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Early Development of *Rhizobium*-Induced Root Nodules of *Parasponia rigida*. I. Infection and Early Nodule Initiation

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Summary

The first of two major steps in the infection process in roots of *Parasponia rigida* (*Ulmaceae*) following inoculation by *Rhizobium* strain RP501 involves the invasion of *Rhizobium* into the intercellular space system of the root cortex. The earliest sign of root nodule initiation is the presence of clumps of multicellular root hairs (MCRH), a response apparently unique among *Rhizobium*-root associations. At the same time or shortly after MCRH are first visible, cell divisions are initiated in the outer root cortex of the host plant, always subjacent to the MCRH. No infection threads were observed in root hairs or cortical cells in early stages. Rhizobial entry through the epidermis and into the root cortex was shown to occur via intercellular invasion at the bases of MCRH. The second major step in the infection process is the actual infection *per se* of host cells by the rhizobia and formation of typical intracellular infection threads with host cell accommodation. This infection step is probably the beginning of the truly symbiotic relationship in these nodules. Rhizobial invasion and infection are accompanied by host cortical cell divisions which result in a callus-like mass of cortical cells. In addition to infection thread formation in some of these host cortical cells, another type of rhizobial proliferation was observed in which large accumulations of rhizobia in intercellular spaces are associated with host cell wall distortion, deposition of electron-dense material in the walls, and occasional deleterious effects on host cell cytoplasm.

Keywords: Infection; Invasion; Multicellular Root Hairs; *Parasponia*, *Rhizobium*.

1. Introduction

That species of the tropical genus *Parasponia*, in the family *Ulmaceae*, bear *Rhizobium*-induced root nodules was discovered and reported first by TRINICK (1973). This remarkable association is the only verified example of a nonlegume becoming effectively nodulated by *Rhizobium* as a natural occurrence (see AKKERMANS and VAN DIJK 1981, for a history of the discovery of this phenomenon).

Limited information on mature root nodule structure has been provided for *Parasponia rugosa* (TRINICK and GALBRAITH 1976) *P. andersonii* (TRINICK 1979), and *P. parviflora* (BECKING 1979). Aside from the statement by TRINICK (1981) that infection threads are found in the root hairs of *Parasponia*, no details of infection or early nodule development have been published.

Rhizobium species isolated from *Parasponia* nodules have been placed in the cowpea miscellany cross-inoculation group (TRINICK and GALBRAITH 1980). The infection process in this large and diverse group appears to range from intercellular penetration of the epidermis at a point where a lateral root emerges in *Arachis* (CHANDLER 1978) and *Stylosanthes* (CHANDLER *et al.* 1982) to the more common mode of infection in legumes, penetration of a root hair and formation of an infection thread, in *Vigna unguiculata* (PUEPPKE 1983) and *Vigna radiata* (BHADURI 1951, NEWCOMB and MCINTYRE 1981). In the present study we describe the infection process and early events in nodule initiation in *Parasponia rigida* Merr. and Perry.

2. Materials and Methods

Air-dried *Parasponia rigida* fruits less than one month old from greenhouse-grown plants were rehydrated overnight in tap water.

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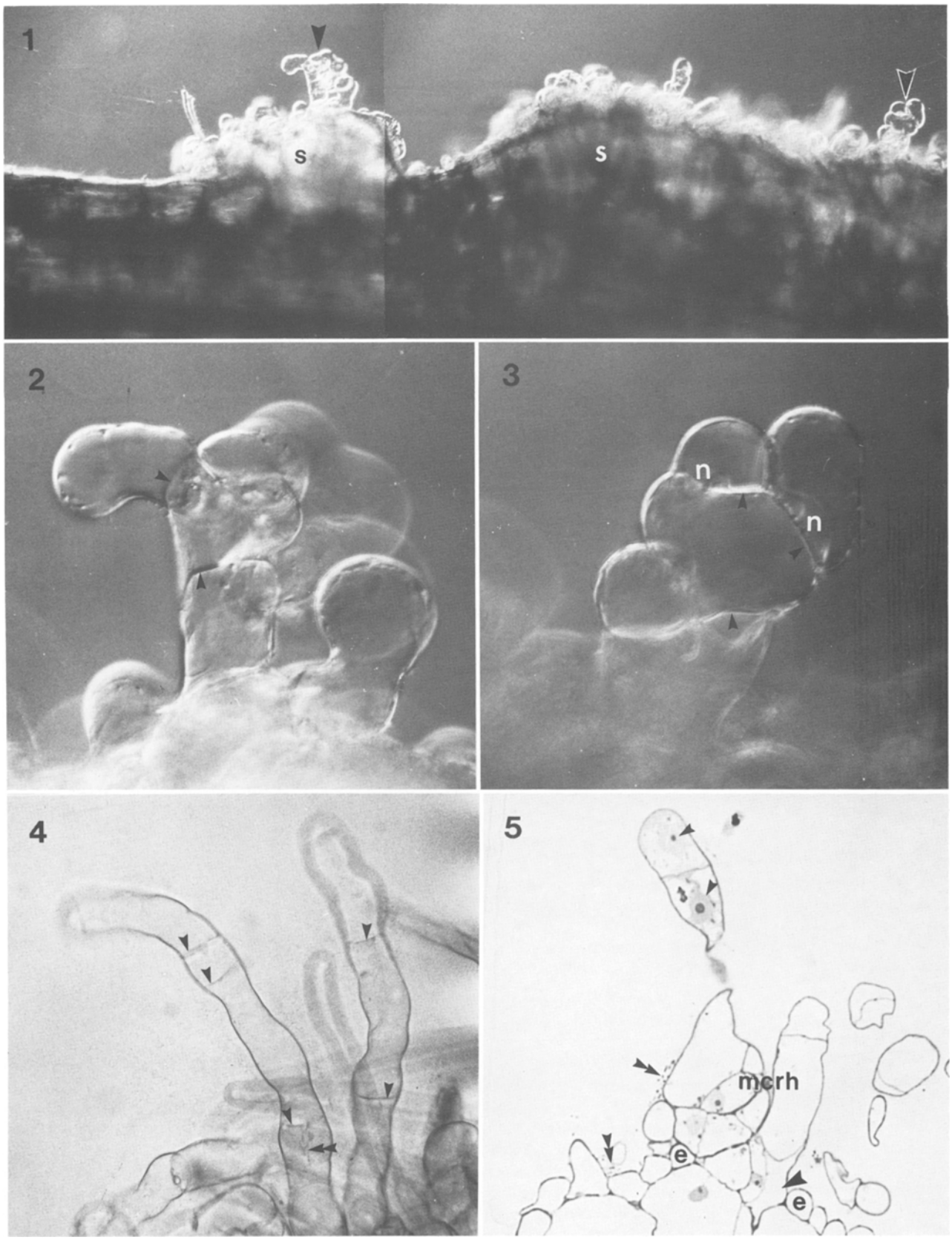


Figs. 1–5. Light micrographs of multicellular root hairs (MCRH) produced in response to inoculation with *Rhizobium*. Fig. 1. Portion of a root (longitudinal surface view) 20 days after inoculation showing root swellings (*s*) and some of the associated MCRH (arrows). Nomarski optics. × 160. Figs. 2 and 3. Higher magnification of MCRH shown in Fig. 1. Internal cell walls (arrows) are positioned in various planes. Some of the individual nuclei (*n*) are visible. Nomarski optics. × 800. Fig. 4. Elongate MCRH with mostly anticlinal walls (single arrows) and an occasional periclinal wall (double arrow). × 350. Fig. 5. Section through a clump of MCRH showing their origin (large arrow) in the epidermis (*e*). Nuclei are clearly visible in the 2 most distal cells of one of the hairs (single small arrows). Accumulations of rhizobia are associated with the MCRH and epidermis (double small arrows). No infection threads were seen in this specimen. × 500

The fleshy fruit coats were removed and the seeds were scarified with a razor blade. They were surface sterilized 20 minutes in 5% aqueous sodium hypochlorite, rinsed 5 times in sterile distilled water, and placed on plates of nutrient agar medium for pregermination in a growth chamber with a 16-hour daylength and 26/19 °C temperature regime. The nutrient medium used for all of these experiments was 1/4-strength nitrogen-free Hoagland's solution (HOAGLAND and ARNON 1950) supplemented with 0.175 mM NH_4NO_3, pH 6.5. Noble agar (Difco) was used at 1% where indicated.

After approximately 14 days, the most vigorous-looking seedlings were transferred aseptically to nutrient agar slants in 25 mm test tubes, capped, and returned to the growth chamber. After 3–4 weeks, when the seedlings were well established, they were inoculated with approximately 0.5 ml of inoculum of the *Rhizobium* strain now designated RP501, originally isolated from nodules of *P. parviflora* in Indonesia (TJEPKEMA and CARTICA 1982). The inoculum was prepared by scraping 5-day-old cultures of RP501 from petri plates containing a solid complex nutrient medium and diluting the cultures in sterile distilled water. Control seedlings were left uninoculated.

For observation and photography of root hairs and very young nodules, young seedlings were removed from the pregermination plates and placed in a modified Fahraeus slide culture assembly similar to that described by NUTMAN (1970). Each slide was placed in a 150 ml Fleaker containing 40 ml liquid nutrient medium. Liquid medium was placed in the slide cell and held there by capillarity. One seedling was placed in each slide cell, and inoculation was carried out as described above.

For structural studies, roots bearing root hairs and young nodules were fixed in a mixture of 2.5% glutaraldehyde and 2.5% paraformaldehyde in 50 mM sodium phosphate buffer, pH 6.8, for 2 hours at room temperature and rinsed several times in fresh buffer. They were post-fixed 2 hours in 1% OsO_4 at room temperature, rinsed, stained *en bloc* 1 hour in 2% aqueous uranyl acetate, and rinsed again before slow dehydration in a graded acetone series. Specimens were infiltrated with a very gradually increasing concentration of Spurr's (1959) low-viscosity resin, vacuum infiltrated in 100% resin for 12 hours, changed to fresh resin, and allowed to polymerize at 60 °C.

Serial 0.5–1.0 µm sections for light microscopy were placed on gelatin-dipped slides and stained in 0.05% toluidine blue O in 1% sodium borate at 60 °C.

Ultrathin sections for electron microscopy were stained 10 minutes in Reynold's (1963) lead citrate before observation on a JEOL 100CX TEM.

3. Results

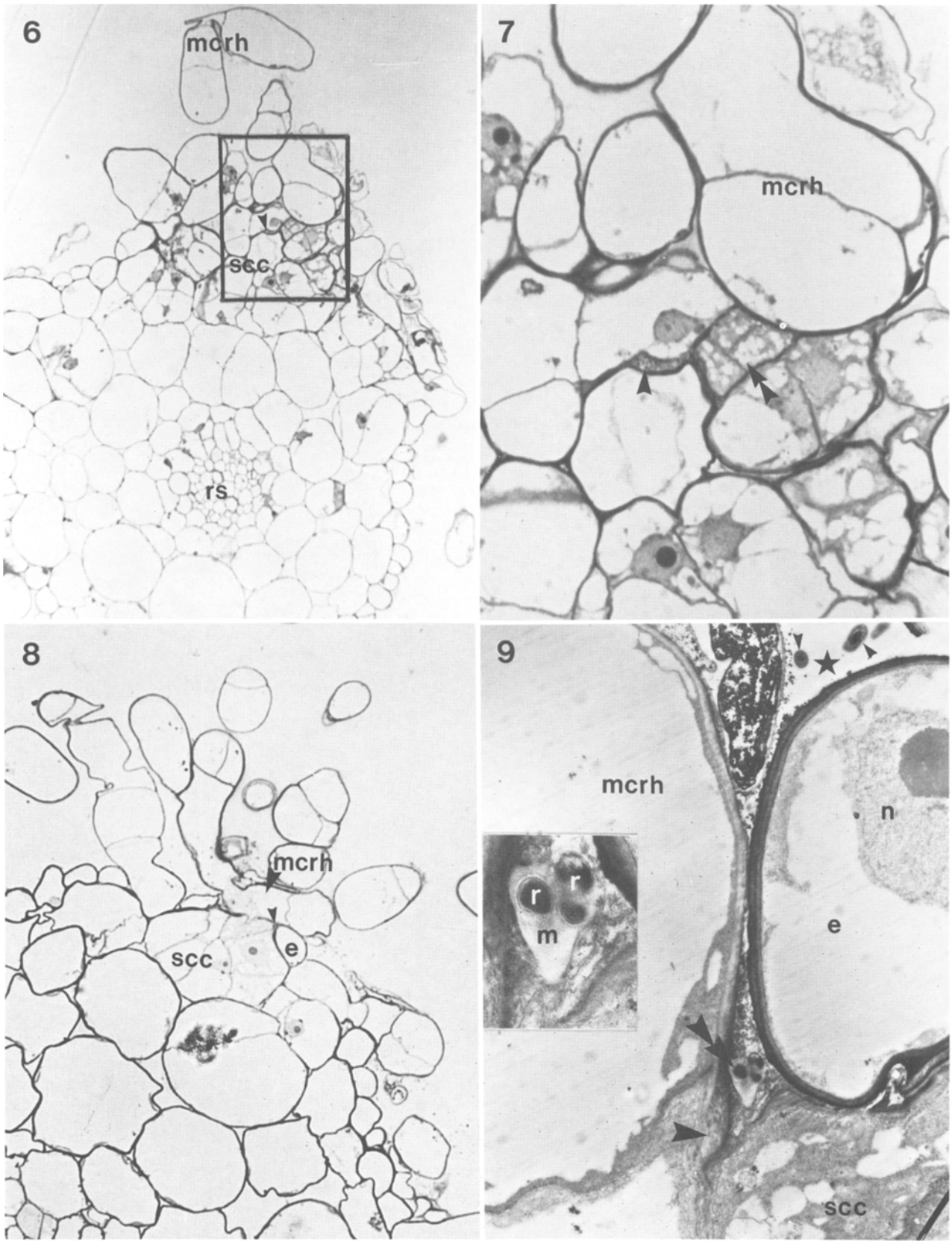
3.1. Response to Inoculation

The onset of nodulation was very irregular, and early signs of nodule initiation were visible to the naked eye from 3 weeks to 3 months after inoculation of the slow-growing seedlings. Because of this unpredictability, exact time course studies were not possible.

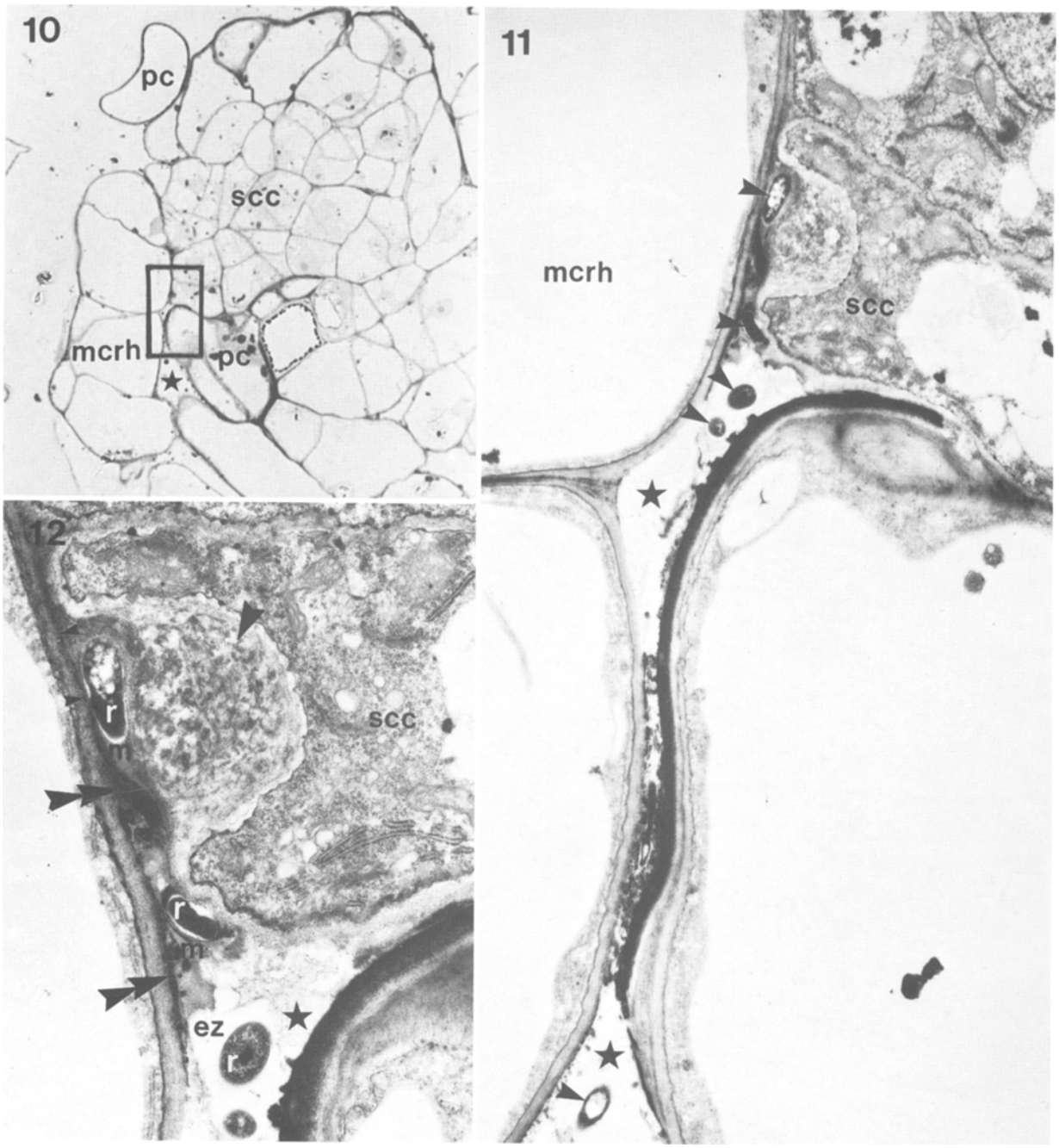
The earliest sign of nodule initiation was the presence of clumps of multicellular root hairs (MCRH) (Figs. 1–5), which were localized and often surrounded by normal, elongate, unicellular root hairs. None of the root hairs exhibited the deformation response usually associated with infection by *Rhizobium*. The appearance of the MCRH ranged from distorted, swollen structures arising from cell divisions in various planes (Figs. 2 and 3) to elongate, septate hairs with mostly anticlinal and occasionally periclinal walls (Fig. 4). Serial sections of clumps of MCRH verified their origin in the epidermis (Fig. 5). MCRH were never observed on uninoculated control seedlings, although abundant normal root hairs were present.

At the same time or shortly after the clumps of MCRH were first detectable with a dissecting microscope, slight swellings of the root, always subjacent to MCRH, appeared (Fig. 1). Although the swellings were occasionally located at the base of a lateral root, they were not necessarily associated with emergent or pre-emergent laterals. Sections of these swellings revealed their origin in the outer cortical cells, which had been stimulated to subdivide at random orientation (Fig. 6). Careful examination of serial 0.5–1 µm sections of several of these swellings failed to reveal infection threads in any of the associated MCRH or cortical cells; however, groups of rhizobia in what appeared to be intercellular spaces were sometimes seen, often near dividing or newly-divided cortical cells (Figs. 6 and 7).

Figs. 6–9. Micrographs of very young nodules and associated multicellular root hairs (MCRH). Fig. 6. Light micrograph of a transverse section through a root in the region of a root swelling, or very young nodule, showing its origin in the outer cortex, and associated MCRH. The planes of division in the subdivided cortical cells (*scc*) appear to be random. Note the parent root stele (*rs*) and lack of association of the young nodule with an emergent or pre-emergent lateral root. Rhizobia are present in an intercellular space (arrow) adjacent to newly divided cortical cells (box, see Fig. 7). No infection threads were seen in serial sections of this nodule. × 380. Fig. 7. Higher magnification of boxed-in area of Fig. 6, showing intercellular rhizobia (arrow) adjacent to newly subdivided cortical cells (double arrow) near a MCRH. × 1,320. Fig. 8. Light micrograph of a section through a very young nodule consisting of a clump of MCRH and a few subdivided cortical cells (*scc*). Rhizobia are present in the intercellular space (small arrow) between a MCRH (large arrow), and epidermal cell (*e*) and the subjacent subdivided cortical cell, as evidenced by Fig. 9. × 450. Fig. 9. Electron micrograph corresponding to a portion of Fig. 8, showing the rhizobia (large double arrow) in the intercellular space between a MCRH, an epidermal cell (*e*), and the subjacent subdivided cortical cell (*scc*). Note the large nucleus (*n*) and prominent nucleolus in the epidermal cell. The wall between the MCRH and the subdivided cortical cell shows an accumulation of loosely fibrillar material (single large arrow) which may be a response to hydrolytic enzyme production by the rhizobia. Free rhizobia (small arrows) with no surrounding matrix are present in the outside space, which is marked by a star. × 5,130. The inset is a higher magnification (× 12,700) of the rhizobia (*r*) and their surrounding moderately electron-dense matrix (*m*) in the intercellular space



Figs. 6–9



Figs. 10–12. Series of micrographs showing intercellular invasion of rhizobia from outside the root. Fig. 10. Light micrograph of a young nodule showing the callus-like mass of subdivided cortical cells (*scc*) and disruption of peripheral cell layers (*pc*) as a result of the cortical expansion. Boxed area, shown at higher magnification in Fig. 11, is the site of rhizobial invasion at the base of a multicellular root hair (*mcrh*) from an area outside the root (star). × 600. Fig. 11. Electron micrograph corresponding to the boxed area in Fig. 10. Rhizobia (arrows) are invading from a pocket of space (stars) which is technically outside the root. The invasion occurs between a basal cell of the multicellular root hair (*mcrh*) and a young subdivided cortical cell (*scc*). × 6,600. Fig. 12. Higher magnification of upper portion of Fig. 11, showing invasion of rhizobia (*r*). The rhizobia appear to have penetrated into the wall (single large arrow) of the subdivided cortical cell (*scc*) rather than progressing by separating two walls at the middle lamella, which is still intact (small arrows). A large accumulation of loosely fibrillar wall material (single large arrow) suggestive of wall loosening is associated with the invading rhizobia. The rhizobia in the wall are surrounded by a moderately electron-dense matrix (*m*) and are associated with a deposit of very electron-dense material (double large arrows). The rhizobia in the nearby outside space (star) do not have a matrix but are surrounded by an electron-translucent zone (*ez*). × 12,730

A correlated light and electron microscopic study was then undertaken in an attempt to elucidate the means by which the rhizobia gained access to the intercellular spaces of the cortex.

3.2. *Intercellular Invasion of Rhizobia*

Sections of a clump of MCRH with just a few subdivided subjacent cortical cells (Fig. 8) revealed a pocket of rhizobia in a space between the base of a MCRH, an epidermal cell, and a young recently divided cortical cell (Fig. 9). The rhizobia in the space were surrounded by a moderately electron-dense matrix and an accumulation of extracellular debris. Free rhizobia in the outside space had no matrix surrounding them.

In Fig. 9, the wall between the MCRH and the adjacent young cortical cell appeared slightly distorted, with an accumulation of loosely fibrillar material in the wall in close proximity to the rhizobia. The distortion may represent loosening of newly-formed wall material in response to enzymatic activity of the rhizobia (HUBBELL *et al.* 1978, MARTINEZ-MOLINA *et al.* 1979, CALLAHAM and TORREY 1981). Further intercellular rhizobial penetration into the cortex was not observed at the light microscope level in the specimen shown in Fig. 8. Ultrathin serial sections of the whole specimen were not attempted.

In a specimen sectioned at a slightly later stage in development, the randomly oriented cell divisions in the outer cortex produced a callus-like appearance (Figs. 10 and 13). At the ultrastructural level, points of rhizobial entry into the cortex via intercellular penetration were observed between basal cells of MCRH and cortical cells exposed to outside space (Figs. 11, 12, 14, and 15). It appears that the proliferation of outer cortical tissue disrupts the peripheral cell layers (Fig. 10), enlarging pre-existing intercellular spaces to such an extent that some of them become continuous with outside space, and underlying young cortical cells are exposed. Rhizobia can then penetrate between the thin, pliable young cell walls (Figs. 11, 12, 14, 15, and 16). This surface disruption is in some ways similar to the disruption caused by lateral root emergence.

Several areas of rhizobial entry were observed within the same specimen, indicating that many separate invasions of the root cortex may occur, each one stimulating more cortical proliferation. The initial invasion may have involved only a few subdivided cells such as those shown in Figs. 8 and 9.

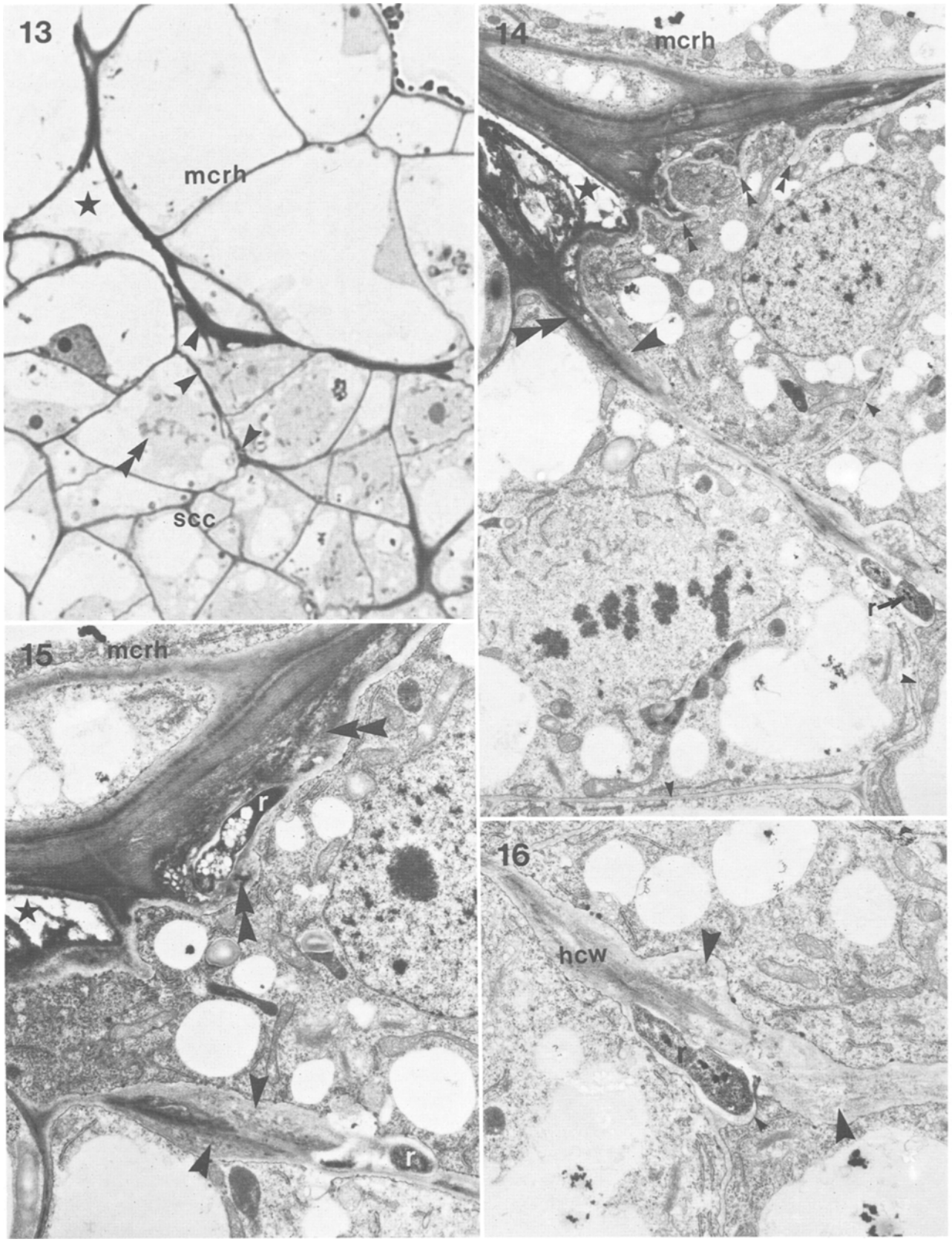
Invading rhizobia may be surrounded by a matrix (Fig. 12). Large accumulations of the loosely fibrillar material described above were present near the points of entry; often deposits of electron-dense material of unknown origin were present also (Figs. 11, 12, 14, and 15). The cell walls appeared to be pliable near these sites and were often much distorted where the accumulations occurred (Figs. 11, 12, 14, and 15). Mitotic activity of the cells immediately abutting invaded intercellular spaces was observed (Figs. 13 and 14).

Continued intercellular invasion of the rhizobia deeper into the cortex did not rely necessarily on separation of the walls at the middle lamellae (Fig. 12), as was suggested by CHANDLER (1978) for *Arachis*. In *Parasponia*, the rhizobia could be found anywhere within the intercellular spaces or the bordering cell walls, sometimes appearing to be in direct contact with the host plasmalemma (Fig. 16). This penetration was probably facilitated by continued loosening of young cell walls, as evidenced by the loosely fibrillar accumulations (Figs. 14, 15, and 16).

3.3. *Rhizobial Proliferation and Infection of Host Cells*

The continued host cortical cell divisions, coupled with rhizobial cell division, facilitated further intercellular invasion of rhizobia deeper into the cortex. In a few instances, moderately large intercellular proliferations of rhizobia were seen (Figs. 17–19). These were associated with large accumulations of a very electron-dense material in the nearby walls and intercellular spaces, and much distortion and disruption of the pliable young host cell walls. The rhizobia were surrounded by an electron-translucent zone and often a matrix (Fig. 17). Host cells in the vicinity of these proliferations of rhizobia subdivided repeatedly, resulting in packets of young cytoplasmically-rich cells nearly surrounded by intercellular rhizobia (Figs. 18 and 19). In some places the rhizobia appeared to be infecting host cells in the form of wide infection threads (Fig. 18). In one case a host cell associated with a large proliferation of intercellular rhizobia appeared to be degenerating (Fig. 19). Its cytoplasm was very diffuse and most cell organelles appeared abnormal in comparison with nearby cells.

It should be noted that the large intercellular proliferations of rhizobia resemble a host-pathogen type of interaction because of the associated host cell wall disruptions, considerable accumulations of dense extracellular material, and occasional deleterious



Figs. 13–16

effects on host cell cytoplasm. In sharp contrast to these events is the more orderly invasion commonly observed in the proliferating cortical tissue. Here, the intercellular rhizobia were most often in single file (Figs. 20 and 21), and their invasion was associated with little or no deposition of densely-staining extracellular material and much less cell wall disruption than observed with the large intercellular proliferations. The host cell walls were thickened where they surrounded rhizobial cells (Figs. 20 and 21), but often retained a finely fibrillar appearance (Fig. 20). Small accumulations of the loosely fibrillar material suggestive of wall loosening and slight amounts of electron-dense wall material were occasionally seen in association with these invasions (Fig. 21). The rhizobia were always surrounded by the electron-translucent zone, but did not appear to have a matrix. As seen in the outer cortex (Fig. 16), the rhizobia were located anywhere in the wall or intercellular spaces, and were sometimes in direct contact with host plasmalemma (Fig. 21). These more orderly strands of intercellular rhizobia could result in true infection of host cells and production of infection threads (Figs. 22 and 23) that probably signify the beginning of a balanced, truly symbiotic relationship.

Where the rhizobia entered a cell, they were surrounded by wall material continuous with the host cell wall, and a membrane continuous with the host plasmalemma (Fig. 22). This thread structure was maintained as the threads grew further into the host cells (Fig. 23). Presumably the host cell manufactures new membrane and wall components of the infection thread. In Fig. 22, the wall material surrounding the entering rhizobium has a slightly different staining property than the host cell wall which may characterize newly manufactured wall material. As the infection threads continued to grow into the host cells in these early stages, the rhizobia remained in single file (Fig. 23). The continued growth of the infection threads did not appear to have any deleterious effects on the host cell cytoplasm (Fig. 23). Rhizobia were not released from any of the infection threads.

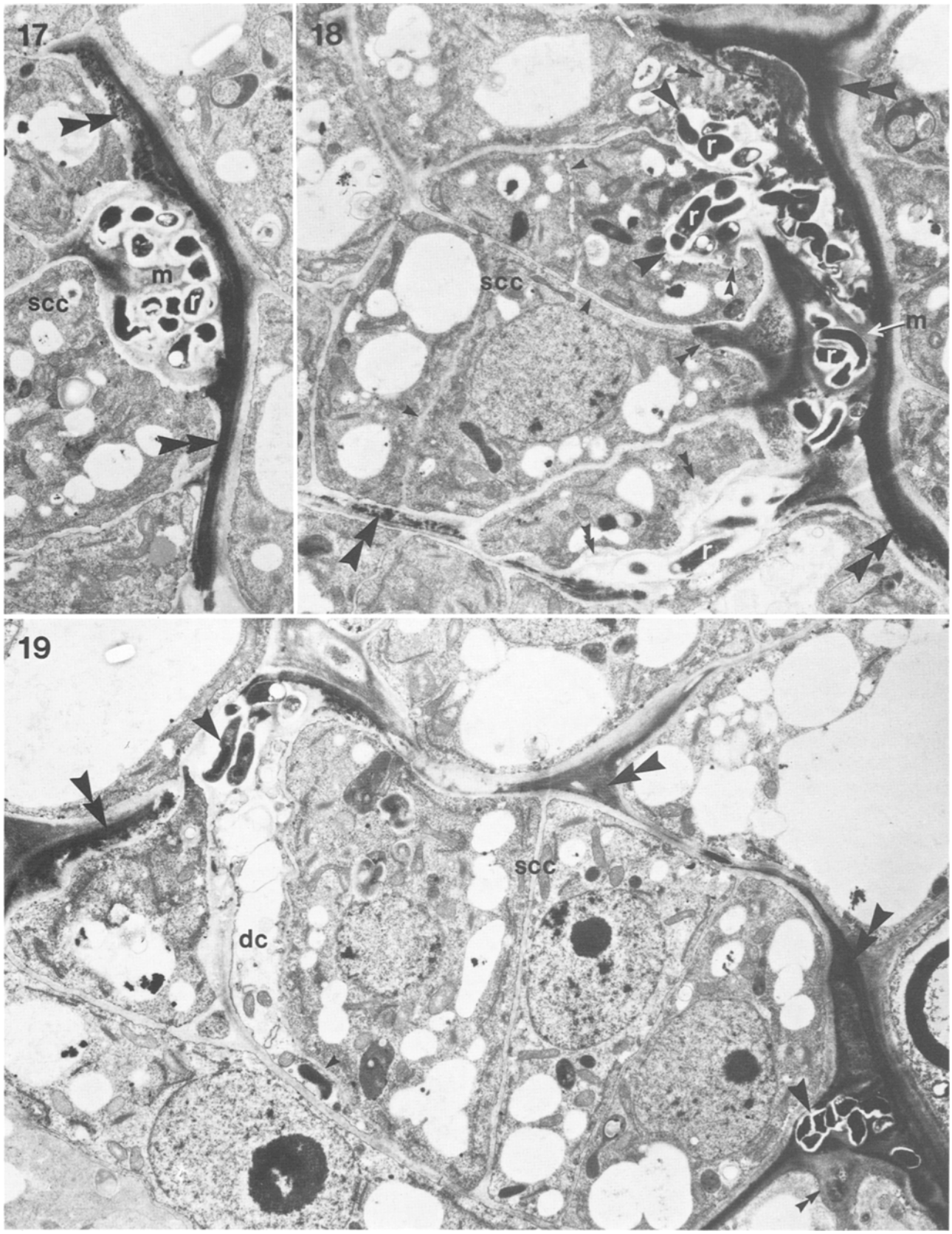
In the callus-like proliferation of cortical tissue seen in Figs. 10 and 13, only a few cells with infection threads in them were seen. Intercellular strands of rhizobia, though much more numerous than infection threads, were only occasionally seen. This indicates that a great amount of mitotic activity can be stimulated by the presence of relatively few rhizobia associated with the host cells in such intimate fashion.

4. Discussion

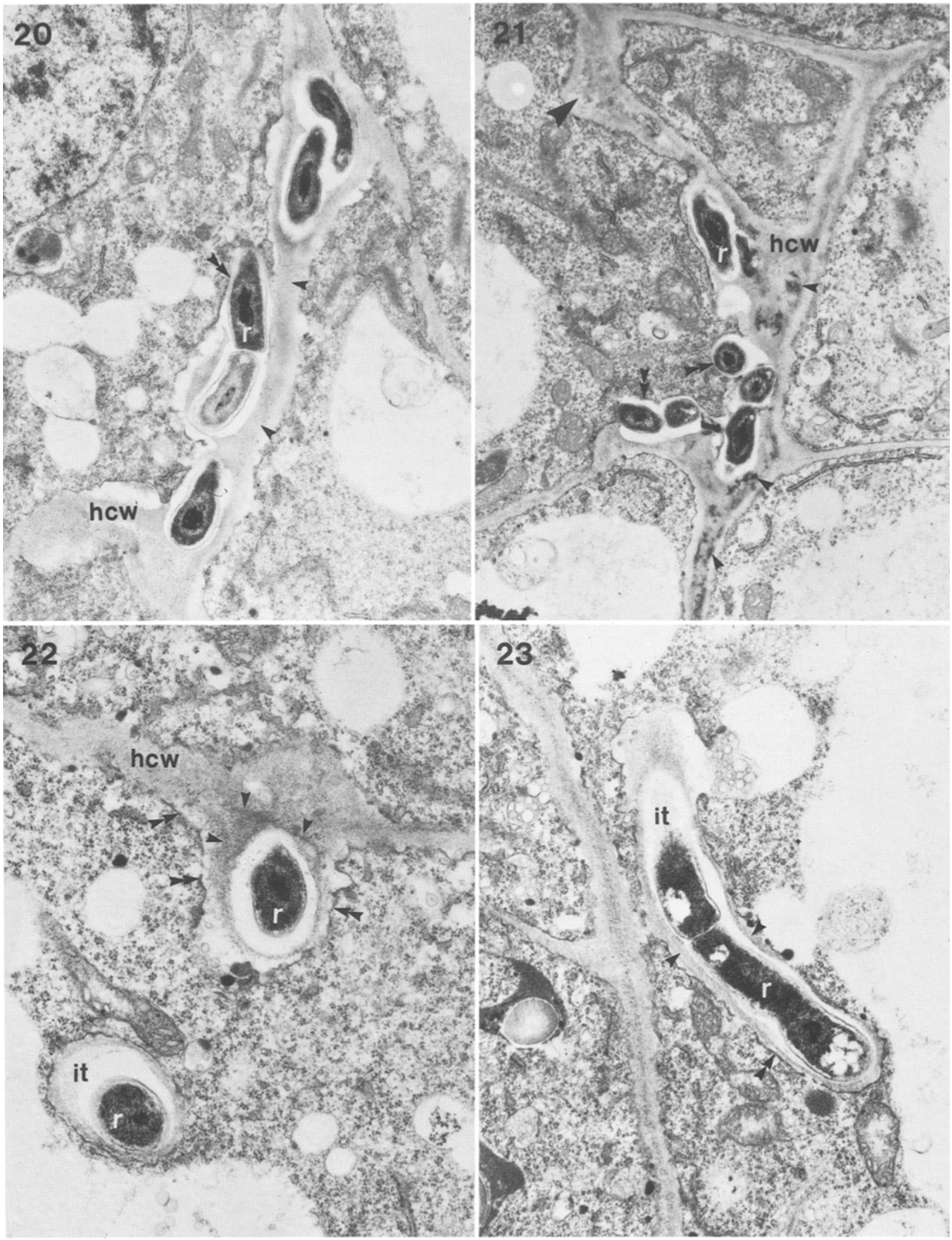
The infection process described above is unlike any legume-*Rhizobium* association thus far described although elements of various infection processes were observed. The steps involved can be listed in their probable order of occurrence. Two major events, each divisible into several steps, are involved.

First is the invasion of *Rhizobium* into the intercellular space system of the root cortex. Infection threads were never observed in the multicellular root hairs (MCRH) nor in any other epidermal cell. The first presence of *Rhizobium* inside the root was observed in an intercellular space at the base of a MCRH (Fig. 9). Bacterial populations external to the root are faced by a continuous impenetrable epidermal layer with no evident breaks, so an access point must be created for rhizobial entry into the root cortex. Stimulation of cell divisions in epidermal root hairs at the root surface or divisions in the outermost cortical cells of the root just beneath the epidermis would serve to stress the radial

Figs. 13–16. Series of sections through a second area of apparent intercellular invasion of rhizobia. These sections are from the same young nodule shown in Figs. 10–12. Fig. 13. Light micrograph of a section of the young nodule, showing the callus-like appearance of the subdivided cortical cells (*scc*). Single arrows mark areas where intercellular rhizobia were observed in subsequent electron micrographs. The rhizobia apparently gained access to the cortex by entering at the base of the large multicellular root hair (*mcrh*) from outside the root (star). One of the cortical cells adjacent to the intercellular rhizobia is in the process of dividing (double arrow). ×1,500. Fig. 14. Electron micrograph corresponding to a portion of Fig. 13, showing the same mitotic cortical cell. Accumulations of loosely fibrillar wall material (single large arrow) suggestive of wall loosening and electron-dense material (double large arrow) are present between cortical cells exposed to outside space (star). Rhizobia (*r*) have invaded further into the cortex along the same wall. The walls of the young cortical cells are very thin (single small arrows), which may facilitate rhizobial invasion. Note the distortions in the wall (double small arrows) in the cortical cell at the base of the multicellular root hair. ×5,400. Fig. 15. Electron micrograph of another section of the area at the base of the multicellular root hair shown in Figs. 13 and 14. Loosely fibrillar wall material (single large arrows) continuous with that shown in Fig. 14 is associated with intercellular rhizobia (*r*). Another rhizobium is seen at the base of the multicellular root hair and is associated with much cortical cell wall distortion and accumulation of electron-dense material (double arrows). Outside space is marked by a star. ×8,300. Fig. 16. Enlarged view of an adjacent section of the intercellular rhizobia seen in Fig. 14. The bacteria may be located anywhere in the host cell wall (*hcw*) and sometimes appear to be in direct contact with host plasmalemma (small arrow). Note the accumulations of loosely fibrillar material (large arrows) associated with the presence of the rhizobium, which is not surrounded by a matrix. ×10,600



Figs. 17–19. Electron micrographs of sections showing large intercellular proliferations of rhizobia. Figs. 17 and 18. Two sections of the same area a few cell layers into the cortex of the nodule in Figs. 10 and 13, showing extensive intercellular proliferation of rhizobia (*r*), which are often surrounded by a matrix (*m*), and are associated with large accumulations of electron-dense material (double large arrows). Nearby host cells are stimulated to divide, resulting in packets of cytoplasmically-rich subdivided cortical cells (*scc*) with very thin new cell walls (single small arrows), which appear to be susceptible to much loosening and distortion (double small arrows) in the presence of rhizobia. In two places, the rhizobia appear to be penetrating host cells in wide infection threads (single large arrows). × 5,600. Fig. 19. Section through another area of intercellular rhizobial proliferation (single arrows), showing a packet of subdivided cortical cells (*scc*) surrounded by rhizobia and accumulations of electron-dense material (double arrows). One cell in contact with intercellular rhizobia has very diffuse cytoplasm with many abnormal organelles in contrast with adjacent cells and appears to be degenerating (*dc*). × 5,400



Figs. 20–23. Electron micrographs of intercellular rhizobia and infection threads observed a few cell layers into the cortex of the nodule shown in Figs. 10 and 13. The rhizobia are surrounded by an electron-translucent zone but no matrix. Fig. 20. A single-file strand of intercellular rhizobia (*r*) located in the host cell wall (*hcw*) and appearing to be in direct contact with the host plasmalemma (double arrows). The host cell wall is thickened where it surrounds the rhizobia but retains a finely fibrillar appearance (single arrows). × 11,390. Fig. 21. Another strand of intercellular rhizobia (*r*) appearing in places to be in direct contact with host plasmalemma (double small arrows). A small amount of electron-dense material (single small arrows) is present in the host cell wall (*hcw*). Some evidence of wall loosening is present (large arrow). × 9,400. Fig. 22. Host cell infection by rhizobium (*r*). Rhizobia penetrate the cell from the host cell wall (*hcw*) and are surrounded by wall material (single arrows) and a membrane continuous with the host plasmalemma (double arrows). The wall material surrounding the entering rhizobium stains slightly darker and appears to be more loosely arranged than the host cell wall, which suggests it may be a newly manufactured thread wall. The infection thread seen in cross-section (*it*) is probably continuous with the one seen entering the host cell. There appear to be no deleterious effects on the cytoplasm. × 20,000. Fig. 23. Infection thread (*it*) in a host cell. The rhizobia (*r*) are in single file and continue to be surrounded by thread wall material (single arrow) and a membrane (double arrows). × 13,400

walls of the epidermis, perhaps even causing a break in the surface and the development of an access point. Some rhizobial strains are known to produce auxins (BADENOCH-JONES *et al.* 1982, WANG *et al.* 1982) and cytokinins (*cf.*, review by DART 1977) *in vitro*. In one study, high levels of cytokinins were detected in the culture medium when both *Rhizobium* and roots of French bean plants were present (PUPPO and RIGAUD 1978). It is possible that an accumulation of rhizobia in the rhizosphere (*e.g.*, Fig. 5) could produce an external hormonal stimulus of sufficient concentration to initiate localized cell divisions in roots of *Parasponia*. The formation of MCRH in *Parasponia* as a response to inoculation with *Rhizobium* has not been reported previously and seems to be unique among *Rhizobium*-root associations. POPHAM and HENRY (1955) described MCRH formation on aerial adventitious roots of *Kalanchoe* unrelated to microbial associations, but MCRH were not observed on subterranean roots. CHANDLER (1978) mentioned that some root hairs of *Arachis* are septate, but this observation was not in relation to inoculation or infection. BECKING (1977) illustrated bicellular root hairs on uninoculated *Alnus* roots. In *Parasponia*, MCRH were never observed on uninoculated plants. The presence of MCRH is clearly a response to inoculation with rhizobia, but the exact course of events leading to their production is not known.

Once in the intercellular space of the epidermis, the rhizobia appear to have multiplied and formed a surrounding matrix. There is evidence of cell wall loosening in this phase, presumably as a result of rhizobial production of cell wall hydrolyzing enzymes (HUBBELL *et al.* 1978, MARTINEZ-MOLINA *et al.* 1979, CALLAHAM and TORREY 1981). The cell wall disruption probably then facilitated further intercellular invasion into the cortex. Cells adjacent to intercellular rhizobial populations were stimulated to divide and enlarge and divide again, forming a callus-like proliferation which further stressed the cell-to-cell association in the root cortex and peripheral cell layers. This created more access points for rhizobial invasion from the outside as well as facilitating intercellular penetration deeper into the cortex. This mode of intercellular invasion would then be somewhat comparable to that in *Arachis* (CHANDLER 1978) and *Stylosanthes* (CHANDLER *et al.* 1982), where emerging lateral roots presumably cause a suitable disruption of the epidermis, allowing rhizobial entry via intercellular space.

The second major event following intercellular invasion is the actual infection *per se* of cortical cells by rhizobia which achieve host cell wall penetration accompanied by thread formation. This stage in the process of infection is most closely analogous to rhizobial penetration at the site of root hair tip curl in infections such as seen in *Trifolium* (CALLAHAM and TORREY 1981). At this stage the host cell accommodates to the rhizobial entry, forming a thread wall around the proliferating rhizobia essentially continuous with the perforated wall. Thread formation appears to cause displacement but relatively little disruption of the cytoplasm.

In *Arachis* (CHANDLER 1978) and *Stylosanthes* (CHANDLER *et al.* 1982) rhizobia gain access to the cortical cells via intercellular invasion and then appear to infect cells by localized cell wall degradation. Rhizobia then enter the host cytoplasm and are enclosed in individual membranes of host origin; further dissemination in the host tissue is by host cell division. Infection threads have not been seen. The situation in *Parasponia* is quite different in that infection of host cells is via continuing infection thread formation, even though the initial invasions of the cortex involve intercellular invasion by freely proliferating rhizobia.

The other type of bacterial-host interaction occurring in the invaded cortex of *Parasponia*, *i.e.*, the large proliferations of intercellular rhizobia and the associated damaging effects on the host cells, may represent a less balanced stage in the evolution of this unusual symbiosis. The deleterious effects on host cell cytoplasm occasionally observed suggests that the invasion and infection process here may be out of control, and the host is responding as it would to a pathogen. It is interesting that both the orderly invasion of intercellular space and the more pathogenic type of interaction can occur in relatively close proximity, and it is possible that the former may lead to the latter if a delicate balance is not maintained. The infection of cells by rhizobia and formation of true infection threads with cooperation from the host most probably represent the beginning of the truly symbiotic relationship in these nodules.

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