplasm (NC). The plasma membrane and the tonoplast are broken. The haustorium is collapsed and electron-dense regions occur close to the wall. Note that the sheath (Sm) around the haustorium is dense and the membranes have formed unusual structures. x21,200

Figure 110. Section of a healthy young haustorium in the adjacent cell to that shown in Fig. 109. x9,900

Figure 111. Section of a necrotic haustorium in a collapsed epidermal cell. Note that the epidermal cell is completely collapsed and contours the necrotic haustorium (H). The cytoplasmic contents of the haustorium and the epidermal cell are almost unrecognisable. Note the wall lesions on the lower epidermal cell wall (arrow). x32,800

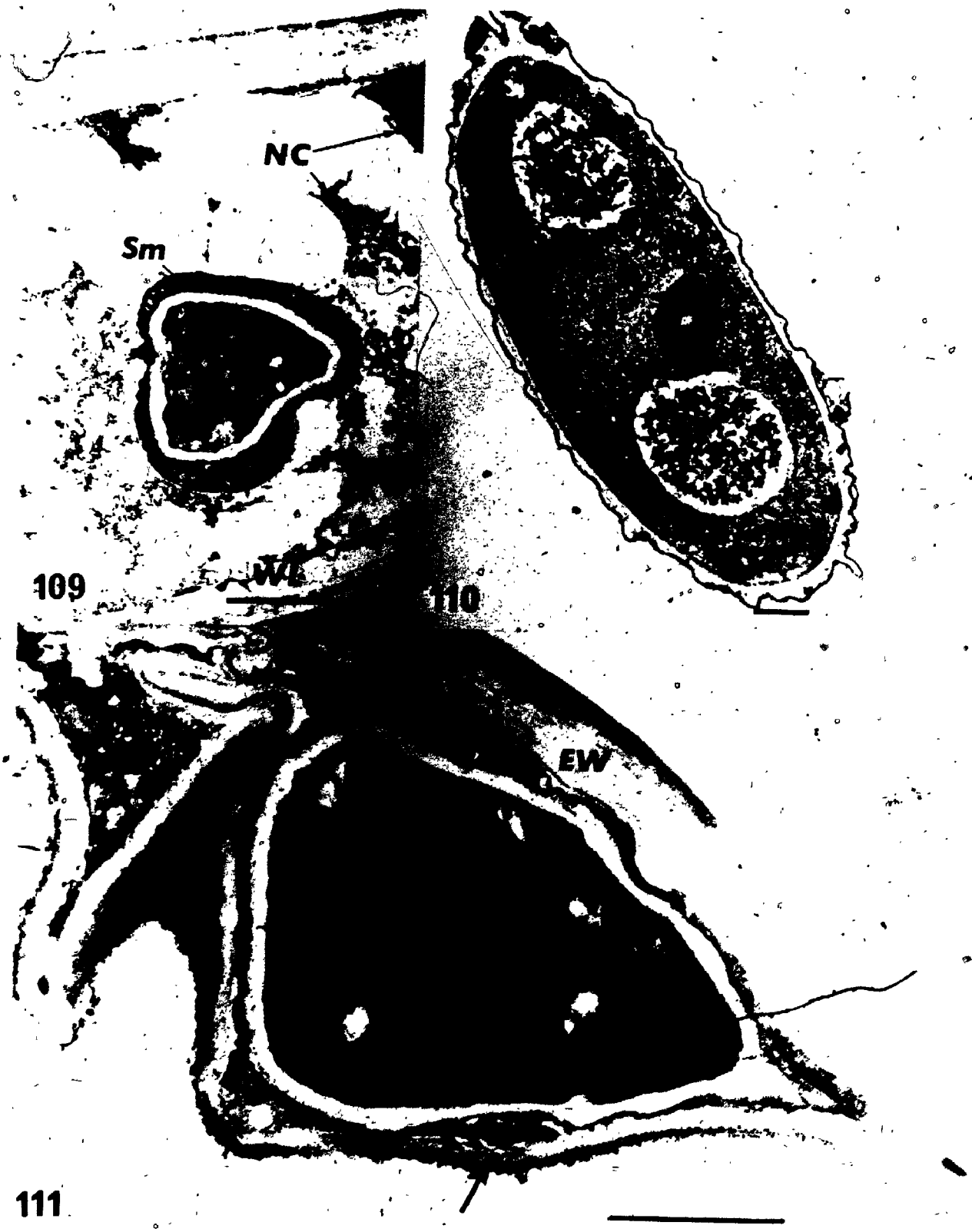

NC
Sm
109
110
EW
111

PLATE 54

Effects of HR on haustoria and epidermal cells

Kwan 120 hr. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 112. Section of a collapsed epidermal cell and a haustorium, close to the penetration site. Note the halo in the wall above the haustorial neck, the neck (Hn) and the haustorial lobes (Hl). The adjacent mesophyll cells are collapsing. x9,200

Figure 113. Section of part of a cell wall between an epidermal and mesophyll cell. Note that dark rings occur in the cell wall during the hypersensitive reaction. x52,200

Figure 114. An enlargement of the section adjacent to that shown in Fig. 112. Note that the sheath (Sm) around the haustorium and the lobes is still present. Intense electron-dense regions (Edm) occur in the haustorial plasma membrane region. The host cytoplasm is disintegrated. x25,000

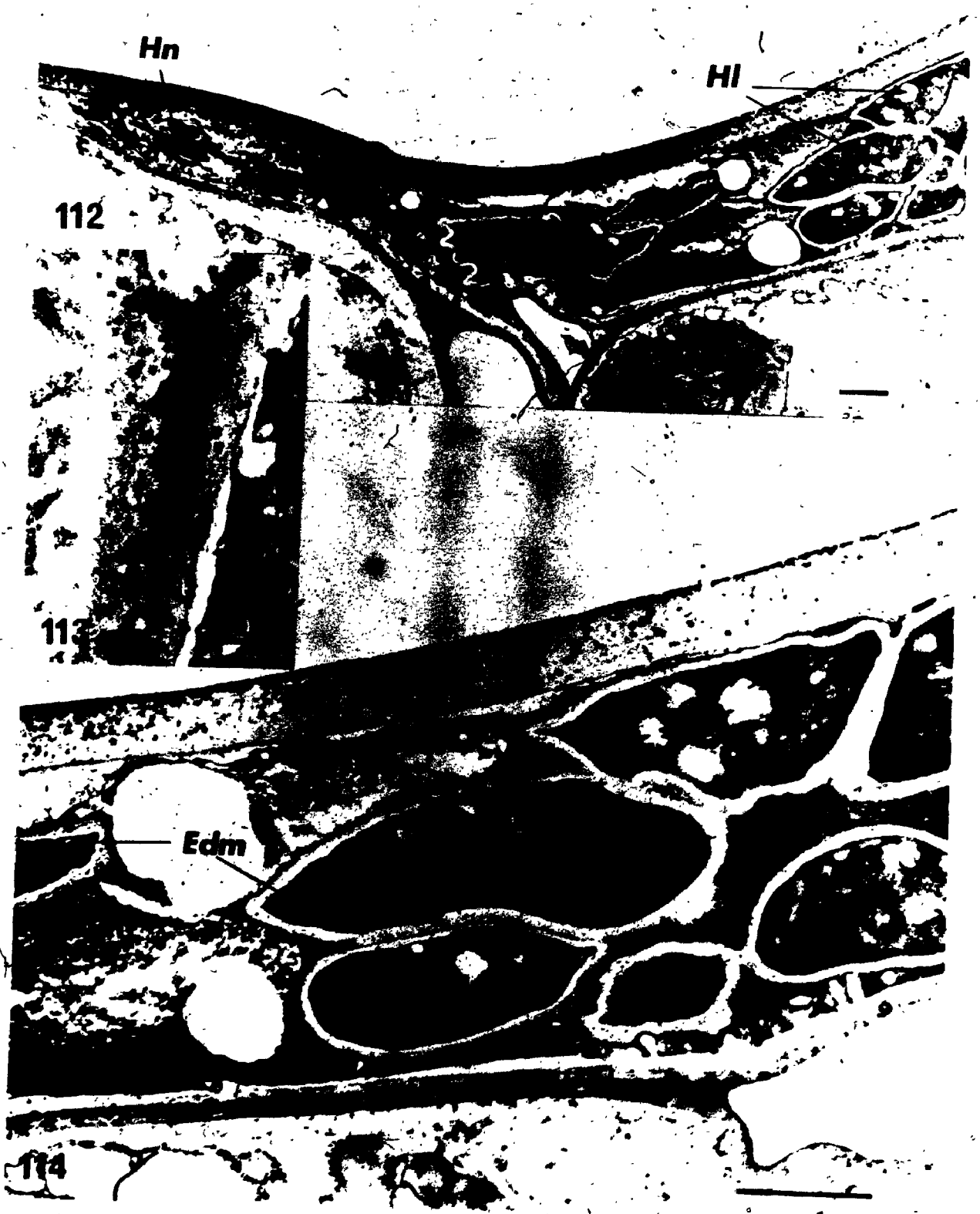

Hn
Hl
112
113
Edm
114

PLATE 55

Effects of HR on haustoria and epidermal cells

Kwan 144 hr. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 115. Section through an epidermal cell and penetration peg. Note the large electron-dense papilla (D) and the aggregations of host or fungal cytoplasm forming convoluted membranous structures. x18,500

Figure 116. Section of necrotic cytoplasm in a collapsed, necrotic epidermal cell. Note the large membranous structure and the presence of other membranes. It is unclear whether these structures, products of necrosis, are of host or fungal origin. x26,500

Figure 117. Section of a haustorium in a collapsed epidermal cell. The lobes of the haustorium (NH) are extremely shrunken and no recognizable cytoplasm is present. Note the extensive wall deposits (WL) on the epidermal cell wall. x49,000

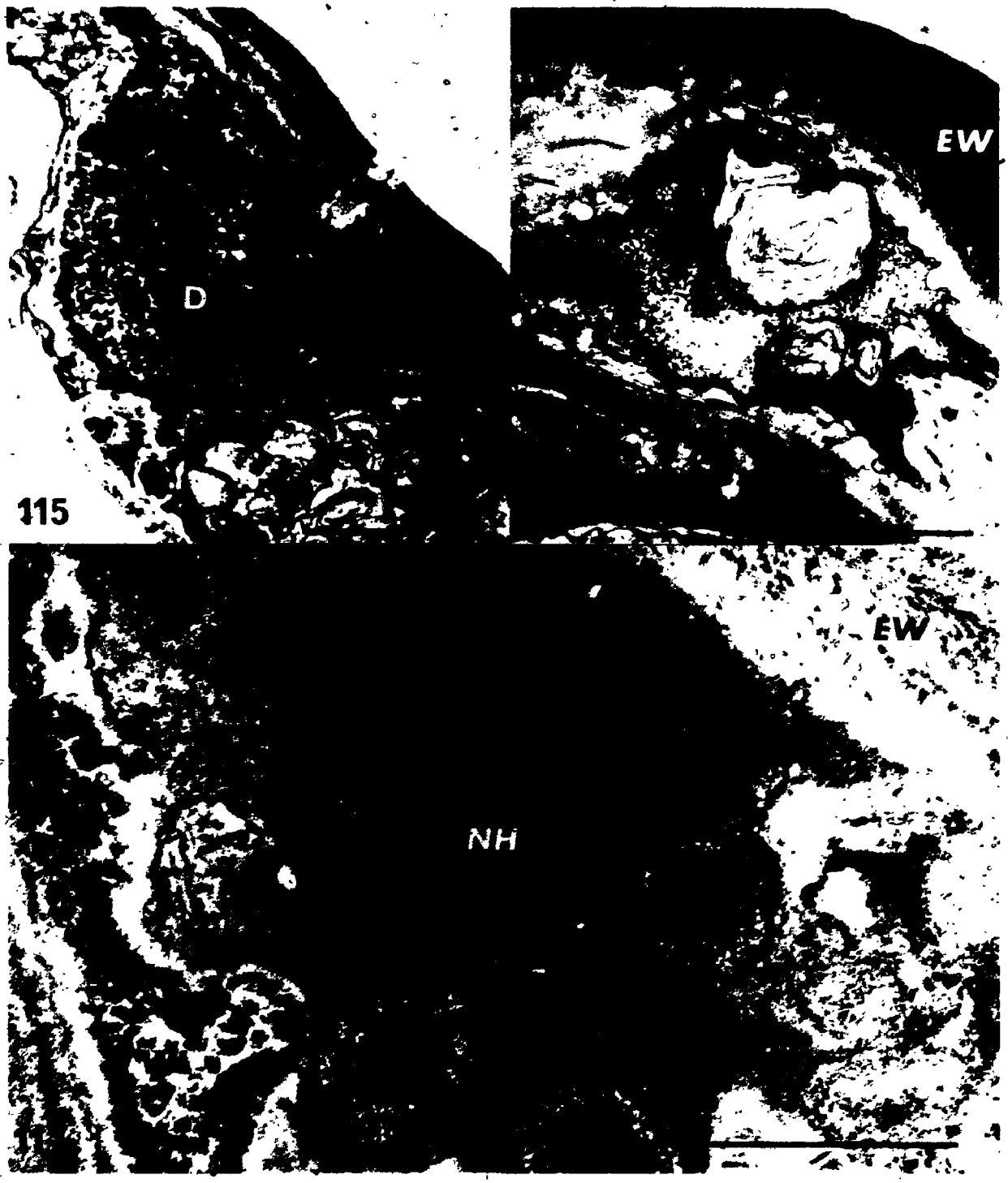

D
EW
115
EW
NH

PLATE 56

Effects of HR on hyphae

Kwan 120 hr. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 118. Transection of a collapsing fungal hypha. Note the coarse, granular cytoplasm, the membrane-free vacuole (V) (arrow) and enlarged mitochondria, some with membranous whorls. Note that the hypha is about half the diameter of those in Fig. 104, 105. x42,600

Figure 119. Oblique section close to the cell wall of a necrotic hypha. Note the electron-dense regions close to the fungal plasma membrane. x25,800

Figure 120. Part of a transection of a collapsing hypha. This figure shows a membrane-free vacuole similar to that in Fig. 118 and a disintegrating mitochondrion, associated with the vacuole (arrow). x56,100

118

119

120

PLATE 57

Effects of HR on hyphae

Kwan 120 hr. Glutaraldehyde followed by osmium. Uranium and lead stained.

Figure 121. Section of a collapsed, necrotic fungal hyphae. Note the infolding of the wall close to the septum (Se) and the electron-dense deposits on membranes. The adjacent cell has collapsed as has the hypha on the left. x65,200

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