3

Observations
3.1 Studies on normal gum-resin producing tissue systems.

3.1.1 Light microscopic studies
Distribution, development and structure of gum-resin producing systems in *Vateria indica*

3.1.1.1 Distribution of ducts and cavities

In young stem, ducts are present only in the pith. They are distributed in a single row towards the periphery of the pith and opposite to the protoxylem of each vascular bundle (Fig. 1). An young stem has about 20-25 vascular bundles and an equal number of gum-resin ducts. The number of these ducts does not increase during the growth of the stem. The pith ducts are circular in outline, vertically elongated, continuous, parallel to the axis and do not anastamose with each other (Fig. 2). At each node a pith duct has its continuation in the leaf. One duct is associated with each leaf trace (Fig. 3).
When the stem has about 1 cm deep secondary xylem, the ducts appear in secondary xylem also (Fig. 4). They are distributed at random with occasional grouping of 3-4 ducts in a tangential row, among the axial parenchyma cells. The xylem ducts are mostly circular in outline, vertically elongated, parallel to the stem axis or traverse obliquely in tangential direction (Fig. 5). Adjacent ducts in a row may anastamose tangentially (Fig. 7), but a network of anastamosing ducts is not formed. The xylem ducts do not traverse the ray parenchyma (Fig. 6). When the duct comes across a multiseriate ray it traverses obliquely along the tangential side of the ray (Fig. 8).

In addition to the ducts, several large cavities are also occasionally observed in the secondary xylem (Fig. 9, 10). The structural differences between ducts and cavities are described under the title "Structure of ducts and cavities". The cavities are observed in 2-3 tangential rows in the late formed secondary xylem. The adjacent cavities of a row anastamose tangentially forming a network of cavities around the multiseriate rays (Fig. 11). The cavities do not traverse the ray cells and hence the multiseriate rays remain as intact islands amidst the cavities.

Tyloses are absent in the vessels of young branches, but they are frequent in an old stem after the formation of gum-resin ducts in the xylem (Fig. 12-14). Tyloses often contain phenolic substances and sometimes they are heavily loaded with them (Fig. 13, 14).
In petiole and mid-vein, several ducts are distributed in the ground tissue inner to the vascular system (Fig. 15, 16, 19). Each leaf has a duct in continuity with the pith duct and the other ducts develop independently in leaf. The ducts are continuous throughout the leaf and all ducts of the mid-vein unite at the base of the petiole (see Fig. 31, 32). The lateral vein has a single duct in the centre of the vascular bundle (Fig. 17), which is connected to a duct of the mid-vein. Thus, a continuity of duct system is established between the stem and the leaf.

In young roots ducts are absent. But after secondary growth, the ducts appear in secondary xylem (Fig. 18). Several ducts are distributed at random among the axial parenchyma cells of the secondary xylem. The ducts are circular in outline, vertically elongated and the adjacent ducts anastamose tangentially. In young roots tyloses are absent; but they are extensively developed in old roots after the formation of ducts (Fig. 18).

3.1.1.1.2 Schizogenous initiation of ducts

The ducts initiate schizogenously in the pith and leaf. Schizogenous initiation of pith ducts is studied ultrastructurally and is described in chapter 3.1.2.1.
3.1.1.1.3 Lysigenous initiation of ducts and cavities

The ducts and/or cavities in the secondary xylem of stem and root develop lysigenously from the axial parenchyma cells.

A few axial parenchyma cells divide transversely forming a group of thin walled initials with prominent nuclei (Fig. 21, 22). Lysis of these cells leads to the formation of a lumen (Fig. 23). Vertically the ducts elongate due to the development of more initials from the axial parenchyma cells and their lysis (Fig. 24, 25).

The cavities develop as the lysis of axial parenchyma cells continues vigorously in vertical and tangential directions. All axial parenchyma cells between the adjacent rays break down forming a network of tangentially anastomosing cavities around the intact rays (Fig. 11).

3.1.1.1.4 Structure of ducts and cavities

In a young stem just below the stem apex, the pith ducts are bordered by distinct epithelial cells with large nuclei and dense cytoplasm (Fig. 26). They are mostly isodiametric with convex inner tangential walls. In lower internodes some of the epithelial cells of the pith ducts undergo lysis followed by disappearance of nucleus and darkening of the cytoplasm (Fig. 27). The epithelial cells which were previously contiguous, separate at radial walls and subsequently they come to lie in the duct lumen.
The pith cells around the ducts undergo periclinal divisions forming a few layers of tangentially flattened sheath cells around the ducts (Fig. 29). When the epithelial cells disintegrate, the sheath cells do not appear to simulate the epithelial cells in morphology, and consequently the pith ducts of an old stem are without a distinct epithelium (Fig. 29).

The ducts in the secondary xylem of the stem and root have a sharp boundary of epithelium (Fig. 28). The epithelial cells are distinct from the neighbouring cells by their shape, size, thin walls and large nuclei. The cavities in the secondary xylem of the stem differ from the ducts in their irregular outline and absence of epithelium (compare Fig. 10 against Fig. 28). Due to extensive lysis of axial parenchyma cells, often the cavities occupy a wide area between the adjacent multiseriate rays.

In stem, often the epithelial cells proliferate into the duct and block the lumen (Fig. 30). Such structures are known as tylosoids which are characteristic of Diptercarpaceae (Chalk, 1983). The tylosoids differ from the tyloses in that they are proliferations of thin walled epithelial cells and do not pass through any pit cavity.
3.1.1.2 Distribution, development and structure of gum-resin producing systems in *Garcinia cambogia*

3.1.1.2.1 Distribution of ducts and cavities

In young stem, gum-resin ducts are present in the pith and cortex (Fig. 33). In old stem ducts are present in pith and secondary phloem, while ducts and cavities are present in the cortex (Fig. 34-37). The ducts are always vertical (Fig. 38), but cavities may be vertical or tangential (Fig. 35-37). The pith ducts do not anastamose, but at the leaf gaps they are continuous with those in the leaf (see Fig. 56). In secondary phloem the ducts are arranged in tangential rows and the adjacent ducts of a row anastamose with each other traversing around the phloem rays (Fig. 40). The cortical ducts are distributed irregularly and do not anastamose. At nodes the cortical ducts are in continuation with those of the leaves (Fig. 39). The cavities in the cortex are isolated and discontinuous. The type of gum-resin producing systems and their interrelations are graphically shown in figure 55. Figure 57 gives a diagrammatic representation of the distribution and inter-connections of the ducts and cavities in the old stem.

From inner secondary phloem to cortex, the ducts show gradual decrease in their number and increase in their cross sectional area (Table 3, Fig. 54). In the inner secondary phloem, the ducts have a comparable size, whereas, the ducts
and/or cavities show wide variation in outer secondary phloem and cortex (Table 3). The percentage of area occupied by duct and/or cavities in the three zones of the bark is shown in figure 53.

In leaf, ducts are present in the mid-vein and lamina. In the mid-vein several ducts are distributed in the ground tissue (Fig. 41). They form a continuous system with those of the pith and cortex of the stem (see Fig. 56). The ducts do not anastamose with each other in the mid-vein, but anastamosis occurs towards the base of the petiole. In lamina a single row of ducts is present placed between the palisade and spongy tissue (Fig. 42).

In root, ducts are present in the secondary phloem, and ducts and cavities are present in the cortex (Fig. 43, 44). In secondary phloem, the ducts are arranged in tangential rows and the adjacent ducts of a row anastamose (Fig. 45). In the cortex the ducts and cavities are distributed irregularly and do not anastamose.

3.1.1.2.2 Development of ducts

The ducts in the stem, leaf and root develop schizontogenously (Fig. 46-50). A group of initials distinguished from the neighbouring cells by their concentric arrangement and conical shape, separate from each other forming an intercalary space (Fig. 46, 47). Further separation of
these cells and their tangential flattening lead to the formation of a duct (Fig. 48-50). The cells lining the duct constitute the epithelium. As the duct enlarges more epithelial cells are derived by the anticlinal divisions of the existing ones.

3.1.1.2.3 Development of cavities

The cavities in the cortex of stem and root develop lysigenously from a group of initials formed from the cortical cells (Fig. 51). The initials are tangentially elongated and narrow with prominent nuclei and thin walls. They are filled with cytoplasm, whereas, the other cortical cells are loaded with polysaccharides and phenolics. Lysis of these cells leads to the formation of a cavity.

3.1.1.2.4 Structure of ducts and cavities

Ducts in all cases are tubular and vertically elongated with circular to oval outline in cross sectional view. They are always bordered by a distinct layer of epithelium. A developing duct has large epithelial cells with their inner tangential wall protruding into the lumen. But in old ducts the epithelial cells become narrow and small due to their anticlinal divisions and tangential flattening (Fig. 52). The cavities are also bordered by distinct epithelial cells. The distinction between the duct and the cavity is based upon their shape. Whilst the ducts form a narrow elongated
tubular system with more or less uniform diameter throughout their length, the cavities are often very large and lack a uniform shape.
Table 3. Showing the variation in number and cross sectional area of ducts and/or cavities from inner secondary phloem to cortex

<table>
<thead>
<tr>
<th>Region</th>
<th>No. of ducts/cavities in 0.5 mm² area</th>
<th>Cross sectional area of ducts/cavities (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner secondary phloem</td>
<td>3.52 1.112</td>
<td>53.78 17.973</td>
</tr>
<tr>
<td>Outer secondary phloem</td>
<td>3.08 1.110</td>
<td>112.78 54.804</td>
</tr>
<tr>
<td>Cortex</td>
<td>1.18 0.823</td>
<td>1116.87 1180.044</td>
</tr>
</tbody>
</table>
3.1.2 Electron microscopic studies
3.1.2.1 Ultrastructure of pith ducts in the stem of *Vateria indica*

3.1.2.1.1 Initiation of the ducts

In the pith, the ducts develop some distance below the shoot apex (see Fig. 20). A group of initials is distinct from the neighbouring cells by their dense cytoplasm and large nuclei (Fig. 58). The middle lamellae among some of the initials break down and the cells separate from each other forming an intercalary space (Fig. 58, 59). Further separation of the bordering cells along their radial walls and their subsequent reorientation lead to the formation of a duct (Fig. 60-63). The lining cells constitute the epithelium. Formation of more epithelial cells, their tangential flattening and lysis of some of them contribute to the widening of the lumen.

3.1.2.1.2 Ultrastructure of the ducts

The epithelial cells are distinct from other pith cells by their dense organelle-rich cytoplasm and large nuclei (Fig. 62-64). They are rich in mitochondria, and have few plastids and dictyosomes. Ribosomes and polysomes are profusely distributed. The epithelial cells have several scattered small vacuoles, whereas, the other pith cells have large vacuoles with a thin parietal cytoplasm (Fig. 63). Plasmodesmatal connections exist between adjacent epithelial
cells and between epithelial and sub-epithelial cells (Fig. 65). Resin component of the gum-resin represented as osmiophilic material in the cytoplasm is found aggregated on either side of the plasmodesmatal opening, indicating intercellular transport of resin.

Many epithelial cells show abundant osmiophilic material in their cytoplasm even during the early stages of duct development (Fig. 60, 61). In the samples studied, the osmiophilic material is not observed in association with any organelles of the epithelial cells. Small osmiophilic droplets arise in the cytoplasm with or without membrane envelopes (Fig. 66, 67), and their number and size gradually increase. Osmiophilic globules are also present in the vacuoles (Fig. 68, 69). Vesicles and multivesicular bodies with osmiophilic contents are found in the cytoplasm, and many of them are observed near the plasmalemma at the inner tangential wall (Fig. 70, 71). They are also observed together with osmiophilic globules in the extracytoplasmic space of the inner tangential wall (Fig. 72-74). Some of the vesicles in association with plasmalemma carry multilamellate structures (Fig. 74). Complex configuration of some vacuoles is observed near the inner tangential wall which seems to indicate the fusion of vacuole with plasmalemma (Fig. 69). Inside the vacuole the degenerating membranes are closely associated with osmiophilic material. Figure 69 suggests a probable deposition of osmiophilic material from the vacuole into the extracytoplasmic space.
The epithelial cells gradually gather large amount of osmiophilic material in the extracytoplasmic space at the inner tangential wall (Fig. 75-77). The osmiophilic material may be stored as several small globules (Fig. 68, 70) or as a single large globule (Fig. 75-77). Presence of mitochondria is usually observed towards this region of cytoplasm (Fig. 72, 74, 75, 77). Number of vesicular structures are also observed between the plasmalemma and inner tangential wall (Fig. 82). Several mitochondria are aggregated towards the wall where the vesicles are collected in the extracytoplasmic space.

During the duct development some epithelial cells undergo autolysis (Fig. 62). Autophagic vacuoles with membrane inclusions and organelles appear in the cytoplasm (Fig. 78, 79). Plastids become darker and degenerate, and the vacuolar system increases (Fig. 80). The cytoplasm becomes more electron dense and subsequently, the whole cell becomes 'dark' (Fig. 80, 81). Ultimately it is detached from the epithelium and lyses in the duct lumen (Fig. 62, 63). The secreted osmiophilic material collects at the periphery of the duct along with the remnants of the lysed cells (Fig. 82).
3.1.2.2 Ultrastructure of pith ducts in the stem of *Ailanthus excelsa*

Normal gum-resin ducts are present in the pith of stem. The epithelial cells surrounding the duct are densely stained, moderately vacuolated and rich in organelles, whereas, the adjacent pith cells are highly vacuolated with thin parietal cytoplasm (Fig. 83, 84). The nucleus of the epithelial cell is spherical to ameboid and has a prominent nucleolus and several conspicuous peripheral heterochromatic regions. The inner tangential wall of the epithelial cell (the wall facing duct lumen) has a loose microfibrillar structure and a sloughed off appearance (Fig. 85). No plasmodesmata are observed in the inner tangential wall, but they occur between epithelial and sub-epithelial cells (Fig. 86, 87).

The cytoplasm of epithelial cell is granular and it contains abundant free ribosomes and polysomes. Plastids, mitochondria, dictyosomes and smooth and rough endoplasmic reticulum (ER) are well represented (Fig. 88-90). Coalescing vacuoles and vesicles are also present (Fig. 91, 92).

Plastids are of various shapes with dense matrix and poorly developed internal membranes (Fig. 93-98). Some plastids are amyloplasts with one or more starch granules in their matrix (Fig. 96-98). Osmiophilic droplets (mostly representing resin component of the gum-resin) of varying electron density are observed in the matrix of plastids.
and amyloplasts (Fig. 93-95, 97, 98). Many osmiophilic droplets in the cytoplasm are in close proximity of the plastids (Fig. 96). Frequently, the rough ER is closely appressed to the envelope of the plastid containing osmiophilic material (Fig. 95).

Mitochondria in the epithelial cells have varying shapes and well developed cristae (Fig. 99-104). Elongated and what appears to be deformed mitochondria are common (Fig. 103, 104). Small osmiophilic droplets are found in the matrix (Fig. 99-101). Occasionally mitochondrial envelope shows close association with osmiophilic globule and ER (Fig. 101, 102).

Osmiophilic globules are also seen associated with ER (Fig. 105-109, 113, 133). The ER cisternae when in contact with osmiophilic globules are often dilated (Fig. 105, 106). The close association of ER to the globule may be at a single site (Fig. 106, 107), or the ER may partially or completely encircle the globule (Fig. 109). Numerous small and large vesicles are derived from ER cisternae, but they have no osmiophilic contents (Fig. 110). Concentric rings of ER cisternae are occasionally found in the epithelial cells (Fig. 111). Cytoplasm is enclosed in the ER rings, and ribosomes are found attached on either side of the ER ring. Sometimes the ER encloses an osmiophilic globule (Fig. 112). A complex configuration of ER associated with the osmiophilic
globule and plasmalemma is also observed (Fig. 113). Several ER cisternae are often oriented parallel to the cell wall near the plasmalemma (Fig. 86, 103, 104, 111, 118).

The dictyosomes appear very active showing close association with numerous vesicles which are apparently budded off from the maturing face of their cisternae (Fig. 114-118). Both smooth and coated vesicles are derived from the dictyosomes. No osmiophilic material is encountered in the cisternae or in the vesicles. Several dense vesicles are sometimes observed in the lumen of vacuoles in the vicinity of dictyosomes (Fig. 118).

Coalescence of several vacuoles, probably leading to their merger is consistently observed in the epithelial cells (Fig. 119, 121, 122). Degenerating membranes, myelin like structures and large osmiophilic globules are present in the vacuoles (Fig. 119-122). The degenerating membranes often are closely associated with osmiophilic material (Fig. 121). Occasionally, osmiophilic globules of similar electron density are found in the lumen of the vacuole and in the cytoplasm closely associated on either side of the tonoplast (Fig. 106).

Large osmiophilic globules of varying electron opacity are scattered in the cytoplasm. Several of them are often aggregated near the inner tangential wall (Fig. 123), and
many of them are found in contact with the plasmalemma (Fig. 125). Association of many vesicles with plasmalemma at the inner tangential wall is also encountered (Fig. 128, 129). The plasmalemma of the epithelial cell is highly invaginated at radial and inner tangential walls (Fig. 124). Many of such invaginations at the inner tangential wall contain smooth and coated vesicles, membraneous structures, myelin like bodies and/or osmiophilic globules.

Small papilllose ingrowths are present in the radial and inner tangential walls of the epithelial cells (Fig. 132-134). The wall ingrowths are enveloped by plasmalemma. The vesicles are associated with the plasmalemma at these wall ingrowths also. Some of the wall ingrowths contain osmiophilic material and electron transluscent areas in the matrix (Fig. 133, 134).

Several microtubules are vertically oriented near the plasmalemma in the epithelial cells (Fig. 135-138). They are not evenly distributed, but occur isolated or in groups.

Occasionally, the epithelial cells undergo lysis. The vacuolar space increases (Fig. 139), and autophagic vacuoles with cytoplasmic debris appear (Fig. 142). Many vacuoles show ruptured tonoplasts (Fig. 142). The mitochondria undergo degeneration followed by the disorganization of their cristae (Fig. 140-142). In more advanced stages of degeneration, the mitochondria have clear matrix with few
degenerated cristae (Fig. 142). The ER cisternae also become distorted. They appear as short distended profiles (Fig. 143, 144). The ER and dictyosome-derived vesicles become abundant in the cytoplasm (Fig. 143, 144). The cytoplasm appears more electron dense and the organelles disappear (Fig. 145). Subsequently the cell is detached and it disintegrates in the duct lumen.
3.2 Experimental studies
3.2.1 Ethephon induced gummosis in *Bombax ceiba*

The plants treated with ethephon started exudation after 10-12 days through the treated holes (Fig. 147). Exudation first started from the plants treated with dilute concentration of ethephon (see Table 4). There was no exudation from control plants. The effect of different concentrations of ethephon on the gum yield is shown in Table 4. Exudation through treated holes stopped after 7-10 days, but the plant yielded gum through any fresh hole or injury made on the stem near the treated region for several days. A fresh hole gives out immediate exudation of copious amount of gum as if the gum was stored in the plant body under some pressure.

The amount of gum exuded from successive levels away from the treated site is shown in figure 146. Even though there was minor variation in the amount of gum exuded from different plants, all the treated plants showed the same tendency - maximum exudation at treated site, less exudation above, and lesser exudation below the site of treatment. At the ethephon treated site there was marked difference in the amount of gum exuded from the treated side and opposite side of the trunk, but away from the treated site, the response to apparent lateral movement of ethephon was irregular (see Fig. 146). The holes made near the treated site gave immediate exudation, whereas, exudation was
delayed from holes made away from the treated site. The fresh gum is yellowish-brown and it gradually turns to red and later becomes opaque and dark brown when dried.

3.2.2 Ethephon induced gummosis in *Sterculia urens*

The ethephon treated plants started exudation 10-12 days after treatment. The effect of different concentrations of ethephon on gum yield is shown in Table 5. The control plants also showed exudation, but the quantity of gum was negligible when compared with that of the treated ones. The exudation through treated holes stopped after about 10 days, but a fresh hole made on the stem near the treated site gave immediate exudation. The effect of treatment was observed on all sides, laterally and vertically. Consequently, the control holes made on the opposite side of the treated plants also gave copious exudation. Vertically the effect of treatment was visible at a higher distance above the treated site than below it. A fresh hole made 100 cm above the treated site gave immediate exudation, whereas, there was no immediate exudation through a hole made 50 cm below the treated site.
Tables 4 and 5 showing the amount of gum collected from holes treated with different concentrations of ethephon and distilled water.

**Table 4. *Bombax ceiba***

<table>
<thead>
<tr>
<th>Active substance of ethephon in 2 ml of injected solution (mg)</th>
<th>0</th>
<th>240</th>
<th>480</th>
<th>720</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of gum exuded (gm)</td>
<td>0</td>
<td>282</td>
<td>378</td>
<td>9.2</td>
</tr>
</tbody>
</table>

**Table 5. *Sterculia urens***

<table>
<thead>
<tr>
<th>Active substance of ethephon in 2 ml of injected solution (mg)</th>
<th>0</th>
<th>96</th>
<th>192</th>
<th>480</th>
<th>768</th>
<th>960</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of gum exuded (gm)</td>
<td>0.5</td>
<td>1.2</td>
<td>2</td>
<td>14.5</td>
<td>64.5</td>
<td>38</td>
</tr>
</tbody>
</table>
3.3 Studies on induced gum/gum-resin producing tissue systems
3.3.1 Distribution, development and structure of traumatic gum cavities in the stem of Bombax ceiba

3.3.1.1 Normal structure of bark and wood

The bark of a normal tree has distinct periderm and well developed phelloderm. Secondary phloem is continuous and stratified into fibrous and non-fiberous tangential zones. Phloem rays are uni to multiseriate. A large number of small irregular cavities are distributed in the cortex and in the phloem rays. These cavities contain colorless slimy mucilage. Wood is soft and diffuse porous. Xylem parenchyma is abundant and paratracheal. Rays are heterogeneous, uni to multiseriate. There are no gum producing cavities or ducts in the secondary xylem. Tyloses are absent.

3.3.1.2 Structure of ethephon treated wood

There are no noticeable structural alterations in the bark upon ethephon treatment. Several layers of traumatic parenchyma cells are formed from the vascular cambium towards the secondary xylem after the treatment (Fig. 148). Some multiseriate rays remain discrete in the traumatic parenchyma, but others lose their identity because of repeated divisions.

After 8-10 days the cambium renews its normal function and the traumatic parenchyma gets embedded in the normal secondary xylem. A large number of vessels in the secondary
xylem on either side of the traumatic tissue are partially or completely filled with tyloses (Fig. 148).

The gum cavities are developed in the traumatic parenchyma. Groups of these cells at irregular intervals accumulate phenolics and PAS positive material and later disintegrate initiating a cavity (Fig. 149). The cells immediately surrounding the cavity divide tangentially and a few layers of cambium-like meristematic cells ( cambiform cells) are formed around the lumen. Further development of the cavity is very characteristic and can be well studied in tangential longitudinal sections.

Once a cavity is initiated, further extension of cavity through the lysis of traumatic parenchyma cells proceeds in a definite circular course (Fig. 150, 151). Consequently, a discrete circular group of intact tissue surrounded by a cavity is formed (see Fig. 160). The circular mass of tissue is surrounded by a few layers of cambiform cells. Layers of these cells increase as more such cells are derived by the periclinal divisions of parenchyma cells in the circle. These cells are characteristically arranged in radial rows and adjoin the intact mass of parenchyma cells in the centre. These cells towards the periphery of the circle undergo active periclinal divisions producing derivatives towards the cavity (Fig. 152). These derivatives enlarge, accumulate phenolics and polysaccharides, and disintegrate augmenting the quantity of gum.
In the traumatic parenchyma several such circular islands of complex tissues are formed, interspersed with the anastamosing cavities (Fig. 159). The cavities contain disintegrating cells and gum (Fig. 152-159). Development of cavities and the formation of the characteristic tissue pattern are shown in figure 160. Eventually, some of the circular islands of traumatic parenchyma also break down forming large cavities. The lysis starts from the central core of parenchyma cells and proceeds centripetally (Fig. 154-157).

A radial longitudinal section from the region of cavities shows distinct villi-like projections of traumatic parenchyma, directed towards the bark amidst the cavities (Fig. 158). The projections have uniform shape and cell arrangement. Their central cells are radially elongated and sometimes in continuity with the multisieriate rays of the secondary xylem. The peripheral cambiform cells are arranged in storied pattern. Towards the tip of the projections all the cells are isodiametric. A 3-dimensional representation of the structure of wood at the region of gum cavities, as seen fifteen days after ethephon treatment is shown in figure 161.
3.3.2 Distribution, development and structure of ethephon induced gum cavities in *Sterculia urens*

In *Sterculia urens*, the stem has normal gum ducts only in the pith and cortex. Gum ducts or cavities are normally absent in the wood (Fig. 162). Wood is characterized by diffuse or occasionally branded parenchyma, broad multiseriate rays and thick walled fibers. Administration of ethephon into the stem induced extensive development of gum cavities in the secondary xylem (Fig. 163-166). The cavities are developed from the axial parenchyma cells formed after ethephon treatment. Upon ethephon treatment the ray initials remain intact, but only axial parenchyma cells are formed from the fusiform initials. The cambium soon renews its normal function and consequently a band of traumatic tissue consisting of only axial and ray parenchyma cells is formed in the outer sapwood (Fig. 167). The axial parenchyma cells undergo active transverse divisions and the derivatives enlarge to form vertical files of isodiametric cells, the cavity initials (Fig. 168, 169). They are mostly thin walled, and have dense cytoplasm and large nuclei (Fig. 168). The cavity initially develops lysigenously from a group of such cells.

The lysis is triggered by the disintegration of a vertical file of cells (Fig. 170). The lysis is mostly preceded by darkening of cytoplasm and disappearance of
nuclei. The lysis of adjacent cells follows and a cavity is developed (Fig. 171, 172). A definite epithelium is not formed. Unlike Ailanthus excelsa and Bombax ceiba the cambium-like meristematic cells are also not formed surrounding the cavity. The lysis of more cells progresses in vertical and tangential directions (Fig. 171, 173). Tangential widening of a cavity is limited by multiseriate rays, which remain mostly intact. Almost all axial parenchyma cells undergo lysis forming a system of tangentially anastomosing cavities (Fig. 175). The cavity is filled with disintegrating cells and gummy substance which show positive staining with PAS. Amidst the anastomosing cavities, the islands of multiseriate rays remain intact (Fig. 175, 177). Nevertheless, at places of extensive cavity formation, some multiseriate rays also disintegrate (Fig. 179). But always ray cells are the last to be affected. In radial longitudinal sections, the cavities appear as vertically elongated system interrupted by multiseriate rays (Fig. 174, 178). A 3-dimensional representation of the cavities showing their distribution and anastomosis is shown in figure 180.

In ethephon treated plants, another tangential row of cavities are observed little inside the sapwood in the banded axial parenchyma (Fig. 176). They are similar to the outer rows of cavities in structure, development and anastomosis. A large number of vessels in the secondary xylem of the treated
plants were plugged with tyloses (Fig. 176). Tyloses were found in the control plants also, but comparatively very few vessels were affected.

3.3.3 Distribution, development and structure of traumatic gum-resin cavities in the stem of Ailanthus excelsa

In Ailanthus excelsa, the stem has normal gum-resin ducts only in the pith. Artificial injuries made on a normal healthy plant do not show immediate response in regard to gum-resin exudation or traumatic cavity formation. But the plants exude gum through old blazes, natural cracks or infected portions of the stem. A fresh injury made on the stem of such a plant gives out exudation immediately or after a few days.

Administration of ethephon into the stem of healthy plants, induced copious exudation of gum-resin after 15 days. There was no exudation from the control plants. Distribution, development and structure of traumatic gum-resin cavities are studied in ethephon treated and naturally infected plants.

3.3.3.1 Normal structure of bark and wood

Stem has distinct periderm. The parenchymatous inner cortex has abundant sclereids. Secondary phloem is continuous and stratified by tangential bands of fibers. Phloem rays are homogeneous, uni to multiseriate, and dilated at the periphery.
Wood is diffuse porous. Vessels are solitary or in radial multiples and clusters. Axial parenchyma is para-tracheal, vasicentric, alliform to alliform-confluent. Rays are broad, uni to multiseriate. Growth rings are not distinct.

3.3.3.2 Structure of ethephon treated stem

There is no noticeable structural alteration in the bark upon ethephon treatment. In the outer sapwood 1-2 tangential rows of traumatic gum-resin cavities are formed close to cambium (Fig. 181). Cavities are absent in the secondary xylem of control plants.

The cavities are developed in the traumatic tissue formed from the derivatives of the vascular cambium in response to ethephon treatment (Fig. 183). Vessels and fibers are absent in the traumatic tissue. In this tissue, the continuity of multiseriate ray is not disturbed, but the axial parenchyma cells have a cambium-like configuration. All the cells in the traumatic tissue have un lignified cells. After some time, the cambium renews its normal function and the traumatic parenchyma is sandwiched between the secondary xylem. The gum-resin cavities develop among the axial parenchyma cells and occupy the entire space between the adjacent rays. The cavities are lined by definite epithelium. The epithelial cells are rich in protein and lipid (Fig. 184). Large lipid droplets are observed in the
cavities also (Fig. 185). A few vessels in the secondary xylem at the vicinity of the cavities are plugged with gum-like material.

3.3.3.3 Structure of infected stem

The infected stem exuding gum-resin, shows fungal hyphae in xylem vessels and parenchyma cells (Fig. 212, 213). Two to five tangential rows of cavities are present in the sapwood (Fig. 182). The cavities of the outer row nearer to the vascular cambium are similar to those formed in ethephon treated plants. They are situated in the un lignified traumatic tissue. The cavities are lined by distinct epithelium rich in protein and lipid. They also contain lipid droplets indicating that they are active in secretion.

But the cavities of the inner rows do not have distinct epithelium, and they are without any contents. When two rows of cavities are apart, normal xylem elements are present between the rows (Fig. 182). Hence tangential bands of un lignified traumatic tissue with cavities are found embedded in the outer sapwood (Fig. 186, 187). The cells of the traumatic tissue remain un lignified for some time. The un lignified state of the traumatic parenchyma cells is supported by fluorescence study. Cells of the traumatic parenchyma are non fluorescent, whereas, the cells on either side give autofluorescence due to lignin (Fig. 188). But the cavities of the inner row towards the middle sapwood
are flanked by lignified cells (Fig. 189). As the wood is characterized by paratracheal, vasicentric parenchyma, the presence of cavities in regular tangential rows indicate that they too were early developed in traumatic parenchyma, and their cells got lignified later. The observations indicate that the vascular cambium responds to infection by producing traumatic parenchyma periodically. The cavities develop in the traumatic parenchyma, and after a period of active secretion they become inactive and the traumatic parenchyma gets lignified.

A large number of vessels in the sapwood are plugged with gummy or granular occlusions of varying composition and fungal hyphae. Development and composition of the occlusions are dealt with in chapter 3.3.4.

3.3.3.4 Development of cavities

A group of initials among the traumatic parenchyma acquire dense cytoplasm and large nuclei (Fig. 190). They undergo anticlinal and transverse divisions and become isodiametric. The centrally situated cell amongst them undergoes lysis initiating a cavity (Fig. 191). The cells surrounding the lumen reorient to form an epithelium around the cavity (Fig. 192). The traumatic parenchyma cells adjacent to the epithelial cells become meristematic and appear cambiform (Fig. 193, 194). The lysis of epithelial cells follows vigorously and the cavity gets enlarged. As
the lysis of epithelial cells progresses, more epithelial cells are derived from the peripheral cambiform cells by anticlinal and transverse divisions (Fig. 194-196). The epithelial cells are remarkably different from the surrounding cells. They have large globular nuclei with one prominent nucleolus and several heterochromatic regions and appear to be rich in cell organelles (Fig. 196, 197). They have dense cytoplasm with several small vacuoles, whereas, the surrounding cells are highly vacuolated.

Initially the epithelial cells are contiguous (Fig. 197), but later they get separated at radial and tangential walls (Fig. 196). Probably the cementing substance between the contiguous cells gets dissolved and subsequently the separated cells show darkened cell wall material. The senescing epithelial cells show dark cytoplasm, elliptical and irregular nuclei (Fig. 199, 200) and ultimately they undergo lysis. There is no sequential direction or orientation in the cells of epithelium regarding lysis. Consequently, often intact cells are left amidst the lysing cells (Fig. 196, 198-200). The contents of lysing cells are released into the lumen (Fig. 201-204). The lysis proceeds vigorously leading to the tangential and vertical enlargement of the cavity (Fig. 205). The tangential widening of the cavity is limited by the multiseriate rays. The cavities do not traverse the rays. Eventually, all the axial parenchyma
cells of the traumatic tissue disintegrate forming a network of tangentially anastomosing cavities (Fig. 206). The multi-seriate rays remain intact like islands, amidst the ramifying system of anastomosing cavities. In radial longitudinal section, the cavities appear as vertically elongated system interrupted by multiseriate rays (Fig. 207). Figure 208 gives a 3-dimensional representation showing the distribution and anastomosis of gum-resin cavities in the secondary xylem.

3.3.4 Development and histochemistry of vascular occlusions in the stem of Ailanthus excelsa

The infected stem exuding gum-resin, shows traumatic gum-resin cavities in the outer sapwood (see chapter 3.3.3 also). Many vessels in the vicinity of the cavities are partially or completely filled with chemical substances and/or with fungal hyphae (Fig. 209-213). The occlusions are mostly gum-like (Fig. 209), sometimes granular (Fig. 210, 211) and rarely fibrous (Fig. 212). The fibrous occlusions appeared to be formed of fungal hyphae along with some granular material.

Fungal hyphae are observed also in some parenchyma cells adjacent to the occluded vessels (Fig. 212). The natural color of the occlusions vary from yellow to dark brown. With toluidine blue 0 the occlusions stain dark blue, green or purple. The fungal hyphae stain purple.
Most of the occlusions give bright yellow fluorescence similar to that of vessel wall and fibers (Fig. 214-217).

The occluding material originates from the parenchyma cells adjacent to the vessel. All the components of the occlusion (see Table 2) except pectin and lignin are observed in the adjacent parenchyma cells also. Figure 218 manifests the mode of secretion. Droplets of dark contents are seen attached to the vessel wall in the lumen. Similarly stained material is seen inside the pit and in the contact cells also. In some cases, while the occlusions remain unstained for a particular compound, the droplets attached to the vessel wall in the lumen, and the contents of the adjacent parenchyma cells show positive staining (Fig. 220). It indicates that new components are being added from the adjacent parenchyma cells into the already formed occlusions (also see Fig. 219).

The compounds detected in the occlusion are lipid, polysaccharide, protein, phenolics, lignin and possibly pectin. Some of the histochemical tests are not too specific and hence responses of the controls are also examined to interpret the results (Table 2). Certain unsaturated lipids, phospholipids and some phenolics are capable to react with periodic acid and give PAS positive reaction (Pearse, 1968; Geier, 1980). But acetylation of 1:2 glycol groups of carbohydrates and the consequent blocking of their oxidation by periodic acid, markedly reduced the Schiff
response. Further, when acetylated sections were subsequently treated with dilute alkali which restores 1:2 glycol groups, the PAS reaction once again took place indicating that polysaccharides are involved in the reaction.

The type of phenols present in the occlusion are not identified. The occlusions give an orange-red staining with nitrous acid reaction, whereas, cherri-red is the characteristic staining for catechol derivatives. But extraction in a solution of 5% KOH in 95% alcohol retarded or considerably reduced the reaction indicating the involvement of phenolics in the reaction.

The occlusions give strong response for pectin with both the tests. Krajčinovič Amine reaction is specific for pectin (cf. Pearse, 1968). Still the presence of pectin in the occlusion is difficult to interpret since the ammonium oxalate extraction did not reduce the staining.

Most of the occlusions give positive staining with phloroglucinol-HCl. The test is not specific for lignin, since it stains hemicellulose and suberin also (Reeve, 1974). But they give strong fluorescence similar to that of vessel wall and fibers, which supports the presence of lignin. Extraction of unbound phenolics does not affect the fluorescence of occlusions, vessel wall or fibers.
The occlusions show wide variation in their chemical nature. Occlusions of all vessels in a given section never show uniform staining for any one compound, and sometimes substances of different chemical nature are observed in the vessel elements in a group (Fig. 221). Many developing occlusions are heterogeneous in composition and different components are seen distinctly in the lumen (Fig. 222, 224). In early stages of many occlusions, only part of the substance shows the presence of any compound. The variation is visible even at the initial stages of development. In some cases the droplets migrating into the vessel lumen are found to be of dissimilar composition (Fig. 223). Fluorescence of the occlusions also shows variation. Most of the occlusions are brightly fluorescent while some are nonfluorescent (Fig. 216). Initially the materials secreted into the lumen remain as distinct droplets (Fig. 225) and later mix and possibly polymerize to form a homogeneous occlusion.

In these experiments the histochemical response of the exudate differs from that of the vascular deposits. The exudate is insoluble in water but soluble in alcohol. The exudate smeared on the slides, shows positive response for lipid and protein, but do not stain for polysaccharides, lignin, phenolics or pectin. With Nile blue the exudate shows prominent red staining with patches of blue, whereas, the occlusions show only blue staining. It shows that the
exudate has mainly neutral lipids, while the occlusions have acidic lipids. Four different sugars are detected in the exudate. Two of them with Rf values 0.38 and 0.47 are identified as glucose and D-arabinose respectively, when compared to the Rf value of the sugar standards, while the other two with Rf values 0.64 and 0.92 are not identified.