III. OBSERVATIONS

1. Structure of Root

The root consists of the following tissue zones; rhizodermis; hypodermis; a cortex consisting of a single layer of exodermis, a broad zone of parenchymatous tissue and an endodermis; and the stele (Fig.4).

Rhizodermis

Although conventionally called epidermis, the term rhizodermis is preferred here in view of the fact that it is not a typical epidermis. The rhizodermal cells are very small and a number of them produce elongated root hairs (Fig.4). The cells of the root hair have fairly thick cell wall especially in the root hair region. Their wall is composed of cellulose (Fig.5), pectic polysaccharides (flame red colour with Acridine orange and light magenta colour with Toluidine blue O) (Figs.6&7), and structural proteins (as detected by Acid fuchsin, Coomassie Brilliant Blue, Mercuric Bromophenol Blue, Fast green FCF) (Fig.8). The cell wall of the other rhizodermal cells are also positive for cellulose, pectic polysaccharides (non-esterified pectins) and structural proteins (the last less intense than in root hair cells). In addition, these cell walls also have phenolic constituents as judged by green colour with Azure B
and Toluidine blue O. The cell walls also contain neutral lipids (Sudan III positive) and free aldehydes (Schiff positive).

Hypodermis

Hypodermis consists of a single layer of small isodiametric cells almost of the size similar to that of epidermal cells. Few of the hypodermal cells, which lie opposite to exodermal passage cells, may also elongate into root hairs. The cell wall of hypodermis shows the same chemical constituents as that of ordinary rhizodermal cells; in addition, a suberized internal layer is often present in the cells (as judged by Sudan III positivity). The cytoplasm of these cells may also have polyphenolics as judged by positivity to Nitrous acid test.

Exodermis

This is considered to be the outermost layer of the cortex with regularly arranged cells (Figs. 5&6). It consist of many thick walled dead and elongated cells and a few thin walled living isodiametric passage cells (Figs. 7,9,10). In all cells, the outer tangential and radial walls are usually thicker than the inner tangential walls (Figs.11&12), although much more impressively in the dead cells. The walls of the dead cells have the following chemical constituents:
Lignin (as judged by positivity to Phloroglucinol, Azure B-green colour, Toluidine blue O-blue-green colour, Schiff's reaction, Maule's reaction and yellow autofluorescence) (Figs. 13-16), phenoloics, lipoidal material (predominantly acidic lipids), cellulose (Fig. 17), pectic polysaccharides (non-esterified pectins) and suberin (Fig. 18). The last substance was detected as an inner layer below the outer lignified wall not only through Sudan III dye but also by the yellow green autofluorescence. A number of exodermal cells also have thick bands of thickenings on the tangential walls (Fig. 15). The passage cells are fairly thin walled with a lining layer of cytoplasm and a DAPI positive nucleus in each cell. These passage cells are located immediately below the root hair cells of the rhizodermis/hypodermis.

Cortex Proper

The cortex proper consists of seven to thirteen layers of cells, the majority of which are parenchymatous, and living with a few idioblastic dead cells distributed here and there. The cells of the cortex are loosely arranged, circular to oval in transectional outline and with large triangular intercellular spaces. The cell walls of living cortical cells have the following chemical components; cellulose (positivity to Chlorazol black E, Potassium iodide
H₂SO₄ and Celluflour), pectic polysacharides (especially non-esterified pectins) prominently abundant in the cell corners (flame colour with Acridine orange, bluish green colour with Alcian blue and majenta colour with Toluidine blue O) (Fig.19), Calcium pectate (positive to Tannic acid - Ferric chloride), basic structural proteins (positive to Fast green FCF and Acid fuchsin) and neutral lipids (red with Nile Blue Sulphate). All the cells have a lining layer of cytoplasm and a prominent but small nucleus. A few of the cortical cells also show raphide bundles.

The most striking components of root cortex are the non living, thick walled and elongated idioblastic cells (Figs.20-28). They are present as strands of 4-10 cells in height; these strands are isolated and discontinuous extending from the layer just below the exodermis and upto the layer below the endodermis. These idioblasts constitute about 2-3% of the total number of cells of the cortex. The walls of these cells are very thick with distinct lamellae (Figs.21&22); they also show a number of slit like apertures (pits) (Fig. 23). They contain a large number of acidic lipids (probably phospho-lipids) [Sudan Black B positive (Fig.25) and dark blue with Nile Blue Sulphate (Fig.24)], high content of total proteins (Coomassie Brilliant Blue, Mercuric Bromophenol Blue, Acid fuchsin positive) (Fig.22), some amount of lignin (mild positivity to Phloroglucinol,
green colour with Azure B and Toluidine blue O, autofluorescence to an yellow colour, and brown colour with Maule's test indicating richness in syringyl-guaiacyl type of lignin) (Figs.20,21,26&27), phenolics, pectic polysaccharides (positivity to Alcian blue, majenta colour with Toluidine blue O, flame red with Acridine orange) probably of the esterified type (Krajcinović amine positive) (Fig.28), and cellulose (positive to Celluloflor, IKI-H₂SO₄ and Chlorazol black E).

Endodermis

It is the innermost layer of the cortex. It is predominantly a single layer excepting in regions opposite to protoxylem poles where it may be two or very rarely three layered (Figs.29 - 43). The endodermis has got two types of cells: one type highly vacuolated and thick walled and other densely cytoplasmic and thin walled. The former are typical endodermal cells and the latter are passage cells located opposite to protoxylem poles. The thick walled endodermal cells have cellulose, some amount of lignin, esterified pectic polysaccharides, phenolics, structural proteins and acidic lipids. However, casparian strips could not be detected. The thin walled passage cells are poor in cellulose, rich in structural proteins (predominantly of the basic type) and acidic polysaccharides; and with no phenolics
or lipids; their cytoplasm contains Sudan Black B positive lipids, free aldehydes, total proteins, phospholipids and acidic polysaccharides.

**Stele**

The stele consists of a central pith of loosely arranged parenchymatous cells leaving large intracellular spaces. The cell walls of these cells have cellulose and pectic poly-saccharides (Figs.31&32), while their cytoplasm is rich in neutral lipids (Fig.33). The xylem is polyarch (Fig.34). Depending on the root, 12-18 arches of xylem may be present. The protoxylem is exarch. Each xylem group has a large metaxylem and 2-3 smaller protoxylem vessels. The walls of xylem elements are lignified (Figs.35-37) and in addition contain cellulose, non-esterified pectins and some phenolics. The lignin present is predominantly of the syringyl guaiacyl type. There are as many phloem groups as there are xylem groups and they alternate with these xylem groups. The phloem cells are living with their cytoplasm rich in neutral lipids, total proteins, RNA and acidic polysaccharides. The cell walls of phloem cells do not have lignin but have cellulose, pectic polysaccharides and some quantity of basic structural proteins (Figs.39&40).

Both xylem and phloem groups are embedded in a sclerenchymatous conjunctive tissue, of which the
peripherally disposed form the pericycle (Figs.38, 41&42). The cell walls of these cells have basic structural proteins, lignin, phenolics, non-esterified pectins (Fig.43) and cellulose.

2. Entry and Spread of the Fungus in the Host Root

All the roots of the orchid become mycorrhizal. As a result of infection the roots do not change appreciably in colour but become irregularly bulged here and there (Fig.3). The bulge corresponds to the sectors of root getting infected.

Fungal mycelia are always abundantly present not only on the surface of the root but also in the adjacent rhizosphere. Throughout the three years of the present study, irrespective of seasons, mycelia were noticed in these two locations and mycorrhizae were seen during all periods. In other words, there was no seasonal periodicity in the incidence of mycorrhizal infection. In addition to fungal mycelia, sclerotia and chlamydospores are also present on the surface of the root and in the adjacent rhizosphere. It has been observed that entry into the host root may be through hyphae of the free mycelium or through hyphae produced by germination of sclerotia or chlamydospores, either through intact root hairs or through partly dehisced root hairs (Figs.44 - 47).
Entry into the root is always through root hairs, with not even a single instance of direct entry through epidermis. The entry into root hairs may be commonly at their tips, often slightly away from the tip or rarely at the base of the root hair. Invariably, only a single hypha enters into each root hair but occasionally two or rarely more may find entry into a single hair. After entry of the fungal hypha, the root hairs usually lose their straight cylindrical nature and become crooked and distorted to various degrees. This distortion may be limited to the tip of the hair alone or may be seen extended variously towards the base of the hair depending upon the locus of entry of the hypha. At the point of the entry, no special vesicular or appressorial or swollen structure could be seen in the hypha. Probably an enzymatic dissolution of the root hair wall is made by the fungus to facilitate its entry.

The hyphae, on entering into root hair, grows up to the base of the hair intracellularly from where they traverse again intracellularly through the hypodermis. Entry into the cortex is always through the passage cells of the exodermis and no instance of fungal mycelium in the thick walled cells of exodermis could be noticed (Fig.48). Entry into passage cells is through the outer tangential walls of these cells. From this location, the hypha finds entry into the cortex proper again intracellularly (Fig.49), branches
repeatedly and some of these branches get into the cortical parenchyma cells (for forming) pelotons. More often, the first entered hyphae enter deep into the root cortex and consequently the first pelotons could be observed in the interior cortex. However, subsequent hyphae may settle down gradually in the middle and outer cortical regions. Cells of root hair, hypodermis and passage cells often show only hyphal filaments and no instance of hyphal clumps or pelotons. In otherwords, the capacity to form pelotons is restricted only to the cortical parenchyma cells. Hyphae were never seen to enter the endodermis or stele, although pelotons could be observed even in the innermost cortical layer.

Infection may range from the occupation of the cortex by the fungus all around the perimetry of the root or may be restricted to certain sectors of the cortex. A detailed study of the percentage of infection made on 100 randomly selected roots all through the year, revealed a minimum of 37.5% and a maximum of 100% with a mean of $65.824\pm16.19$. In otherwords, the percentage of infected cells was fairly high, in this species.

3. Pelotons

Pelotons are loosely arranged fungal mycelial networks inside the cortical parenchyma cells (Fig.50). In cross
sections of root, these appear as spherical balls of mycelia (Figs.51 & 52). Pelotons are always formed in the cortical parenchyma cells of the root (Fig.5). Very rarely peloton like structures are seen in the dead cortical idioblasts (Fig.53). These idioblasts were often living cells at the time of fungal infection but subsequently become dead. A careful examination of infected root cortex reveals that two types of hyphae are often present in the parenchymatous cells, one forming pelotons and the other not participating in peloton organization. These non-pelotonic hyphae often remain closely adpressed to the cell wall of the host cell (Fig. 54). However, these hyphae are connected to the pelotonic hyphae by very short, narrow bridge-like hyphae whose number may be one to a few in each host cell. In addition to location in the host cell and participation in peloton organization, these two hyphae also differ from one another in several other respects as detailed on page 21.

Initially, the individual hyphae that make up the pelotons can be differentiated easily from one another; also the peloton almost completely fills up the entire interior of the cortical cells (Figs.55 & 56). However, subsequently it gets contracted with the loss of recognition of its individual hyphae leading to a decrease in size and finally to an almost total digestion of it by the host cell (Fig.24). Generally, fresh pelotons are noticed in the outer
cortical region and the older ones towards the inner cortex but this distributional specificity is often lost subsequently leading to a situation where cortical cells irrespective of their location may contain fresh or old pelotons.

The hyphae of the fresh pelotons are fully filled with cytoplasm and with very little vacuolation. With age, the cytoplasm becomes gradually restricted to a lining layer inside the cell wall and with increased vacuolation. Two nuclei could be distinctly seen in each hyphal cell. They get stained to a bluish colour with Nile Blue Sulphate indicating that they are rich in nucleophosphates. They are also very prominently stained with Fast green FCF indicating their richness in histones, and with DAPI indicating their good DNA content. The cytoplasm is rich in RNA, total insoluble polysaccharides, free aldehydes, basic proteins, moderate amount of total proteins, high content of carboxylated acidic polysaccharides and fairly good amount of lipids and predominantly of neutral lipids. It has a moderate activity of acid phosphatase and ATP-ase and a moderate amount of total proteins and polyphenols; proteins rich in tyrosine, tryptophan and phenylalanine could not be
detected in the cytoplasm (Fig.76). A fairly high activity of malate dehydrogenase (Fig.77), succinate dehydrogenase (Fig.78), starch phosphorylase (Fig.79), glucose-6-phosphatase (Fig.80) and β-glucosidase (Fig.81) could be detected in the cytoplasm of young pelotonic hyphae.

The pelotonic hyphal walls have chitin (not illustrated), good amount of acidic polysaccharides (Fig.50) and cell wall proteins (Figs. 68-73). Although they stain intensely with Chlorazol black E (Fig.82) and Cellufluor (Fig.5), the cell wall may not contain much cellulose since these two tests are not exclusively specific to cellulose and since the very specific IKI-H2SO4 treatment did not produce good colouration (Fig.83). The non-cellulosic polysaccharides detected in cell wall include both esterified and non-esterified pectins (Fig.84-86), whose proportion may vary with the age of the pelotonic hyphae (see page 21). The fresh pelotonic hyphae also lack phenolics in their cell walls.

The pelotonic hyphae soon undergo the process of digestion by the host cell. During this process, as already stated, the pelotons become reduced in transectional size, with loss of identity of the individual hyphae. During the initiation of digestion process, the distinction between pelotonic and non-pelotonic hyphae of each host cell becomes
sharper. The changes described above for pelotonic hyphae are not noticed in the non-pelotonic hyphae. In some cortical cells where there is no initial distinction at the time of initiation of digestion a peripheral part of each peloton gets segregated from the rest of the ball and positions itself close to the host cell wall (Figs. 87-89). This hyphal portion suddenly develops the accumulation of phenolics in its cell wall thereby probably becoming immune from digestion. The absence of phenolics in the cell walls of the pelotonic hyphae has already been indicated. Moreover a strikingly prominent sheet of material gets slowly invested around the pelotonic hyphae entering into digestion (Fig. 90). Initially this sheet of material is formed in patches all around the periphery of the pelotons (Fig. 91) but soon becomes a complete layer (Fig. 92). This material gives a very strong positive reaction to callose, a 1,3-β-glucan. The presence of callose could be categorically demonstrated by not only the Lacmoid blue dye (Figs. 91 & 92), but also through Aniline blue staining (Fig. 93) and fluorescence (not illustrated). In addition, this layer also fluoresces with Cellufluor since callose also is known to fluoresce with this chemical. In other words, the pelotonic hyphae undergoing degeneration are separated from the rest of the environment of host cell by the insulating and isolating callose layer.
During digestion, the peletonic hyphae lose their structural integrity. There is an increase in phospholipids during digestion, but during very late stages these could not be detected in pelotons but in the host cell (Figs.53,54, 64 & 65). In the cell walls, there is a greater degree of non-esterification of pectins during digestion (Figs.84&85). There is also an increase in total proteins (Figs.68&72), neutral lipids (Fig.94) and total insoluble polysaccharides (Fig.95). There is an increased activity of peroxidase (Figs.96&97). A gradual loss of chitin (not illustrated) and cellulose (Figs.82&83) of the hyphal cell walls was also noticed during digestion.

The non-pelotonic hyphae remain intact when the pelotonic hyphae undergo digestion. They remain poor in basic proteins (Fig.98), although may have enough of total proteins (Fig.99). These hyphae form the source material for the organisation of a fresh peloton in the same host cell (Figs.100-103). When the digestion process is going on in the original peloton, a fresh peloton is already being organised in the same host cell very rapidly (Figs.100-103). In some cells, it is so rapid that three generations of the pelotons could be seen in the same host cell (not illustrated).
4. Peloton-Containing Cortical Cells

The host cell undergoes an overall enlargement of about 3-5% over its initial size due to fungal infection and peloton formation. This is reflected in the slight bulging of the infected root (already drawn attention to). Although striking changes could not be detected in host cells consequent to peloton formation, there are a few noticeable events. The most important of these, is the change in the host cell nucleus. It continues to increase in size, some times up to thirty fold with a concomitant increase in DNA and histone content and becomes polytenic (Figs.104-109). It may remain spherical all through or may undergo distortions in shape. An instance of distinctly lobed nucleus is shown in figure 90. Its affinity for Trypan blue (Fig.110), Toluidine blue O (Fig.111), Azure B (Fig.14), DAPI (Fig.104), Methyl green (Fig.112), Fast green FCF (Fig.105), Auramine O (Fig.6), Orange G-Aniline blue (Fig.64), Mercuric Bromophenol Blue (Fig.106) and Coomassie Brilliant Blue (Fig.113) increases steadily.

The host cell cytoplasm is rich in acidic polysaccharides; there is a moderate amount of total proteins, total lipids and acidic lipids (phospho lipids); the last (one) increases strikingly during peloton digestion. Esterase activity could not be detected (not
illustrated), but ATP-ase was present moderately in comparison to the intense activity detected in cells that are awaiting fungal entry. The cytoplasm of the cells, during digestion (process), often shows a number of small red fluorescent particles as seen with differential interference contrast microscopy (Fig.114). The same particles revealed white fluorescence with DAPI (not illustrated), indicating probably their DNA nature. If so, these particles must be cytoplasmic DNA. These particles are especially concentrated near the cell walls of the host cell.

The host cell walls are rich in total insoluble polysaccharides, acidic polysaccharides and structural proteins (the last excepting in three cell junctions); a moderate amount of neutral lipids and phenols have also been detected in the cell wall. Intense activity of acid phosphatase was found in the three cell corners of the cell walls, although activity could also be detected to some extent in other regions. A similar trend of the activity is also shown by the enzyme peroxidase.

5. The Fungus

The mycorrhizal fungus associated with *Spathoglottis plicata* roots was an anamorphic species of *Rhizoctonia*. The fungus was never observed to produce basidia and basidiospores inside the root. Since the fungus has
binucleate hyphae, it has been tentatively placed under *Rhizoctonia repens* renamed by Moore (1987) as *Epulorhiza repens* whose sexual stage corresponds to *Tulasnella calospora*. The identification was based on Sneh et al. (1991). No attempt has been made here to describe the fungus as obtained in PDA cultures.

The present study has revealed that the path of the fungus into the host root is not an one way traffic. It has been repeatedly observed that non-pelotonic hyphae from the cortical region of host root often retraced their path and enter into the passage cells from where they traverse into those root hairs which have not been in the sources of primary infection. These hairs may be distinguished from those serving for primary entry by their straight and cylindrical morphology (Fig.115). These hyphae enter into such root hairs and start producing shorter hyphal cells (Fig.116). They may remain unbranched or often undergo branching (Figs.115-120); the two branched hyphae run parallel to one another in host root hair (Figs.117-120). One or both of these hyphal branches soon produce the so-called "chlamydospores" (Saksena and Vaartaja 1961). These chlamydospores are either terminally produced in these hyphae or are intercalary, may be produced singly or more commonly in chains resulting in beaded or moniliform appearance (Figs.121-130). These chlamydospores are
pyriform, oval, spherical, club shaped or cuneate, the terminal ones often showing a beak. In a number of instances a group of chlamydospores are formed so close to one another, that all of them together become sclerotia-like structures (Figs. 45 & 47). The hyphae producing the chlamydospores are rich in total proteins, RNA, carboxylated polysaccharides, phenolics and PAS positive granules. The cell walls are rich in acidic polysaccharides, structural proteins, phenolics and chitin. Each hypha has two nuclei which are very prominent and fairly rich in DNA content. All these hyphal characters are repeated in the chlamydospores produced by them.

Those root hairs in which chlamydospores are produced soon "dehisce" in a characteristic manner. The dehiscence takes place in a spiral fashion (Fig. 131), initiated at the tip of the hair and extending towards the base, (Figs. 132 & 133) releasing the spores/spore aggregates (Fig. 134). Depending upon the locus of the chlamydospore aggregates in the root hair, dehiscence may stop at any place from the tip of the root hair (Fig. 135). Root hairs destined to dehisce often show a slight spiral twisting of a hair with lines of dehiscence already evident as translucent areas in the cell walls. In those root hairs where the number of chlamydospores formed is one or a few, root hairs may not dehisce (Figs. 136 & 137). In such cases,
the chlamydospores so formed may remain intact but the hyphae bearing them are lost beyond recognition; these spores germinate to produce fresh infection hyphae (Figs.138&139), which re enter into the cortex to form fresh pelotons.
Fig. 1  *Spathoglottis plicata*, the study material in bloom. x 1

Fig. 2 Underground part of the plant enlarged to show bulbs and the root produced from them. Note that all the roots are of the same type. x 1

Fig. 3 Part of mycorrhizal root enlarged. It is irregularly swollen. x 10
Fig. 4  T.S. of root stained with Cellufluor and observed under UV light, to show the rhizodermis (with root hairs), hypodermis, exodermis, cortex and stele. x 56.

Fig. 5  Part of a T.S. of root stained with Cellufluor and observed under UV light. Note the regularly arranged layer of exodermal cells and the restriction of fungal pelotons to the cortex. The cell walls of all cells fluoresce to a blue colour indicating the presence of cellulose. x 130.

Figs. 6&7  T.S. of root stained with Auramine O - the pectic polysaccharides of cell walls of rhizodermal, hypodermal and cortical cells flame red in colour. The thick walled lignified cells of exodermis autofluoresce to an yellow colour. The passage cells of exodermis contain thin walls. Note fungal hyphae in the passage cells. Fig. 6. x 190, Fig. 7. x 475.
Fig. 8  T.S. of root stained with Coomasie Brilliant Blue. Note that the cell walls of root hairs and cortical cells including idioblasts have proteins (blue to violet colouration). x 325.

Figs. 9&10  L.S. of root and surface view of exodermal layer observed respectively after staining with Auramine O and Acridine Orange. The thick exodermal cells as well as the cortical idioblasts autofluoresce to an yellow colour indicating the presence of lignin in their walls. The passage cells are non-lignified and are isodiametric. Figs. 9&10. x 56.
Figs. 11-13 T.S. of roots respectively stained with DAPI, Schiff’s reagent and Phloroglucinol. In Fig. 11 all cell walls contain cellulose and in other 2 Figures the walls of thick walled exodermal cells, especially the outer tangential and radial, are lignified. Fig. 11. x 475, Fig. 12. x 150, Fig. 13. x 325.
Figs.14-17 T.S. of roots stained respectively with Azure B, Toluidine blue O, Schiff’s reagent and IKI-H₂SO₄. The cell walls of the exodermal cells are lignified (as indicated by greenish blue colouration in Figs.14&15 and brown colouration with Schiff’s reagent and IKI-H₂SO₄). They also show banded thickenings in Fig.15. In Fig.17 the cellulosic walls of cortical cells are stained to blue colour and the lignified walls of cortical idioblast to a brown colour. Fig.14&17. x 150, Fig.15. x 325, Fig.16. x 650.
Fig. 18  L.S. of the outer part of the root showing the features of exodermal cells. The dead cells of exodermis show cell walls with two layers outer fluorescing to an yellow colour indicating its lignin nature and inner thick layer a green fluorescence indicating its suberin nature. The preparation was stained with Auramine O. x 130.

Fig. 19  T.S. of root to indicate the presence of polysaccharides in the cortical cell walls as stained to a blue colour with Alcian blue. x 150.

Fig. 20  L.S. of root cortex stained with Toluidine blue O to show the cortical idioblasts. The walls stained to a blue colour indicating the presence of lignin. x 65.

Fig. 21  T.S. of portion of root cortex showing lignified idioblastic cells stained to a blue colour with Toluidine blue O. Note the lamellated nature of cell wall. x 325.
Fig. 22  T.S. of a portion of root cortex stained with Mercuric Bromophenol Blue. The idioblast cell has thick lamellated wall showing postivity to wall proteins (blue colour). x 650.

Fig. 23  L.S. of portion of root cortex stained with Auramine O and observed under fluorescence microscope. The cell wall of the idioblast shows numerous slit like openings (pits). x 475.

Fig. 24  T.S. of root cortex showing idioblast. The walls stain to a blue colour with Nile Blue Sulphate to indicate the presence of acidic lipids in them. x 325.
Fig.25-28 T.S. of portions of root cortex stained respectively with Sudan Black B, Maule's reagent, Azure B and Krajcinovic amine, thereby indicating the presence of lipids (Fig.25), lignin (syringyl-guaiacyl type) (Figs.26 & 27) and esterified pectins (Fig.28) in the cell walls of the idioblast. Figs.25-27. x 325, Fig.28. x 650.
Figs. 29-31 Portions of the stele stained respectively with Trypan blue, IKI-H2SO4 and Alcian blue. Note that the endodermis consists of 2 types of cell, one thick walled and arranged in a single layer and the other (passage cells) in irregular 2 layers opposite to the protoxylem poles. The passage cells also have strong cytoplasmic contents and are fairly thin walled. In Fig. 31. the phloem cells have cell walls positive to pectic polysaccharides. Fig. 29. x 325, Figs. 30&31. x 150.
Figs.32-35 T.S. of roots to show features of the stele. The cell walls of endothelial cells as well as the stelar cells have non-esterified pectins as indicated by red colour with Ruthenium Red (Fig. 32). Fig.33 illustrates a root T.S. stained with Nile Blue Sulphate. Note the presence of neutral lipids (red colour) in the cytoplasm of pith, phloem, and endodermal passage cells. The blue colour in the other endodermal cells and the thick walled cells of the stele indicates the presence of acidic lipids in their walls. Fig.34 illustrates the polyarch nature of root xylem, all lignified cells autofluoresce to an yellow colour in this preparation as well as in the preparation shown in Figs.32&33. x 150, Figs.34&35. x 130.
Figs.36-39 T.S. of roots to illustrate features of the stele. The lignified cells of endodermis (other than passage cells), pericycle, xylem and conjunctive tissue are illustrated in these preparations. The pith as well as phloem cells are not lignified. In Fig. 39, the cell walls of all cells show the presence of basic proteins. Fig.36 root stained with Maule's reagent, Fig.37 with Phloroglucinol-HCl. Fig. 38. Autofluorescence and Fig.39. Fast green FCF. Figs. 36,37&39. x 150, Fig.38. x 475.
Figs. 40-43 Preparations of root transfer sections. Fig. 40. Stained with Coomassie Brilliant Blue. Note the protein rich cytoplasm of endodermal passage cells and phloem cells and the protein rich cell walls of other endodermal cells and peripheral pith cells. In Fig. 41 shows a transection of root observed under fluorescence microscope, under Differential Interference contrast, lignified walls autofluoresce. Fig. 42. T.S of root stained with Rhodamine B lignified cells autofluoresce while cells rich in pectic polysaccharides stained to a flame red colour (phloem, hypodermis and epidermis). Fig. 43. illustrates T.S. of root stained with Krajcinovic amine reaction - the cells of the stelar region (other than phloem) have esterified pectins in this cell walls. Fig. 40. x 325, Figs. 41&42. x 130, Fig. 43. x 150.
Figs. 44-47 Infection of host-root through root hairs by the mycorrhizal fungus. Hyphae of the fungus are produced from sclerotia as shown in Fig. 47 (or) from mycelia at the root surface. Note the disfiguration of root hairs of the entry of fungal hyphae partially. Fig. 44. x 150, Fig. 45. x 650, Figs. 46 & 47. x 325.
Figs. 48-50 Fungi in the root cortex. In Figs. 48 and 49 fungal hyphae are seen in the passage cells of exodermis from where they are seen entering into cortex proper intracellularly. Fig. 50. L.S. of root cortex showing the formation of fungal coils or pelotons in the root cortical cells. Note that the hyphae are rich in acidic polysaccharides as indicated by red colouration with Toluidine blue O. Fig. 48. x 325, Figs. 49 & 50. x 650.
Figs. 51-54 Fungal pelotons. Fig. 51. T.S. of root cortex stained with Acridine Orange showing loosely arranged hyphae of fresh pelotons with increasing compactness in older pelotons. Fig. 52 a cortical cell showing loosely arranged hyphae of fungal peloton stained with Toluidine Blue O. The red colouration indicates their richness in acidic polysaccharides. Fig. 53. T.S. of root cortex showing fungal pelotons. Occasionally pelotons may be seen in the idioblastic cells of cortex (arrow). Fig. 54. T.S. of root cortex stained with Nile Blue Sulphate. Note the distinction of hyphae into pelotonic (single arrow) and non-pelotonic (double arrow) categories. Note the richness of these two hyphae in lipids (red) Note also the cross connections between these two types of hyphae. Fig. 51. x 190, Figs. 52 & 54. x 650, Fig. 53. x 150.
Figs. 55-58 Fungal pelotons. Fig. 55. Root cortex stained with Acridine Orange. The pelotons almost completely fill up the cell interior. Fig. 56. A peloton stained with Acid fuchsin, for the demonstration of the presence of total proteins in the hyphae. Fig. 57. Pelotons stained with Methyl green-Pyronin. The strong violetish red colouration of the hyphae indicate the richness of these hyphae in RNA. Fig. 58. Root cortex T.S. showing pelotons rich in total insoluble polysaccharides as indicated by Periodic acid–Schiff staining. Fig. 55. x 190, Fig. 56. x 650, Figs. 57&58. x 150.
Figs. 59–62 Fungal pelotons. The pelotons are respectively rich in free aldehydes (Schiff's positivity, Fig. 59), basic proteins (positivity to Fast green FCF, Fig. 60), acidic polysaccharides (positivity to Alcian blue, Fig. 61) and total lipids (positivity to Sudan III, Fig. 62). Figs. 59, 60 & 62. x 150, Fig. 61. x 325.
Figs. 59-62 Fungal pelotons. The pelotons are respectively rich in free aldehydes (Schiff's positivity, Fig. 59), basic proteins (positivity to Fast green FCF, Fig. 60), acidic polysaccharides (positivity to Alcian blue, Fig. 61) and total lipids (positivity to Sudan III, Fig. 62). Figs. 59, 60 & 62. x 150, Fig. 61. x 325.
Figs. 63-66 Fungal pelotons. Fig. 63. Pelotons stained with Sudan Black B to indicate the presence of total lipids in the hyphal cytoplasm. Figs. 64 & 65. Pelotons stained Orange G-Aniline blue to show that fresh pelotons have predominantly neutral lipids (brownish yellow colouration) while during digestion of pelotons these are lost with an increase in acidic lipids like phospholipids (blue colour). Fig. 66. Pelotons stained for acid phosphatase activity. Fresh pelotons have milder activity than those subjected to digestion. Fig. 63. x 650, Figs. 64-66. x 150.
Figs. 67-70 Fungal pelotons. Fig. 67. pelotons stained for Acid phosphatase activity. Note the moderate activity. Figs. 68-70. pelotons respectively stained with Chloramine-T, Mercuric Bromophenol Blue and Coomassie Brilliant Blue. Fresh pelotons are all positive to total proteins, both in cytoplasm and cell wall. Figs. 67, 68 & 70. x 150, Fig. 69. x 325.
Figs. 71-75 Fungal Pelotons. Figs. 71-73. Pelotons stained for total proteins with Amido Black (Fig. 71) and Acid fuchsin (Figs. 72 & 73). The pelotons are rarely rich in total proteins both in cytoplasm and cell wall. Figs. 74 & 75. respectively stained with Sulphanilic acid and Nitrous acid to indicate the moderate presence of polyphenols. Figs. 71, 72, 74 & 75. x 150, Fig. 73. x 650.
Figs. 76-79 Fungal Pelotons. Fig. 76. Pelotons subjected to Xanthoproteic reaction to indicate that proteins rich in Tyrosine, Tryptophan and Phenylalanine could not be detected in the pelotons. Figs. 77-79. Pelotons respectively stained for the activities of Malate dehydrogenase, Succinate dehydrogenase and Starch phosphorylase. Figs. 76-79. x 150.
Figs. 80-83 Fungal Pelotons. Figs. 80 & 81. Pelotons respectively stained for the activities of Glucose-6-Phosphatase and β-Glucosidase. Figs. 82 & 83. Pelotons stained respectively with Chlorazol black E and IKI-H₂SO₄. Fresh pelotons are stained intensively with Chlorazol black E, but with digestion the intensity is gradually decreased. With IKI-H₂SO₄ the pelotons are not coloured blue indicating the paucity of cellulose in the hyphal cell walls. Figs. 80-82. x 150, Fig. 83. x 325.
Figs. 84-87 Fungal Pelotons. Fig. 84. Pelotons stained with Ruthenium Red. Young pelotons are mildly positive for non-esterified pectins whereas older pelotons are increasingly positive for the same. Some cells (arrow) show both the primary and secondary pelotons. Fig. 85. Pelotons stained with Krajcinovic amine reaction. Fresh pelotons are fairly rich in esterified pectins but with digestion the amount of the same gets reduced. Fig. 86. Pelotons stained with Tannic acid-Ferric chloride to detect the degree of esterification of pectins in the pelotons. Fresh pelotons are esterified to great extent than older ones. Fig. 87. T.S. of a cortical cell showing the segregation of pelotonic (single arrow) and non-pelotonic (double arrows) hyphae. In one of the cells the peripheral hyphal part of the peloton under going digestion is differently stained from rest of the pelotons. This preparation was stained with Alcian blue. The large nucleus stained to a blue colour is also seen in one of the cells. Figs. 84-87. x 150.
Figs. 88–93 Successive fungal peloton formation. Fig. 88. T.S. of two cortical cells stained with Toluidine blue O. The peloton undergoing digestion is seen as a solid reddish mass in the centre of the cells while non-pelotonic hyphae which form the initials for a fresh pelotons are stained to greenish blue tinge indicating their richness in phenols. Fig. 89. T.S. of root cortex stained with Toluidine blue O. Fresh as well as older pelotons are seen. In one of the cell indicated by an arrow the remnants of old pelotons are seen as a reddish mass while the fresh peloton forming hyphae are greenish; the large host nucleus is stained to a bluish colour. Fig. 90. A peloton undergoing digestion is stained with Alcian blue. Note that the pelotons are surrounded by a layer of Alcian blue positive material. Note also the non-pelotonic hypha and a large host nucleus towards one side of peloton. Fig. 91. T.S. of cortical cell enclosing a peloton which is to undergo digestion - callose appears in patches (blue colour regions with Lacmoid staining) to insulate these peloton from the rest of the host cell cytoplasm. Note the abnormally large highly lobed host nucleus (arrow). Fig. 92. A peloton undergoing digestion separated from the rest of the host cell cytoplasm by a fully formed sheet of Lacmoid blue positive callose layer. Note also the large host cell nucleus. Fig. 93. T.S. of root cortex showing pelotons successively formed. Pelotons stained with Aniline blue. The older pelotons in the same host cells are brownish in colour and are separated from the fresh pelotons by a Aniline blue positive material. Figs. 88, 90, 91& 92 x 650, Fig. 89. x 325, Fig. 93. x 150.
Figs. 94-97 Fungal pelotons subjected to digestion. Fig. 94. Pelotons stained with Nile Blue Sulphate indicating the richness of acidic lipids during the digestion process. Fig. 95. A peloton undergoing digestion stained with Periodic acid-Schiff reagent showing high positivity to total insoluble polysaccharides. Note also the large host cell nucleus. Figs. 96 & 97 pelotons stained for the activity of Peroxidase during the activity. Figs. 94&95. x 650, Figs. 96&97. x 150.
Figs. 98-100 Figs. 98&99 Root cortical cells showing pelotons undergoing digestion and fresh non-pelotonic hyphae stained for total proteins. Fig. 100 T.S. of root cortex showing cells with both older and fresh pelotons. The non-organization of fresh pelotons. Figs. 98&99. x 650, Fig. 100. x 150.
Figs. 101-103 Successive formation of pelotons in the same host cell: Respectively stained with Toluidine blue O, Aniline blue and Toluidine blue O. Note in Figs 101 & 103 the older pelotons stained to a reddish colour and stained to a greenish tinge; the latter contain phenolics. Note also the prominent host cell nucleus in some of the cells. Fig.101. x 325, Fig.102. x 150, Fig.103. x 650.
Figs.104-107 Fig.104. T.S. of root hair stained with DAPI. Note that the host nucleus becomes increasingly larger in peloton containing cells. Fig.105 T.S. of root cortex showing highly enlarged host cell nucleus stained for nucleo proteins with Fast green FCF (arrow). Figs.106 & 107 host cells containing pelotons stained respectively by Mercuric Bromophenol Blue and Azure B to indicate the highly prominent host cell nuclei rich in total proteins and DNA respectively. Fig.104. x 130, Figs.105&107. x 650, Fig.106. x 325.
Fig.108-110 Host cells containing fungal pelotons showing the presence of highly enlarged and prominent nuclei, stained respectively with Toluidine blue O, Methyl green-Pyronin and Trypan blue. The first two figures indicates the richness of the nuclei in DNA. Fig.108. x 650, Fig.109. x 150, Fig.110. x 325.
Figs.111-113 Prominent and highly enlarged nuclei of host cell containing pelotons stained respectively with Toluidine blue O, Methyl green-Pyronin and Coomassie Brilliant Blue. Figs.111&113. x 325, Fig.112. x 150.
Figs. 114-119 Fig. 114. T.S. of root cortex examined under Differential Interference Contrast. Note the numerous small reddish colour fluorescent particles towards the cell wall in cortical cells containing pelotons. These particles are probably lipoidal materials. Figs. 115-119. Root hairs showing entry of fungal hyphae from within the host root. Fig. 114. x 190, Fig. 115. x 325, Figs. 116-119. x 650.
Figs. 120–127 Behaviour of fungal hyphae in root hairs leading to the formation of chlamydomospores.  
Figs. 120–127. x 650.
Figs. 128-130 Behaviour of fungal hyphae in root hairs leading to the formation of chlamydospores. Figs. 128-130. x 650.

Figs. 131&132 Root hairs dehiscing in a spiral fashion from tip downwards resulting in the discharge of chlamydospores into the rhizosphere. Figs. 131&132. x 325.
Figs.133-139  Fig.133: Root hair showing chlamydospires dehiscing spirally. Figs.134&135. Chains of chlamydospires in root hairs about to be released by them. Figs.136-139. Stages in the germination of chlamydospires in root hairs that have not dehisced to release the spores. Figs.133&139. x 325, Figs.134-138. x 650.