Ultrastructure of Host and Nonhost Reactions to Cowpea Rust

Michele C. Heath

Postdoctoral Research Fellow, Department of Plant Pathology and Plant Genetics, University of Georgia, Athens 30601.

I thank I. B. Heath for advice with electron microscopy, and M. S. Fuller for use of the electron microscope facilities.

Journal Series Paper No. 1101.

Accepted for publication 12 July 1971.

ABSTRACT

An ultrastructural survey of resistant reactions induced by the cowpea rust, *Uromyces phaseoli* var. *vignae*, revealed that the nonhost, *Phaseolus vulgaris*, responded to each infection hypha by the deposition of electron-opaque material on and within the surrounding host cell walls. These deposits apparently prevented haustorial formation in 90% of infection sites. In contrast, no signs of resistance were detected in either of the two immune cowpea cultivars until the formation of the first haustorium. The subsequent reaction of one of these cultivars closely resembled that already described for another immune cowpea, and included a distinctive response of the host plasmalemma surrounding the haustorial body. In the other, haustorial development was retarded, and all host membranes bore small areas of electron-opaque material. In both immune cultivars, these initial responses were quickly followed by the rapid and simultaneous disorganization of both haustorium and host cell. In contrast with these immune responses, the cowpea cultivar giving an intermediate (necrotic fleck) reaction showed no signs of resistance during the initial stages of penetration or haustorial formation. The eventual slow disorganization of invaded cells involved a dissection of the peripheral cytoplasm, followed by a gradual disintegration of most of the cell membranes. Haustorial disorganization did not immediately follow that of the host cell.

In both the resistant host cultivars and the nonhost, death of the haustorium and the haustorial mother cell did not result in the immediate death of the intercellular mycelium. It is possible that starvation was the primary cause of the cessation of fungal growth.

Phytopathology 62:27-38.

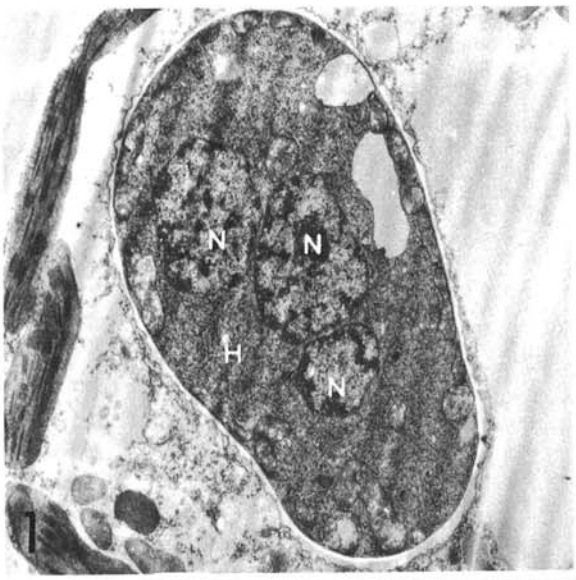
Additional key words: Vigna sinensis.

In an earlier ultrastructural investigation (9) of the interaction between an immune cowpea cultivar (*Vigna sinensis* [Torner] Savi 'Queen Anne') and the rust *Uromyces phaseoli* (Pers.) Wint. var. *vignae* (Barcl.) Arth., an unusual membrane response was one of several distinctive features which had not been reported for the less vigorous types of resistant reaction examined in other plants (4, 18, 21, 23). Whether these features were typical only of the immune response or whether they were peculiar to all types of resistance induced by this species of rust was not known. Thus, the following ultrastructural survey was undertaken to compare the different reactions to this pathogen. It was hoped that some basic features might be revealed which would help elucidate the biochemical events involved in resistance to rust infection.

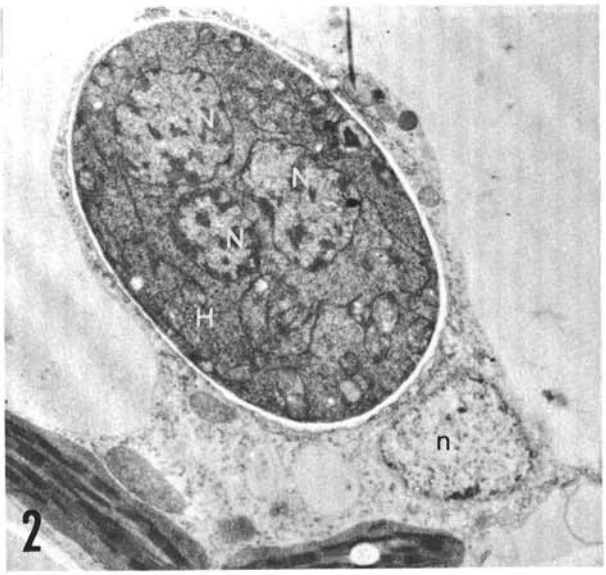
In previous ultrastructural studies, there has been considerable confusion in the nomenclature of structures associated with the fungal haustorium, mainly through the varied use of the term "sheath". As discussed in an earlier paper (8), this term was first applied to structures observed with the light microscope to surround haustoria of both rusts and powdery mildews, and its use has persisted in subsequent ultrastructural work in spite of the fact that the structures associated with rust haustoria are structurally (and probably functionally) different from those associated with haustoria of the powdery mildews. In this work, Berlin & Bowen's (2) terminology is followed, which applies the term sheath to that structure originally designated as such in early studies of rust infections, and uses "encapsulation" to describe the region of uncertain origin between the haustorial wall and the host plasmalemma. This terminology has been used in preference to that proposed by Bracker (3) based on the haustorial apparatus of *Erysiphe* spp.

MATERIALS AND METHODS.—Cowpea cultivars Cream 40 (susceptible), Purple Hull Pinkeye (intermediate), Dixie Cream (immune), and Calico Crowder (immune), and a bean, *Phaseolus vulgaris* L. 'Pinto' (nonhost), were grown and inoculated with a single race of *U. phaseoli* var. *vignae* as described by Heath (8).

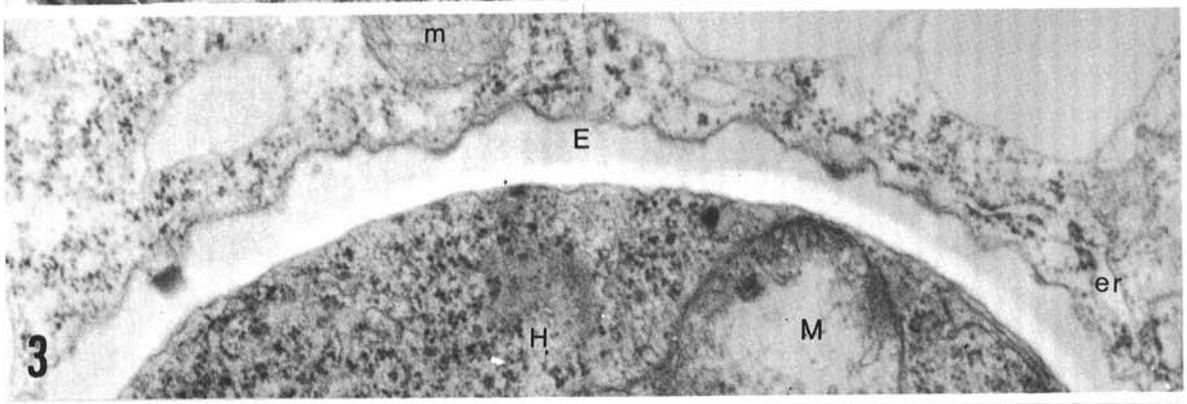
The characteristics of fungal growth in each cultivar were first examined with the light microscope using whole mounts of stained and cleared leaf tissue (8). In all combinations, haustoria were formed between 12 and 24 hr after inoculation. Therefore, material was prepared for electron microscopy 19-22 hr after inoculation, when no haustorium was more than 10 hr old. In addition, leaves of the cowpea cultivar, Purple Hull Pinkeye, were fixed 8 days after inoculation after the macroscopic appearance of host necrosis. Pieces of infected leaf were vacuum-infiltrated with 2.5% glutaraldehyde in 0.07 *M* phosphate buffer, pH 7.0, containing 0.1 *M* sucrose, and were left for 2-3 hr at room temperature (22 C). After four 15-min washes in the buffered sucrose alone, the material was postfixed for 2 hr in similarly buffered sucrose containing 1.0% osmium tetroxide. The tissue was dehydrated in an ethanol series, passed through epoxypropane, and embedded in Epon 812. Ultrathin sections were cut parallel to the leaf surface so that the path of each infection



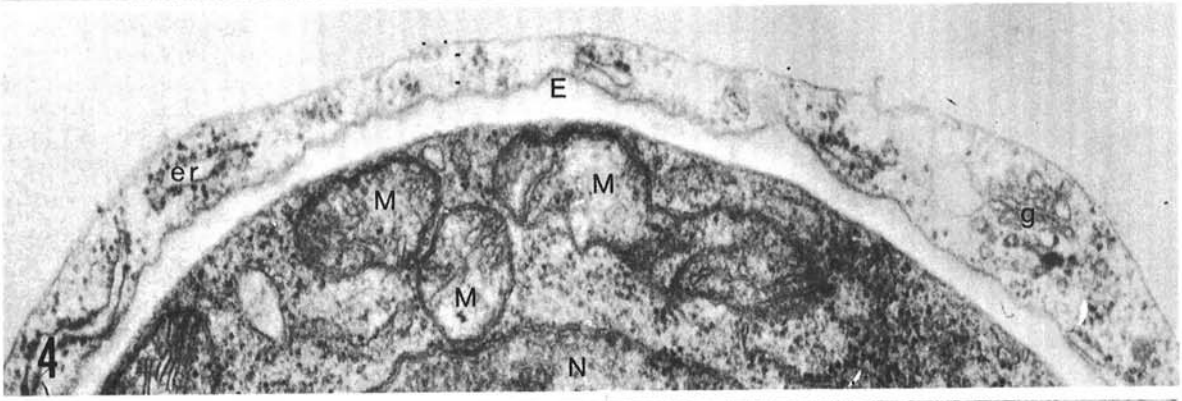
1



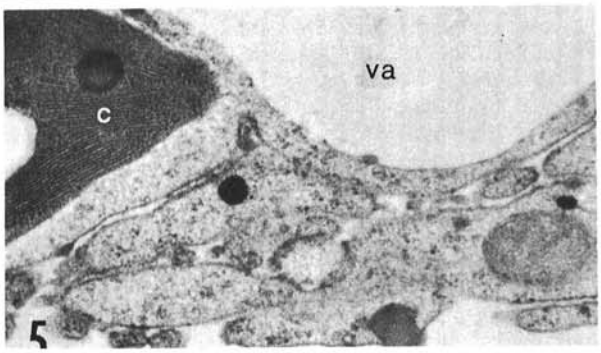
2



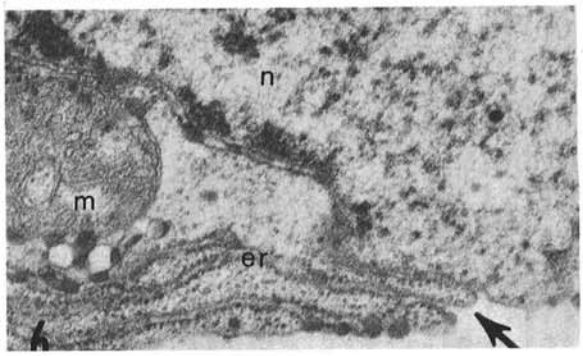
3



4



5



6

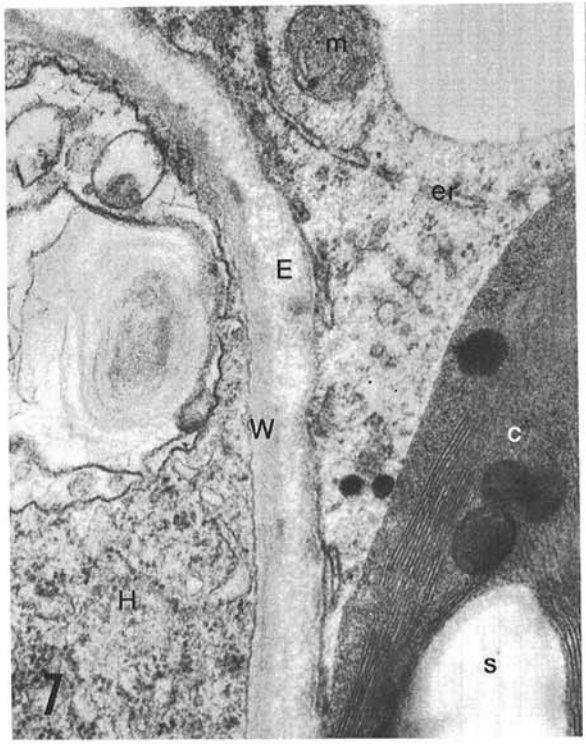
hypha could be followed. Sections were usually stained with uranyl acetate and Reynolds' (16) lead citrate before examination.

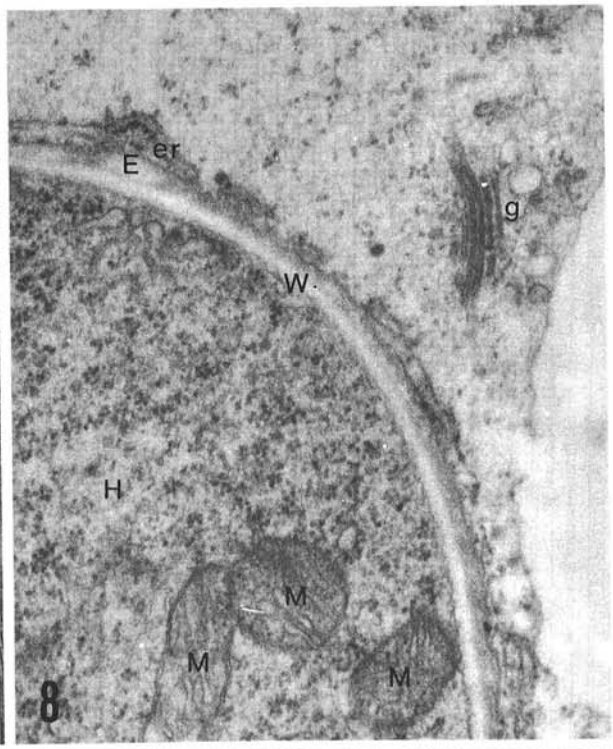
RESULTS.—*Susceptible reaction (cultivar Cream 40).*—Only two infection sites were examined in this cultivar, but in both, the fungal and host ultrastructure showed the same features described in detail elsewhere for another susceptible cowpea cultivar, Early Ramshorn (9). Under the light microscope, the first haustorium formed by each infection hypha apparently did not induce any extensive deposition of material around the point of host wall penetration. Examination of the two infection sites prepared for electron microscopy supported this observation. Such deposits were also absent in the susceptible cultivar, Early Ramshorn, at a comparable stage of infection (8, 9), but have been commonly reported in older, susceptible rust infections of other plants (6, 7, 14); perhaps such host reactions increase in frequency with age of infection. As commonly found in cells invaded by rust fungi, the young haustorium was surrounded by an aggregation of host cytoplasm and organelles, but was separated from the host by an encapsulation (Fig. 1) bounded by a host membrane continuous with the plasmalemma at the region of host wall penetration. This encapsulation was more variable in width, ranging from 30 to 100 nm (Fig. 3), and was more electron-translucent around the body of the haustorium than around the haustorial neck (a similar situation is shown in Fig. 24). As found in other susceptible rust interactions (5, 7, 21, 23), profiles of rough endoplasmic reticulum accumulated in the host cytoplasm adjacent to the haustorium (Fig. 3), but there were no other obvious signs of cytoplasmic activity additional to that observed in neighboring uninvaded cells.

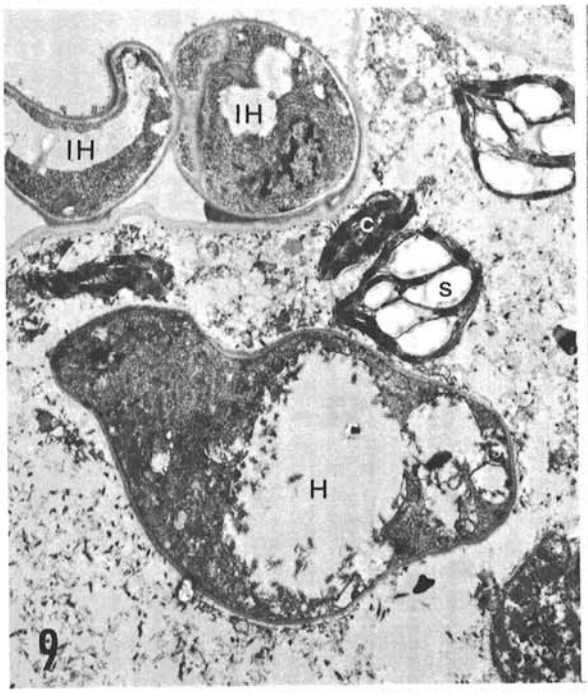
Intermediate reaction (cultivar Purple Hull Pinkeye).—Out of 23 cultivars of cowpea tested, 22 were either immune or fully susceptible. Purple Hull Pinkeye was the only one which responded to infection by the formation of a small macroscopic fleck about 1-3 mm in diam. Light microscopy revealed no differences in fungal growth between this reaction and the susceptible until the onset of host necrosis, which occurred 4-5 days after inoculation. Thus, it was not surprising that by 21 hr after inoculation, both fungus and host appeared ultrastructurally similar to the susceptible combination (compare Fig. 1, 2 and 3, 4) except for a slight deposit of electron-translucent material around the point of entry of one of the two haustoria examined at this stage. This material was similar to that described below for the immune reactions (Fig. 12). Such a host reaction was apparently rare, as it was not observed in the many infections of comparable age seen with the light microscope, and was not present in any of the numerous invaded cells examined with the electron microscope at the later stage in infection.

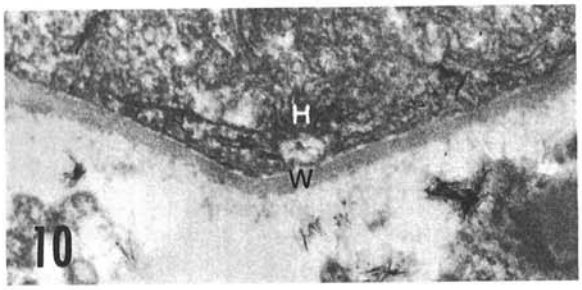
Under the light microscope, dead host cells were first observed at the center of each infected area. Further necrosis then occurred fairly rapidly after haustorial formation by the still-growing hyphae at the lesion edge. Uninvaded cells, particularly those at the infection center, also browned, and a macroscopic brown fleck became visible 5-6 days after inoculation. Usually, fungal growth ceased before sporulation, but a few small pustules were occasionally observed. When the edges of two nonsporulating flecks were examined with the electron microscope 8 days after inoculation, many of the intercellular hyphae were still ultrastructurally similar to those observed in susceptible tissue (Fig. 9) and were, therefore, presumably still alive when fixed. Nearly all the host cells examined in this region showed signs of disorganization, but did not appear to be collapsing. Many of these cells contained no observable haustoria, but it was impossible to determine whether or not invasion had occurred in unsectioned portions of the cell. In those haustoria-containing cells showing only slight disorganization, the tonoplast and plasmalemma were apparently intact, and the majority of organelles were still recognizable. A characteristic feature, however, was the apparent dissection of the cytoplasm around the periphery of the cell (Fig. 5-6). The origin of these packets of cytoplasm remains uncertain, but at least some appeared to be derived by the fusion of host endoplasmic reticulum and other membranes with the plasmalemma (Fig. 6). Clear examples of such fusion are rare in both plant and animal cells, and its presence here probably reflects a considerable change in the properties of the membranes involved. At a later stage in disorganization, the membranes of the

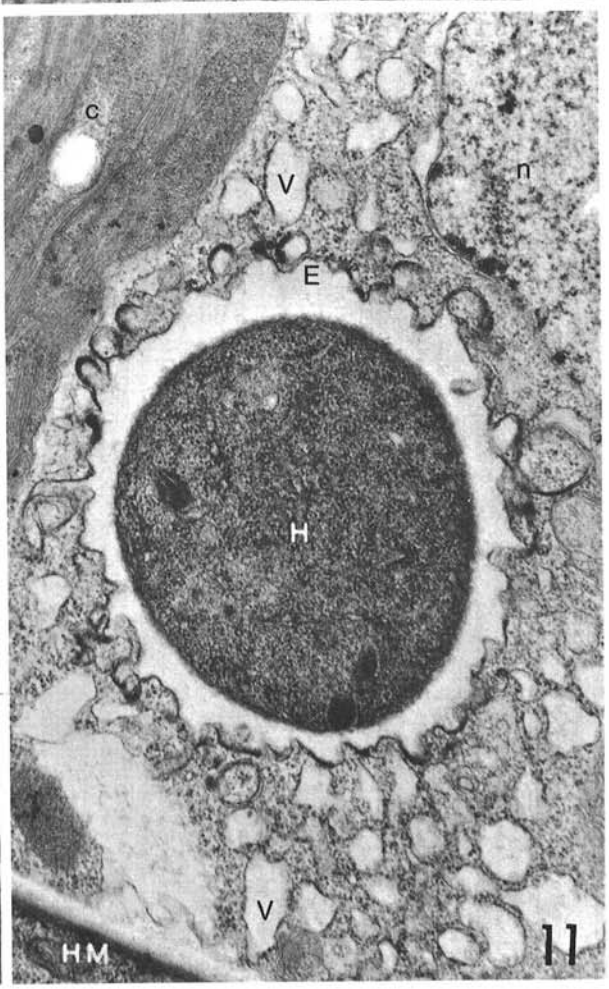
Fig. 1-6. 1) Transverse section through the body of the first haustorium formed by an infection hypha in the susceptible cultivar Cream 40; 21 hr after inoculation. (X 7,700) **2)** Transverse section through the body of the first haustorium formed in cultivar Purple Hull Pinkeye (intermediate in resistance). Notice the similarities in appearance of both haustorium (H) and host cell to those of the susceptible reaction in Fig. 1; 21 hr after inoculation. (X 7,500) **3)** Detail of Fig. 1. The wall around the young haustorium (H) in all cultivars is at first difficult to distinguish. Here it may be represented by the more electron-translucent component of the encapsulation (E). Notice the profiles of rough endoplasmic reticulum (er) in the adjacent host cytoplasm. (X 45,200) **4)** Details of Fig. 2. The appearance of both host and fungus is similar to that shown by the susceptible reaction in Fig. 3. (X 42,900) **5)** Intermediate reaction 8 days after inoculation, showing the dissection of the cytoplasm around the periphery of the host cell, which is the first sign of disorganization in this reaction. (X 33,000) **6)** Similar stage in disorganization to that shown in Fig. 5. Here the peripheral packets of cytoplasm are apparently being formed by the fusion (arrow) of the host endoplasmic reticulum (er) and the nuclear membrane with the plasmalemma. Notice the small drops of electron-opaque material on many of the membranes. These increase in size as disorganization continues. (X 40,200) c = chloroplast; E = encapsulation; er = host endoplasmic reticulum; g = Golgi body; M = fungal mitochondrion; m = host mitochondrion; N = fungal nucleus; n = host nucleus; va = host vacuole.


m
er
E
W
c
H
s
7


E
er
g
W
H
M
M
M
8


IH
IH
c
s
H
9


H
W
10


c
V
n
E
H
V
H.M.
11

tonoplast, plasmalemma, nucleus, mitochondria, microbodies, and endoplasmic reticulum all lost their integrity, and drops of lipidlike material appeared on those fragments still remaining (Fig. 6). This process appeared to take place fairly slowly, as there were large numbers of cells in each infection site showing intermediate stages in such disorganization. Chloroplast grana persisted longer than other cell membranes, and were commonly still associated with starch grains at the latest observed stages in cell death (Fig. 9).

As reported by Ehrlich & Ehrlich (4) for a resistant reaction induced by *Puccinia graminis tritici*, the host plasmalemma surrounding the haustorium remained intact, or nearly so, for some time after the remainder of this membrane had disorganized. Compared with its appearance 21 hr after inoculation, the enclosed encapsulation tended to be more electron-opaque, and was sometimes granular in appearance (Fig. 7). However, it did not remain as a discrete layer once the bounding plasmalemma had broken and could only occasionally be distinguished as a diffuse area when the membrane eventually disappeared (Fig. 10). In contrast, the haustorial wall remained distinct through all stages in host necrosis and, apart from an increase in vacuolation, the haustorial cytoplasm appeared relatively normal in all host cells except those showing complete disorganization of cytoplasm and organelles (compare Fig. 7, 8, 9, 10). When it eventually occurred, the disorganization of the haustorial cytoplasm was characterized by a gradual loss in definition of the fungal organelles. Disorganization of the haustoria and haustorial mother cells did not result in any change in appearance of the rest of the infection hypha.

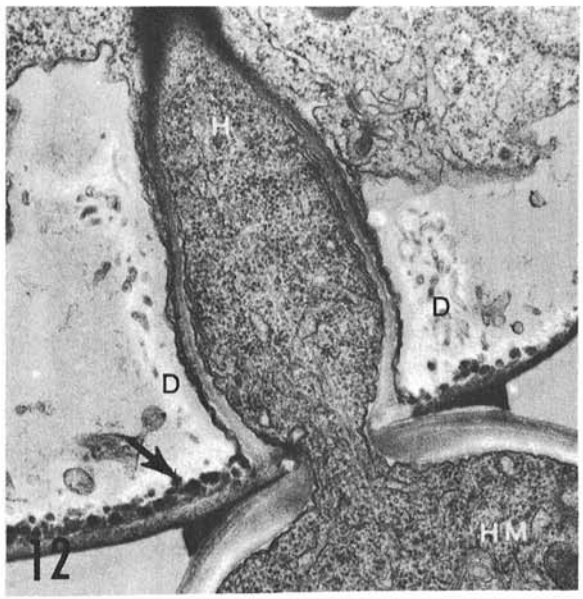
Immune reactions (cultivars Calico Crowder and Dixie Cream).—Fungal growth in both these cultivars, as in all immune cowpeas (8), was similar to that in susceptible plants until the formation of the first haustorium. This apparent lack of any marked interaction between fungus and host before haustorial formation was further corroborated by the lack of any unusual ultrastructural cytoplasmic activity in host cells bordering infection hyphae, and by the normal appearance of the fungal cytoplasm.

In Calico Crowder, as in the immune cultivar, Queen Anne (9), resistance was first manifested at the time of host penetration when a deposit of material was formed around the point of entry of the haustorium (Fig. 12). Although only two penetration regions were sectioned in this investigation, earlier studies with the light microscope showed that these deposits are present in all invaded cells, and that they react histochemically as callose (8). Under the electron microscope, this material was mainly electron-translucent and contained vesicular membranous material (Fig. 12). In one of the two deposits, a more electron-opaque, granular layer could be distinguished against the host cell wall (Fig. 12). Compared with the susceptible or intermediate reactions, the host cytoplasm surrounding the haustorium contained more profiles of rough endoplasmic reticulum and more membrane-bound vesicles (Fig. 13). Some of the latter may have been formed by the activity of Golgi bodies, as many were associated with these organelles. However, the most striking feature of the host response was the distinctive reaction of the host plasmalemma surrounding the haustorial body. In this region, the membrane was associated with areas of densely staining material, and was so greatly convoluted that the enclosed encapsulation varied from 30 to 350 nm in width (Fig. 13). This membrane reaction closely resembled that already described for the immune cultivar, Queen Anne (9).

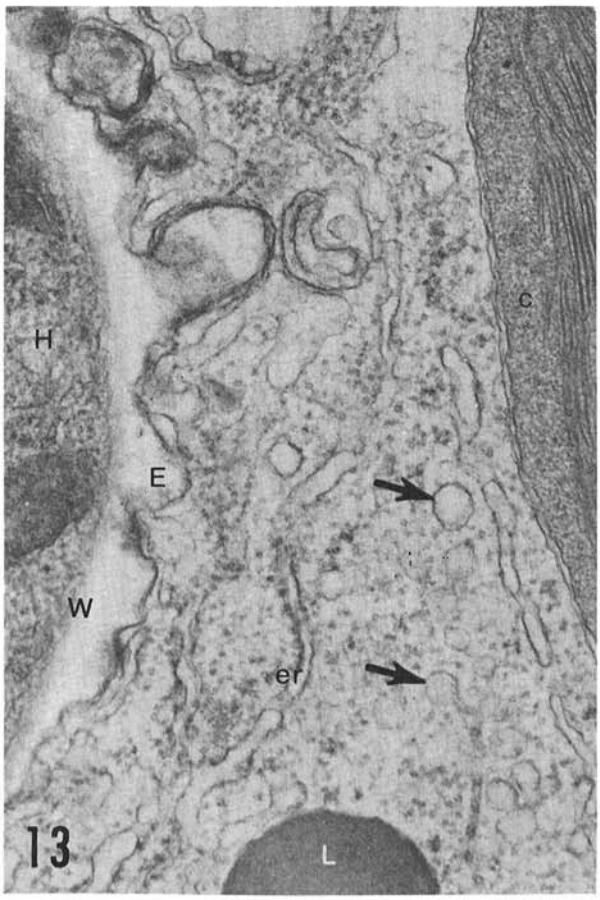
In spite of the apparently vigorous response of the invaded cell, the young haustorium appeared ultrastructurally similar to those of comparable age in susceptible tissue. A densely staining "neckband" (7) (similar to that shown in Fig. 24) was present along the haustorial neck bridging both haustorial wall and encapsulation and extending for a short distance along both fungal and host plasmalemmas. Similar neckbands have been seen in the susceptible and intermediate reactions described above, but were consistently absent in the immune cultivar Queen Anne (9).

The initial reaction of the immune cultivar, Dixie Cream, differed considerably from that already described for Queen Anne (9) and Calico Crowder. In the eight haustoria examined, no deposits of material

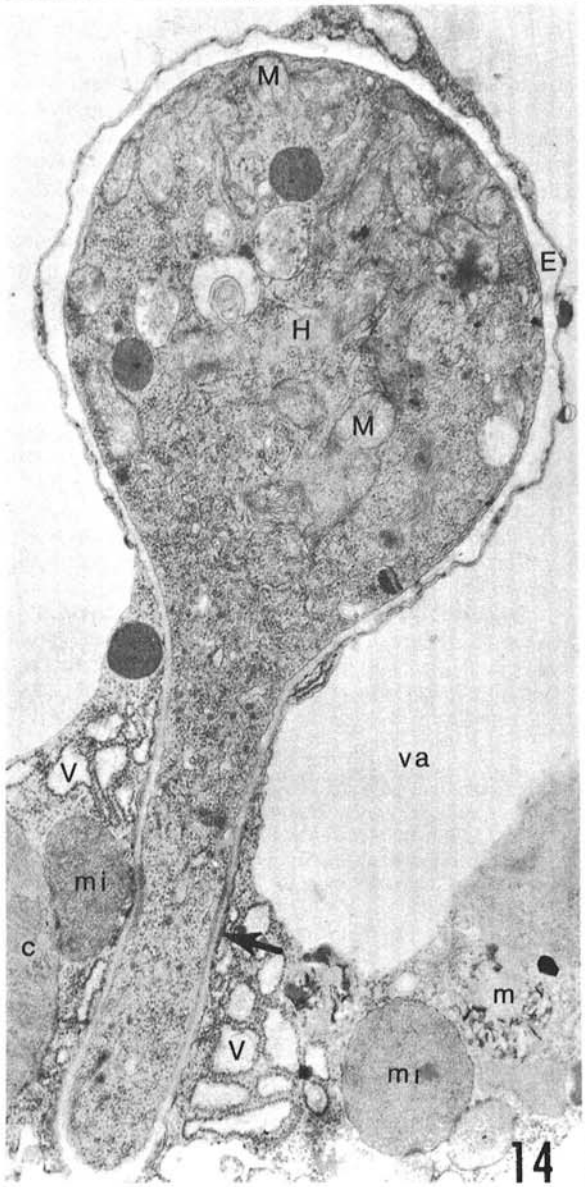
Fig. 7-11. 7) An early stage in host cell disorganization in the intermediate cultivar, Purple Hull Pinkeye, 8 days after inoculation. The host cytoplasm appears sparse, but the cell membranes are still intact. The haustorium (H) appears normal. (X 39,800) **8)** A later stage in host disorganization than that in Fig. 7. The host cytoplasm is almost void of organelles except for chloroplast grana and a few Golgi bodies (g). The host plasmalemma around the haustorium (H) is mainly intact, and still associated with endoplasmic reticulum (er). The haustorial cytoplasm still appears normal (compare with Fig. 4, 7) (X 38,400); **9)** A completely disorganized host cell in Purple Hull Pinkeye. All organelles have disintegrated except for the chloroplast grana. The haustorial (H) cytoplasm has also disorganized, but the adjacent intercellular hyphae (IH) still appear healthy. (X 4,550) **10)** Detail of Fig. 9 showing the intact haustorial wall (W). The surrounding host plasmalemma has finally disappeared, and the encapsulation may be represented by the diffuse material adjacent to the fungal wall. (X 41,500) **11)** Transverse section through the body of the first haustorium formed by an infection hypha in the immune cultivar Calico Crowder. Both haustorium (H) and haustorium mother cell (HM) appear normal, but the host cytoplasm is full of vesicles (V), some of which appear bordered with ribosomes. Notice the much-convoluted nature of the host plasmalemma bordering the encapsulation (E) and the associated areas of electron-opaque material; 22 hr after inoculation (X 27,600); c = chloroplast; E = encapsulation; er = host endoplasmic reticulum; g = Golgi body; H = haustorium; HM = haustorial mother cell; IH = intercellular hypha; M = fungal mitochondrion; m = host mitochondrion; n = host nucleus; s = starch grain; V = vesicle; va = host vacuole; W = haustorial wall.



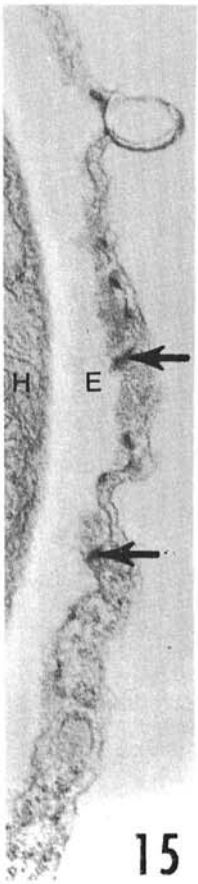
12



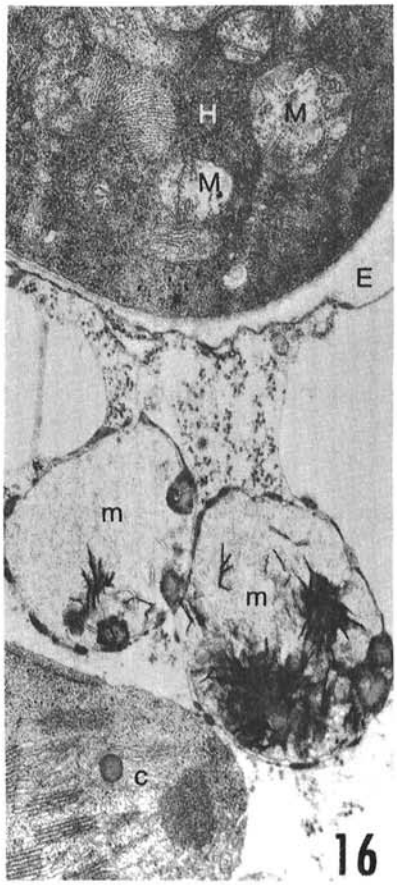
13



14



15



16

were observed around the base of the haustorial neck (Fig. 14, 17). As early as 19 hr after inoculation, only one example was found where extensive disorganization of host or haustorium had not yet taken place (Fig. 14). Here there were none of the signs of host reaction described for the other two immune cultivars, although there was an aggregation of large membrane-bound vesicles bordered with ribosomes around the haustorial neck, and the matrix of each mitochondrion appeared less electron-opaque than in neighboring cells. Even those responses normally seen in susceptible tissue appeared absent, as the host cytoplasm was not aggregated around the haustorium, and there were few profiles of rough endoplasmic reticulum surrounding the slightly undulated encapsulation membrane. Nearly all host membranes, however, bore small electron-opaque areas visible before section staining (Fig. 15). No such structures were seen in uninvaded neighboring cells, although there was an accumulation of rough endoplasmic reticulum and a slight deposit of electron-translucent material, bearing membrane inclusions, in that neighboring cell connected to the invaded cell by plasmodesmata. Similar reactions are commonly observed next to necrotic cells in many cultivars (see below), and are probably a response towards materials released during cell death. Thus, their presence here may well be further evidence that the invaded cell was unhealthy in some way.

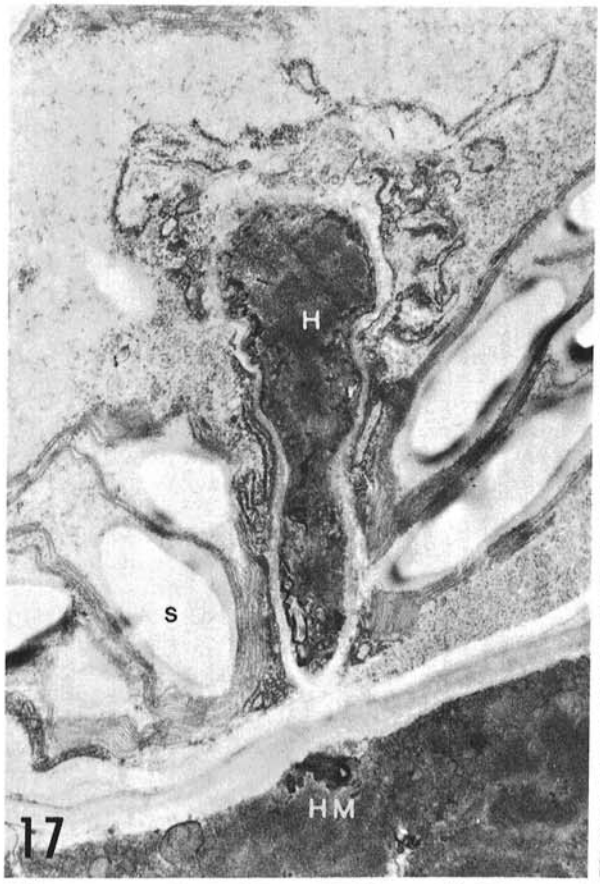
Although the haustorial cytoplasm appeared normal, measurements suggested that the haustorial body was only approximately half the volume of those in all other cultivars at a similar stage of development. Other haustoria in more disorganized cells were of similar dimensions, suggesting that no further development of the haustorium took place before the onset of host cell death. In no case did the usual migration of nuclei from haustorial mother cell to haustorium take place. Thus, haustorial development appeared to have been slowed or arrested in this cultivar.

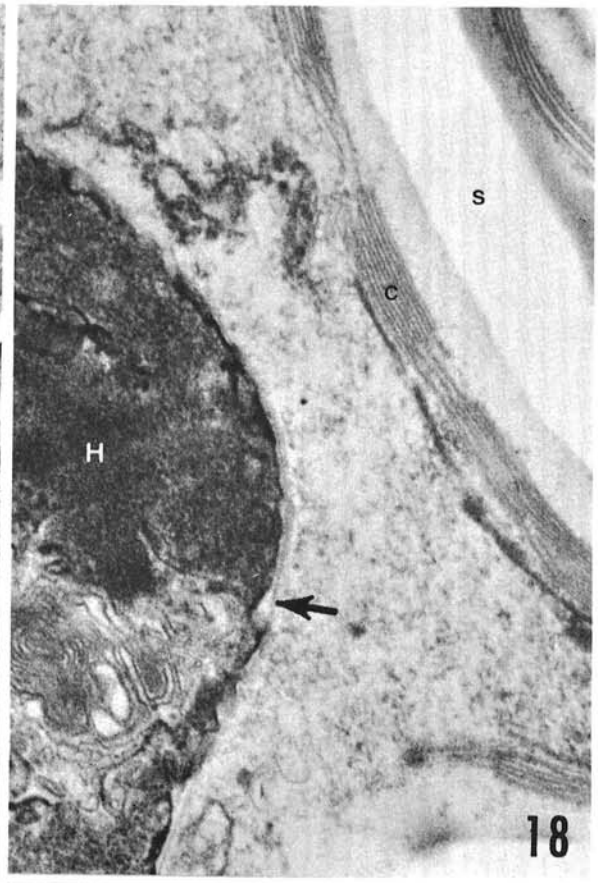
Although there was a slight increase in electron-opacity of the host plasmalemma about halfway along the haustorial neck (Fig. 14), no clear neckband was found in this haustorium or in the two others in dying cells sectioned through the neck region (Fig. 17).

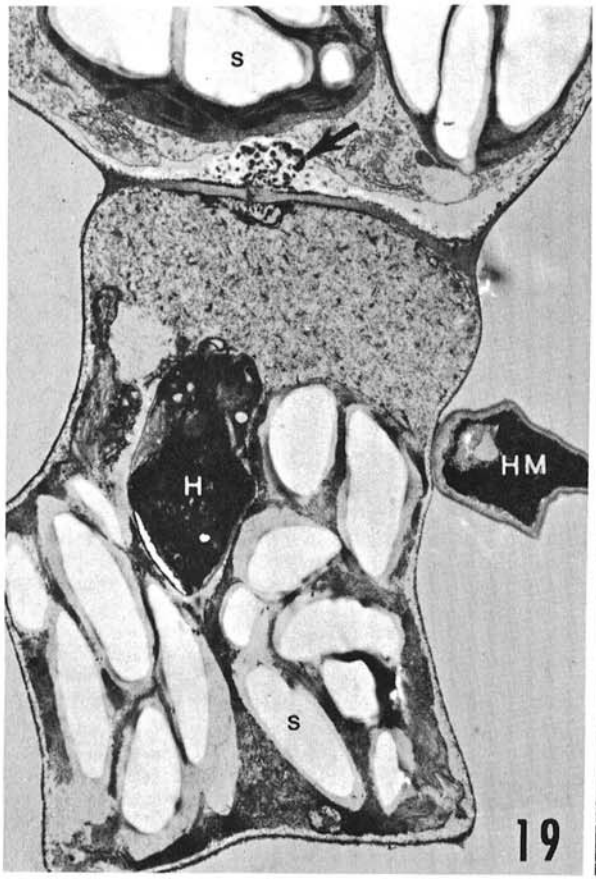
In both immune cultivars, as was the case in about 60% of the invaded cells in Queen Anne (8), the initial host reaction to haustorial formation was quickly followed by the disorganization of the host cell. In Dixie Cream, only one example was found of an early stage in such cytoplasmic disorganization (Fig. 16). The ground cytoplasm of the host appeared sparse, and the electron-opaque areas of the membranes, particularly those of the mitochondria, were more pronounced than in the stage described above. Mitochondria of both host and haustorium were noticeably swollen. All other invaded cells in this cultivar appeared almost completely disorganized (Fig. 17, 18, 19), and closely resembled those necrotic cells observed in Calico Crowder and Queen Anne (9). The lack of intermediate stages suggests that in all these immune cultivars, the process of host cell disorganization occurred rapidly after its initiation. This is in contrast to the much slower process observed in the intermediate response. In all the immune cultivars, the host plasmalemma, including that portion around the haustorium (Fig. 18), the tonoplast, and the nuclear and mitochondrial membranes, were the first to disintegrate (Fig. 17). This process was not accompanied by the appearance of lipidlike material similar to that seen in the intermediate response. A characteristic feature of Dixie Cream alone was the persistence of ribosome-studded membranes which were possibly remnants of the large vesicles observed before cell necrosis (Fig. 17). These were still often recognizable even at an advanced stage in the cell collapse which closely followed cytoplasmic disorganization in all immune cultivars. As in the intermediate response, the chloroplast grana were the most persistent of cell membranes in all immune reactions (Fig. 17, 18, 19).

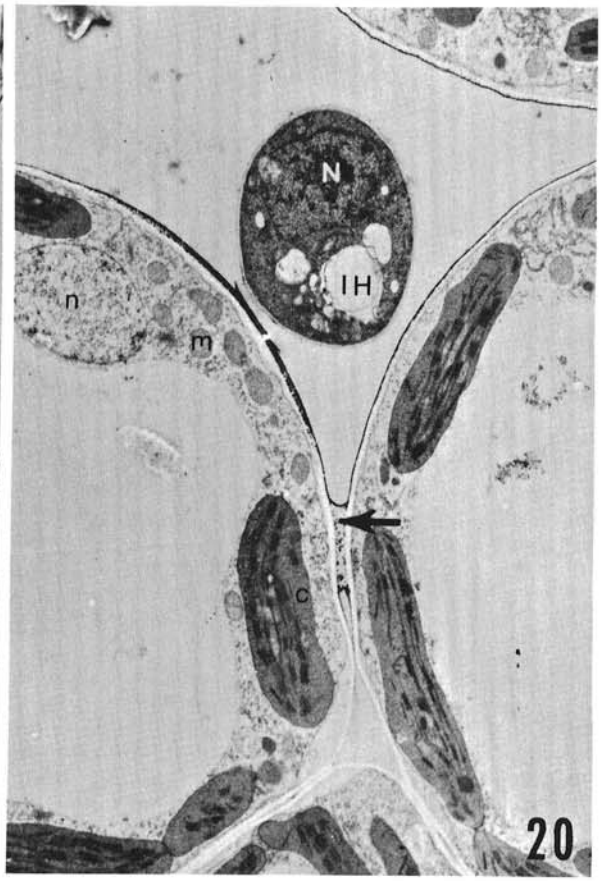
In all immune cultivars, the haustorium and haustorial mother cell appeared necrotic in all host cells showing any degree of disorganization. The cytoplasm of these fungal structures was extremely electron-opaque (Fig. 17, 18, 19), and the organelles were hard to distinguish, although their broken outlines could sometimes be detected. The remainder

Fig. 12-16. 12) Oblique section of the neck of a young haustorium formed in the immune cultivar Calico Crowder showing the region of host wall penetration with a deposit (D) of electron-translucent, membrane-containing material around the point of entry of the haustorium (H). Electron-opaque granular material (arrow) was sometimes associated with these deposits; 22 hr after inoculation. (X 27,400) **13)** Another haustorial body (H) in Calico Crowder showing the convoluted host plasmalemma associated with areas of electron-opaque material. The adjacent host cytoplasm contains abundant rough endoplasmic reticulum (er) and membrane-bound vesicles (arrows). (X 56,500) **14)** Longitudinal section through the first haustorium formed by an infection hypha in immune cultivar Dixie Cream. Both host and haustorial cytoplasm look relatively normal apart from the accumulation of ribosome-studded vesicles (V) around the haustorial neck and the electron-translucent appearance of the host mitochondrial matrix (m). No neckband can be detected (see Fig. 24), but an increase in electron-opacity of the host plasmalemma (arrow) can be seen halfway along the haustorial neck; 19 hr after inoculation. (X 21,900) **15)** Detail of Fig. 14 showing the electron-opaque areas (arrows) on the host plasmalemma surrounding the haustorium (H). Similar areas were present on most membranes of the host. (X 75,000) **16)** First stages in host and haustorial disorganization in immune cultivar Dixie Cream. The host cytoplasm has become sparse, and the mitochondria of haustorium (M) and host (m) are swollen. The electron-opaque areas on the host mitochondrial membranes have increased. The dark crystals in the host mitochondria are probably fixation artifacts present before section staining, and were sometimes found in healthy tissue; 20 hr after inoculation (X 28,400); c = chloroplast; E = encapsulation; er = host endoplasmic reticulum; H = haustorium; HM = haustorial mother cell; M = fungal mitochondrion; m = host mitochondrion; V = vesicle; va = host vacuole.


H
S
HM
17


S
C
H
18


S
H
HM
S
19


N
IH
n
m
C
20

of each infection hypha, however, appeared normal at this stage, although fungal growth usually proceeded no further than the formation of the first haustorium (8).

In both immune cultivars examined here, as in Queen Anne (9), cell death and collapse were accompanied by the accumulation of rough endoplasmic reticulum in the neighboring cells, where they were in contact with the invaded cell. Such an accumulation was usually noticeable first in the region of plasmodesmata. Later, deposits of material were formed similar to those formed around the base of the haustorial necks in some reactions (Fig. 19). These deposits also react histochemically as callose (8). Light microscopy has shown that these neighboring cells occasionally become necrotic, presumably through the action of toxic materials released from the dead invaded cell (13). However, at the early stage of infection examined here, no signs of such necrosis were detected.

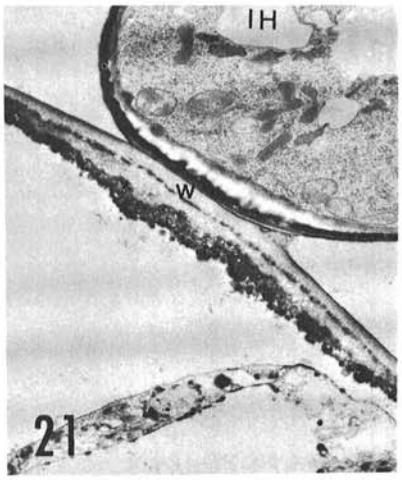
Nonhost reaction (bean cultivar Pinto).—Although a few infection hyphae shrivelled and died soon after entering the leaf, the majority grew at a rate comparable to that in the susceptible cowpea cultivars, and eventually formed haustorial mother cells. About 30 infection hyphae were examined with the electron microscope 21 hr after inoculation, and all appeared ultrastructurally similar to those in susceptible tissue (Fig. 20, 21, 22, 23). However, within 6 hr after entering the leaf, the host cell walls in the vicinity of each infection hypha appeared to transmit less light, under the light microscope, than did their neighbors. Under the electron microscope, these walls had electron-opaque granular material deposited on and apparently within them (Fig. 20, 21, 22, 23) which closely resembled that occasionally found associated with callose-containing deposits in immune cowpea cultivars. In the bean, however, this material was much more widespread, and appeared to decrease the flexibility of the walls, as many broke during the fixation and embedding processes (Fig. 20, 23). Similar breakage was extremely rare in noninoculated portions of the leaf. These deposits, which appeared electron-opaque even before section staining, were thicker where the infection hypha touched the cell wall (Fig. 21, 22). Next to the haustorial mother cells, where such deposits were especially massive, the granular material was often interspersed with less electron-opaque areas to give a layered appearance in cross section (Fig. 23). Next to these large deposits, the fungal walls also commonly contained granules of electron-opaque material (Fig. 22).

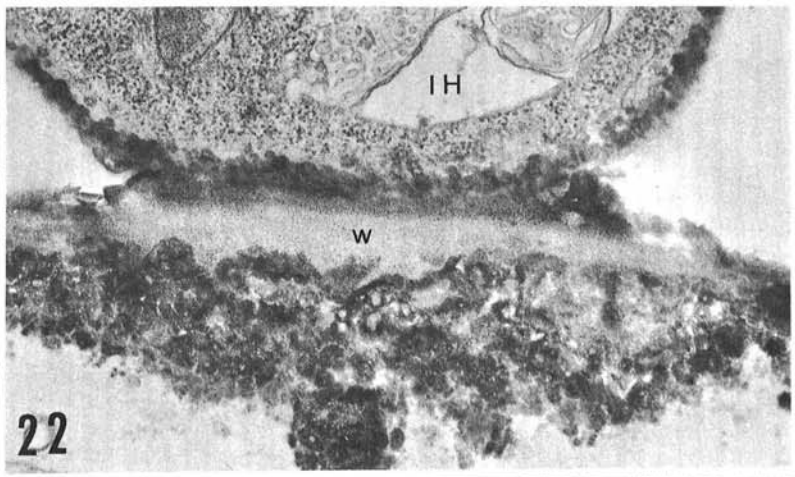
In addition to that associated with the cell wall, electron-opaque material was also found in the intercellular space at the junction between two affected host cells (Fig. 20). Similar material has occasionally been seen near the callose-containing deposits formed in the immune cowpea cultivars, Calico Crowder and Queen Anne (9).

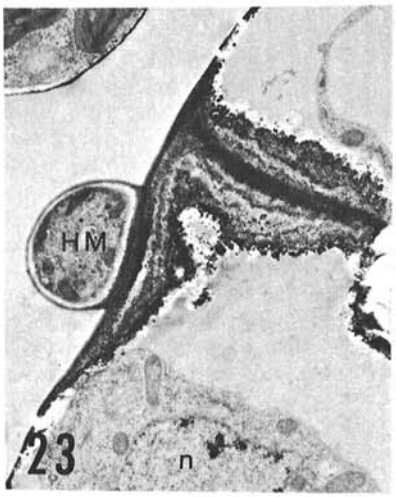
Light microscopy showed that only 10% of all infection hyphae formed haustoria. Three haustoria were observed with the electron microscope, and in each, the deposit of electron-opaque material adjacent to the haustorial mother cell was slight or undetectable (Fig. 24). Each haustorium appeared similar to those in susceptible cowpea cultivars (Fig. 24). Each possessed a neckband (Fig. 24); in one, nuclear migration had taken place. Each haustorium was separated from the host cell by the usual encapsulation bordered by a host membrane continuous with the plasmalemma at the base of the haustorial neck. At low magnifications, this membrane appeared thicker around the haustorial body than in other parts of the cell (Fig. 24), and at higher magnifications, this thicker portion had a more diffuse appearance compared with other areas of this membrane (Fig. 25). However, preliminary observations of several bean cultivars infected with bean rust (*Uromyces phaseoli* var. *typica*) suggest that this may be a characteristic feature of the early stages of all types of reaction by the bean. Rough endoplasmic reticulum accumulated in the host cytoplasm around the haustorium (Fig. 24, 25), and there appeared to be an increase in the number of membrane-bound vesicles. Otherwise there were no marked differences in cytoplasmic activity of the invaded cell when compared with its uninvaded neighbors.

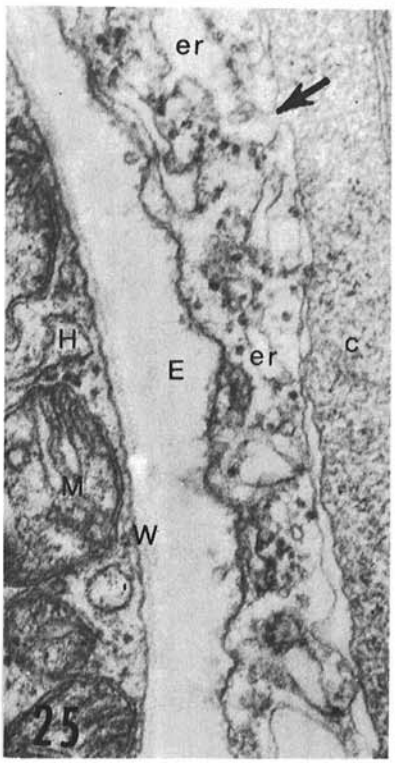
Light microscopy showed that host cell death occurred soon after haustorial formation. However, because of the difficulty in locating any of the few haustoria formed, it was not possible to find any stages in host or haustorial necrosis. Presumably, host cell death was closely linked with that of the haustorium, as in the immune cowpea cultivars, as the haustorial mother cell collapsed at the first signs of browning of the host cell. Light microscopy also revealed that occasionally one or two uninvaded cells

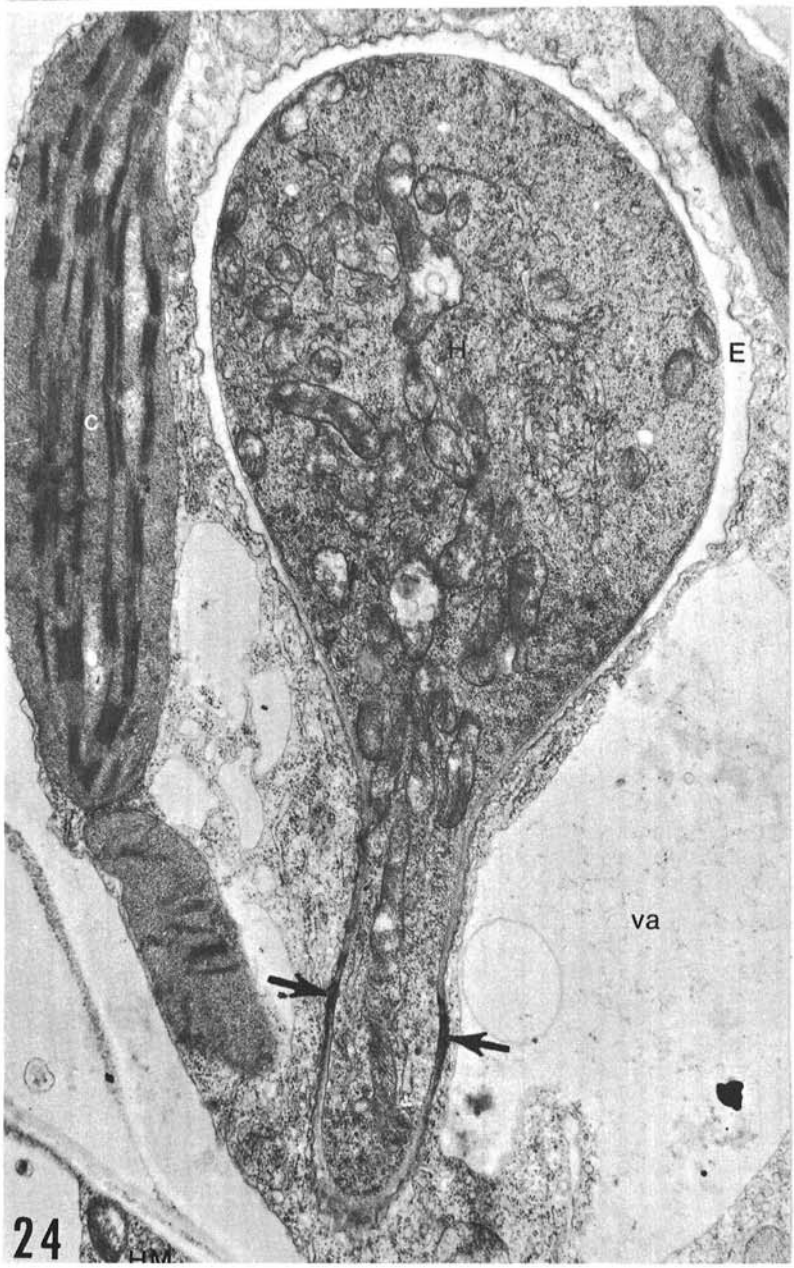
Fig. 17-20. 17) A stage in necrosis of host cell and haustorium in immune cultivar Dixie Cream. The cytoplasm of the haustorium (H), haustorial mother cell (HM), and host are all disorganized, but host cell collapse has not yet taken place. Ribosome-studded membranes remain around the haustorium. The main part of the haustorial body is out of the plane of section; 19 hr after inoculation. (X 19,750) **18)** Detail of the body of the haustorium shown in Fig. 17. The organelles of the haustorium (H) cannot be distinguished. The haustorial wall (arrow) is just detectable, but no recognizable encapsulation remains. (X 37,500) **19)** A later stage in host and haustorial necrosis than that shown in Fig. 17. Considerable collapse of the host cell has taken place. A deposit of electron-translucent membrane-containing material (arrow) has formed in the adjacent cell against a plasmodesmata; 20 hr after inoculation. (X 7,160) **20)** An early stage in the infection of the nonhost bean cultivar Pinto. The infection hypha (IH) has induced an electron-opaque deposit on all adjacent host cell walls and in the intercellular space (arrow). These deposits are less extensive than many observed; 21 hr after inoculation (X 6,060); c = chloroplast; H = haustorium; HM = haustorial mother cell; IH = infection hypha; m = host mitochondrion; N = fungal nucleus; n = host nucleus; s = starch grain.


IH
W
21


IH
W
22


HM
23
n


er
H
E
er
C
M
W
25


C
H
E
va
24
HM

adjacent to infection hyphae became necrotic. However, apart from an accumulation of rough endoplasmic reticulum adjacent to the wall deposits, the majority of cells examined 21 hr after inoculation showed no ultrastructural signs of abnormality. Only one example (Fig. 21) was found where the cell membranes appeared broken and were associated with drops of lipidlike material similar to that seen in the intermediate cowpea response.

DISCUSSION.—One of the objectives of this investigation was to determine whether the haustorium-induced reaction of the host membrane, observed in one immune cowpea cultivar, Queen Anne (9), was typical of all highly resistant reactions to cowpea rust. A similar membrane reaction was indeed found in the immune cowpea, Calico Crowder, but did not occur in the other immune cultivar, Dixie Cream. In addition, the few haustoria formed in the nonhost, *Phaseolus vulgaris*, induced a host reaction initially indistinguishable from the susceptible response. Thus, the distinctive reaction of the host plasmalemma does not appear to be a consistent indication of a high level of incompatibility between host and pathogen.

There were, however, other features which did clearly distinguish the different types of reaction induced by this fungus. The nonhost reaction differed strikingly from the reactions of all cowpea cultivars in that a response was detectable in all cells bordering the infection hypha. The resulting change in wall ultrastructure apparently prevented the formation of a haustorium in 90% of infection sites. Light microscopy has shown that similar reactions can be detected in all of six bean cultivars so far examined (*unpublished data*). Presumably, this difference in response by host and nonhost reflects a difference in sensitivity to the secretions of the infection hypha. Between the nonhost and the immune cowpea cultivars, this difference may only be one of degree, as similar-appearing reactions were occasionally observed in the immune cultivars at the region of host cell penetration. It is possible that in the immune hosts, the concentration of materials per host cell can only reach a sufficiently high level during penetration to induce the observed response; presumably it is then too late to prevent the formation of haustoria. No such reactions were ever observed in susceptible cultivars or in that giving an intermediate response. Although some other plants resemble the cowpea in their lack of response before haustorial penetration (1, 10, 13), many do not (15, 17, 19, 20, 22). Perhaps this reflects a difference in the degree of adaptation of the rust to its host.

The reaction of the bean to cowpea rust appears remarkably similar to that described by Leath & Rowell (11, 12) from light microscope studies of corn infected with *Puccinia graminis*. They, too, concluded that the change in appearance of the host cell walls prevented haustorial formation. The interesting feature revealed here by the electron microscope was the presence of a seemingly identical reaction of the fungal wall where it touched a particularly massive host wall reaction. This observation suggests the diffusion from the host of some substance capable of inducing similar changes or deposition of materials in the walls of both organisms. In their investigation, Leath & Rowell (11) found that many hyphae ceased to grow before contact with the host cell walls. Only a few such cases were seen in the nonhost bean and, at least by 21 hr after inoculation, the majority of infection hyphae appeared ultrastructurally normal. Even when some toxic material was present, therefore, most of the infection hyphae were insensitive. Possibly the close taxonomic relationship between the cowpea rust fungus (*U. phaseoli* var. *vignae*) and the bean rust fungus (*U. phaseoli* var. *typica*) accounts for this lack of sensitivity.

For the various types of resistance to *P. graminis*, Stakman (19, 20) concluded that the differences between immunity and the less vigorous types of resistance are only of degree. Whereas this may be true for the differences between the reaction of the bean and the immune cowpea cultivars, the ultrastructural observations described here suggest that there are more fundamental differences between the immune response and the intermediate reaction. In all of the three immune cultivars examined in this work and previously (9), the first signs of resistance were detectable within a few hours after the formation of the first haustorium. No such reactions were observed in the cultivar giving the intermediate (necrotic fleck) response even when the initial compatibility between host and parasite began to break down. The rapidity with which the invaded host cell died and the ultrastructural details of host cell disorganization were similar in all the immune

Fig. 21-25. 21) An early stage in the infection of the nonhost bean, cultivar Pinto, showing an infection hypha (IH) touching a necrotic cell. A deposit has formed on the host wall (w) and also on the fungal wall where the two touch; 21 hr after inoculation. (X 13,380) **22)** Another infection hypha (IH) in Pinto bean. A thick, granular, electron-opaque deposit has formed on the host wall (w) and also in the adjacent fungal wall; 21 hr after inoculation. (X 29,900) **23)** An especially large, layered deposit formed in Pinto bean adjacent to the tip of a haustorial mother cell (HM); 21 hr after inoculation. (X 5,490) **24)** One of the few haustoria (H) formed in Pinto bean. The wall deposit adjacent to the haustorial mother cell (HM) is barely detectable. The haustorial ultrastructure and host reaction appear similar to those in susceptible tissue. Notice the neckband (arrows) around the haustorial neck; 21 hr after inoculation. (X 16,900) **25)** Detail of Fig. 24. The host plasmalemma bounding the haustorial body (H) is more diffuse than in other regions of the cell. Notice the apparent fusion (arrow) of the host endoplasmic reticulum (er) with the chloroplast membrane. This was the only observed example of such fusion in any cultivar, and its significance is unknown (X 71,200); c = chloroplast; E = encapsulation; er = host endoplasmic reticulum; H = haustorium; HM = haustorial mother cell; IH = infection hypha; M = fungal mitochondrion; n = host nucleus; W = fungal wall; w = host wall.

cultivars, but quite different in the intermediate reaction. Presumably, these processes also differed biochemically, since haustorial death did not immediately follow the onset of host disorganization in the intermediate response as it did in all the immune cultivars so far examined with the electron microscope. This close assocation between haustorial necrosis and host cell death also appears to be present in the 12 other immune cowpea cultivars examined with the light microscope (8). Thus, there appear to be at least two basic types of varietal resistance to rust infection in the cowpea. It remains to be seen whether such differences exist in other plants with differential types of varietal resistance to rust infection. To date, no other immune reactions have been examined with the electron microscope, and there have been only four published investigations of the intermediate types of response (4, 18, 21, 23). These all show an initial lack of any significant unusual response to haustorial formation, and in at least two of these combinations (18, 23), host cell death does not necessarily cause the immediate death of the haustorium. Thus, the intermediate response in the cowpea does appear to be basically similar to that induced by rusts in other plants.

Both host and nonhost responses to cowpea rust were similar in that the death of the haustorium and haustorial mother cell did not result in the immediate necrosis of the rest of the intercellular mycelium. Whether any fungistatic substances were present will not be known until the potential growth of the infection hypha in the absence of any haustoria has been determined. It may be that starvation, rather than some toxic product, is the primary cause of the cessation of fungal growth.

Because of the practical difficulties in examining rust infections a few hours after the formation of the first haustorium, relatively few haustoria were examined in each cultivar. It should, therefore, be mentioned that even where a large number of infection sites were examined in this and other work (9), there was no variation between individuals in such basic features as the presence of a membrane reaction or the association of host and haustorial death. Thus, the features reported here are probably truly representative of the reactions concerned.

LITERATURE CITED

1. ALLEN, RUTH F. 1923. A cytological study of infection of Baart and Kandred wheats by Puccinia graminis tritici. J. Agr. Res. 23:131-152.
2. BERLIN, J. D., & C. C. BOWEN. 1964. The host-parasite interface of Albugo candida on Raphanus sativus. Amer. J. Bot. 51:445-452.
3. BRACKER, C. E. 1967. Ultrastructure of fungi. Annu. Rev. Phytopathol. 5:343-374.
4. EHRLICH, H. G., & M. A. EHRLICH. 1962. Fine structure of Puccinia graminis tritici in resistant and susceptible host varieties. Amer. J. Bot. 49:665 (Abstr.).
5. EHRLICH, M. A., J. F. SCHAFER, & H. G. EHRLICH. 1966. Association of host endoplasmic reticulum with haustoria of Puccinia graminis f. sp. tritici. Phytopathology 56:876 (Abstr.).
6. EHRLICH, M. A., J. F. SCHAFER, & H. G. EHRLICH. 1968. Lomasomes in wheat leaves infected by Puccinia graminis and P. recondita. Can. J. Bot. 46:17-20.
7. HARDWICK, N. V., A. D. GREENWOOD, & R. K. S. WOOD. 1971. The fine structure of the haustorium of Uromyces appendiculatus in Phaseolus vulgaris. Can. J. Bot. 49:383-390.
8. HEATH, MICHELE C. 1971. Haustorial sheath formation in cowpea leaves immune to rust infection. Phytopathology 61:383-388.
9. HEATH, MICHELE C., & I. B. HEATH. 1971. Ultrastructure of an immune and a susceptible reaction of cowpea leaves to rust infection. Physiol. Plant Pathol. 1:277-287.
10. HILU, H. M. 1965. Host-pathogen relationships of Puccinia sorghi in nearly isogenic resistant and susceptible seedling corn. Phytopathology 55:563-569.
11. LEATH, K. T., & J. B. ROWELL. 1966. Histological study of the resistance of Zea mays to Puccinia graminis. Phytopathology 56:1305-1309.
12. LEATH, K. T., & J. B. ROWELL. 1969. Thickening of corn mesophyll cell walls in response to invasion by Puccinia graminis. Phytopathology 59:1654-1656.
13. LITTLEFIELD, L. J., & SANDRA J. ARONSON. 1969. Histological studies of Melampsora lini resistance in flax. Can. J. Bot. 47:1713-1717.
14. LITTLEFIELD, L. J., & C. E. BRACKER. 1970. Ultrastructure of Melampsora lini infection in flax. Phytopathology 60:1300 (Abstr.).
15. MARRYAT, DOROTHEA C. E. 1907. Notes on the infection and histology of two wheats immune to the attacks of Puccinia glumarum, yellow rust. J. Agr. Sci. 2:129-138.
16. REYNOLDS, E. G. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17:208-212.
17. ROTHMAN, P. G. 1960. Host-parasite interactions of eight varieties of oats infected with race 202 of Puccinia coronata var. avenae. Phytopathology 50:914-918.
18. SHAW, M., & M. S. MANOCHA. 1965. The physiology of host-parasite relations. XV. Fine structure in rust-infected wheat leaves. Can. J. Bot. 43:1285-1292.
19. STAKMAN, E. C. 1914. A study on cereal rusts: physiological races. Minn. Univ. Agr. Exp. Sta. Bull. 138. 56 p.
20. STAKMAN, E. C. 1915. Relation between Puccinia graminis and plants highly resistant to its attack. J. Agr. Res. 4:193-200.
21. VAN DYKE, C. G., & A. L. HOOKER. 1969. Ultrastructure of host and parasite in interactions of Zea mays with Puccinia sorghi. Phytopathology 59:1934-1946.
22. ZIMMER, D. E. 1965. Rust infection and histological response of susceptible and resistant safflower. Phytopathology 55:296-301.
23. ZIMMER, D. E. 1970. Fine structure of Puccinia carthami and the ultrastructural nature of exclusionary seedling-rust resistance of safflower. Phytopathology 60:1157-1163.