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STRUCTURAL AND BIOCHEMICAL CHANGES IN THE BOLL WALL IN SUPPORT OF THE NAWF5+350 HEAT UNITS RULE

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Abstract

Terminating insecticide applications at the end of a season is a very important management decision which in the COTMAN monitoring program relies on identification of the last effective boll population (NAWF=5) and the accumulation of an additional 350 heat units. Earlier evidence showed that bollworms and boll weevils stop feeding on bolls at NAWF5+350HUs, however, evidence of changes in the boll wall with age (at NAWF=5 + 350 HU's) to support this premise is lacking. The cotton (*Gossypium hirsutum* L.) fruit wall structural and biochemical features were analyzed for four weeks after anthesis. At approximately 350 heat units after anthesis, the endocarp (inner layer of the fruit wall) cells possessed an increased concentration of phenolic materials, thickened cell walls, and increased lignification of cell walls. These structural changes coincide with the increased resistance to physical penetration during this time of development. In addition, the mesocarp cells had an increased concentration of tannin material, and the exocarp (outer layer) cells contained cytoplasmic microfibrils which may be oxalate crystals. Also the percent composition of free sugars of the total fruit dry weight decreased with time. These observations help to explain the reported loss of interest by insects in the fruits as feeding sites at about 350 heat units after anthesis. These results provide increased confidence in timing of insecticide termination at this stage of fruit development as is used in the COTMAN crop monitoring program.

Introduction

The development of nodes-above-white flower (NAWF) is a key component of the COTMAN crop monitoring program (Danforth and O'Leary, 1998). When a crop reaches NAWF=5, an additional 350 Heat units (threshold 60F) are accumulated before the last effective boll population (at NAWF=5) are deemed safe from bollworm and boll weevil attack and insecticides can be terminated (Bourland et al., 1992). However, evidence of changes in the boll wall with age (i.e., at NAWF=5 + 350 HU's) is lacking to support this premise. Bagwell (1995) reported that punctures of the fruit made by bollworms and the percentage of fruits damaged by larvae declined with heat-unit accumulation after anthesis. He suggested that the endocarp appears to be the major site of resistance to insect penetration of the fruit, because at around 15-20 days after anthesis lignification of the fruit wall occurs, although the evidence for this is lacking. The apparent hardness of the endocarp is thought to prevent penetration of the fruit by boll weevils (Cook, 1906). The current study was designed to provide evidence of changes in the boll wall that discourage insect feeding to support that "NAWF5+350HU rule" in COTMAN.

The cotton fruit wall contains many features that may be involved in the decline in insect feeding at 350 heat units after anthesis Oosterhuis and Jernstedt, 1999). Van Iersel et al. (1995) reported that the fruit wall (pericarp) was composed to three layers: exocarp (outer fruit wall), mesocarp (parenchymatous middle layer), and endocarp (schlerenchymatous inner layer,) in addition to a large number of club-shaped structures consisting of almost entirely fibers present near the surface of the fruits. Bondada et al. (1993) showed that the boll wall was composed of tightly packed parenchymatous cortical cells with little or no intercellular air space. The cotton fruit wall consists of radial bundles as evidenced by occasional mechanical fibers located near the surface which contribute to the relative thickness of the capsule wall (Baranov and Maltzev, 1937). These authors showed that the tangentially aligned endocarp cells were transformed into fibrous schlerenchyma tissues of many layers at maturity. They also observed the schlerenchyma fibers at maturity provided support for the fruit wall tissue.

The objectives of this study was to document anatomical changes in the cotton fruit wall with time after anthesis and relate these to changes in the resistance to physical penetration of the cotton fruit wall with time as measured using a modified penetrometer. In addition, the study investigated possible changes in the concentrations of carbohydrates, lignin, tannin, and phenolic acid in the boll wall with time after anthesis.

Methods and Materials

Cotton (*Gossypium hirsutum* L.) cv. Deltapine 20 was hand-planted in early May in 1996 and 1997 in a Captina silt loam (Typic Fragiudult) at the Arkansas Agricultural Research and Extension Center in Fayetteville. Rows were spaced 0.9 m apart and plots were 4 rows wide and 5 m long with ten plants per meter. The experiment was arranged in a randomized complete block with three treatments and three replications. All plots received fertilizer and pesticide applications following the cotton production recommendations for Arkansas. Pre-plant fertilizer consisted on N-P-K applied at 45-30-75 kg/ha.

Additional nitrogen was applied at pinhead square at a rate of 56kg/ha. After planting, Temik was applied in furrow at planting at 4.48kg/ha and Terrachlor Super X was applied pre-plant at 7.84 kg/ha. Furrow irrigation was applied as needed.

Fifty white flowers were tagged in each plot at first flower and at anthesis of the NAWF=5 (five nodes above the first white flower) growth stage. COTMAN (Danforth and O'Leary, 1998) records were taken weekly to follow crop development and to determine physiological cutout (NAWF=5) and the last effective boll population. To calculate accumulated heat units (HU's), the maximum and minimum temperatures for each day were added, divided by 2, and the growth threshold temperature of 15ºC (60ºF) subtracted. Thereafter, fruits were sampled at weekly intervals for 4 weeks (i.e., at 7, 14, 21, and 28 days after anthesis) with fruit age monitored in calendar days and heat units accumulated after first flower and after NAWF=5. Ten fruits from three replications were harvested on a weekly basis to observe and to record structural and biochemical developmental changes in the cotton fruit wall.

Measurements were made at select times after anthesis of boll size, volume, length, breadth and dry matter. Mesaurements of resistance of the boll wall to physical penetration was made with a modified penetrometer (UC firmness tester, Amtek Hinder Spring, Hatfield, PA). In addition, determinations of phenolic acid concentration, condensed tannin concentrations, and boll wall carbohydrates were made using HPLC. Light and transmission electron microscopy was used to study changes in the boll wall cross sections at weekly intervals after anthesis.

Results and Discussion

Effect of Boll Wall Age on Physical Penetration

Resistance to physical penetration of the boll wall increased slowly with ontogeny up to about 3 weeks after anthesis, after which the resistance increased sharply (Figure 2). This increase coincided with an accumulation of approximately 350 heat units after anthesis in support of the theory proposed by Bagwell (1995) and used in COTMAN program (Danforth and O'Leary, 1998). Bagwell (1995) reported that punctures of the fruit made by bollworms and the percentage of fruits damaged by larvae declined with heat-unit accumulation after anthesis. Our research provided evidence for the decrease in feeding by bollworms at NAWF5 + 350HU's, i.e. increased physical strength of the boll wall.

Phenolic Concentration in the Boll Wall with Increasing Age

There was no clear trend of phenolic acid concentration in the boll wall with time (Figure 3).

Tannin Concentration in the Boll Wall with Increasing Age

Tannin concentration in the boll wall decreased slowly with time after anthesis (Figure 4), but with no apparent changes coinciding with the time of 350 heat unit accumulation.

Carbohydrate Concentrations in the Boll Wall with Increasing Age

There was no clear trend of carbohydrates in the boll wall with time (data not shown).

Changes in Boll Wall Anatomy with Increasing Age

The exocarp, mesocarp and endocarp of the cotton boll wall are clearly visible by light microscopy at 21 days after anthesis (Plate 21). Also visible are some sclerenchyma, parenchyma and a vascular bundle. In addition, there is some tannin material noticeable.

The exocarp (outer layer of the boll wall) at 7 after anthesis is shown in Plates 2. By 14 days after anthesis, the exocarp cells of the boll wall showed thin continuous strands of microfibrils in the cytoplasm (Plate 3 &4).

The mesocarp at 7 days after anthesis are shown in (Plate 5). At 14 days after anthesis, the mesocarp cells showed electron-dense ring-shaped tannin-filled tonoplast in the vacuoles. By 21 days after anthesis, the mesocarp cells of the boll wall showed thickened cell walls and clear evidence of tannins in the vacuole (Plate 7).

The 5-layered endocarp of the boll wall at 7 days after anthesis is shown in Plate 8. At 14 days after anthesis, the lignified cell walls and tannin material were clearly evident (Plate 9). By 21 days, the cell walls of the endocarp had become well lignified and tannin depositions in the cell vacuoles were clearly evident (Plate 10). In addition, the electron micrograph of endocarp cells at 21 days showed large numbers of mitochondria in the cytoplasm an a compound plasmadesmata (Plate 11 & 12). Plate 12 shows the junction with the xylem of a compound plasmadesmata between the massive cell walls of the xylem, as well as tannin deposition. This is in contrast with the situation in the endocarp cells 7 days after anthesis (plate 13).

The anatomical changes in the endocarp that occur at about 21 days after anthesis support the reported decrease in bollworm feeding at this stage. In addition, the deposition of tannin material in the endocarp cells also support these findings. There may not have been a clearly defined increase in tannin in the boll wall at 21 days (Figure 3), but there appears to have been a net increase in tannins in the inner part of the boll wall (i.e., endocarp cells) and a decrease in the exocarp cells.

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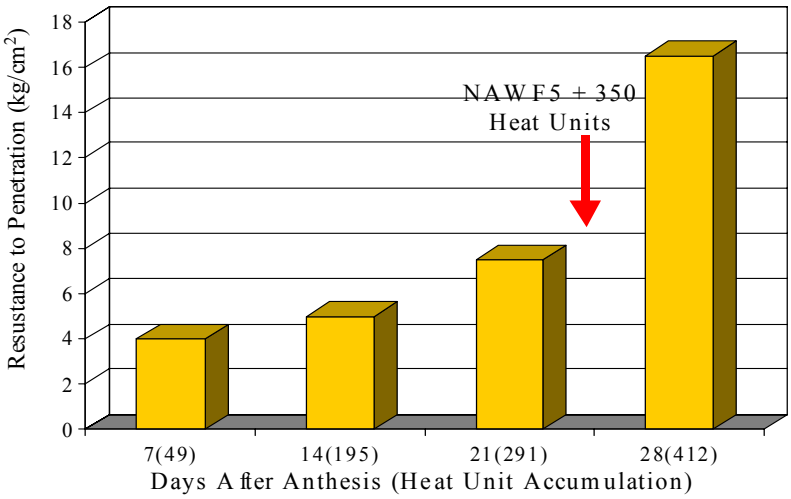

Figure 1. Changes in resistance to physical penetration of the boll wall with days after NAWF5 + 350 heat units . The accumulated heat units after cutout (NWAF=5) is shown for each sampling date.

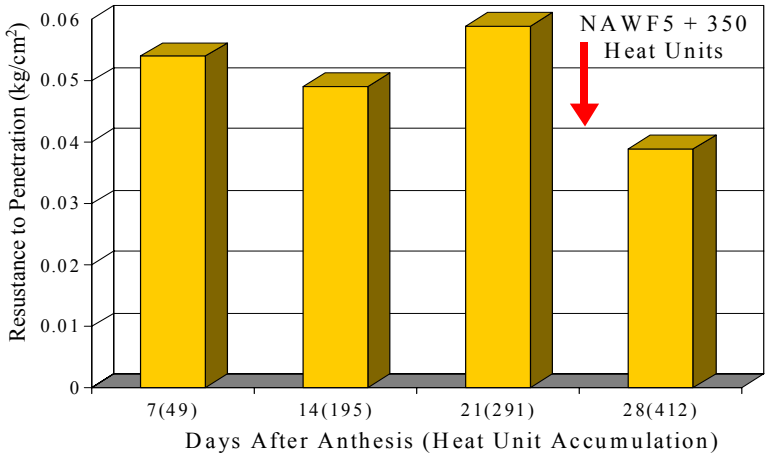

Figure 2. Changes in Phenolic Acid Concentration of the Boll Wall with Days after Anthesis (and accumulated Heat Units).

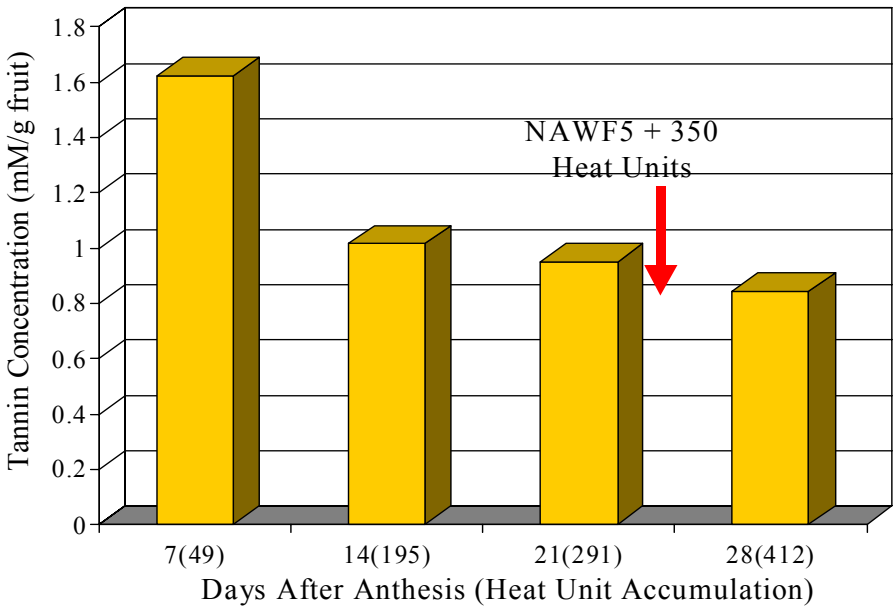

Figure 3. Changes in Tannin Concentration of the Boll Wall with Days after Anthesis (and accumulated Heat Units).

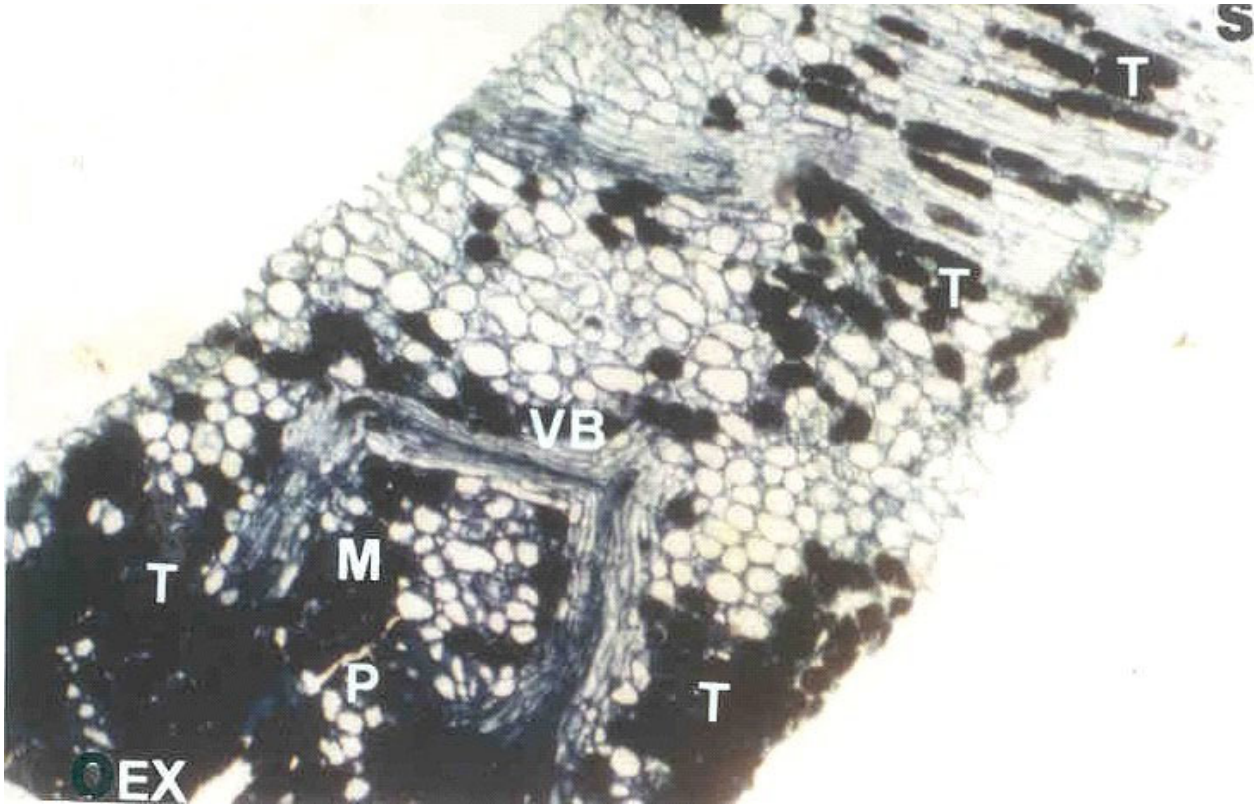

Plate 1. Light micrograph of a cross-section of the cotton boll wall at 21 days after anthesis showing the exocarp, mesocarp and endocarp. Stained with 1% Toluidine (X40). EX = exocarp, M=mesocarp, T = tannin material, S = sclennchyma endocarp, P = parenchyma, VB = vascular bundle.

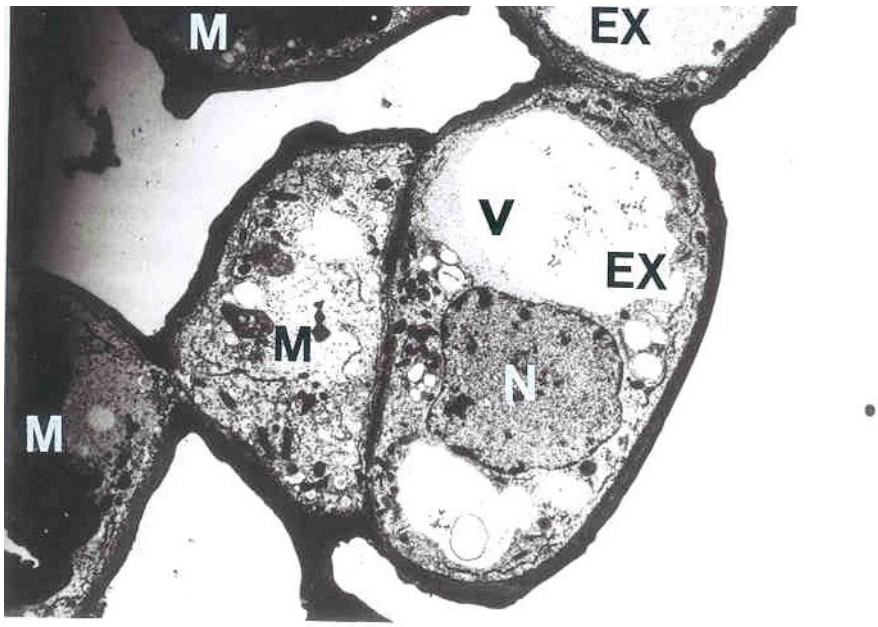

Plate 2. Electron micrograph of an exocarp cell (EX) of the boll wall 7 days after anthesis.

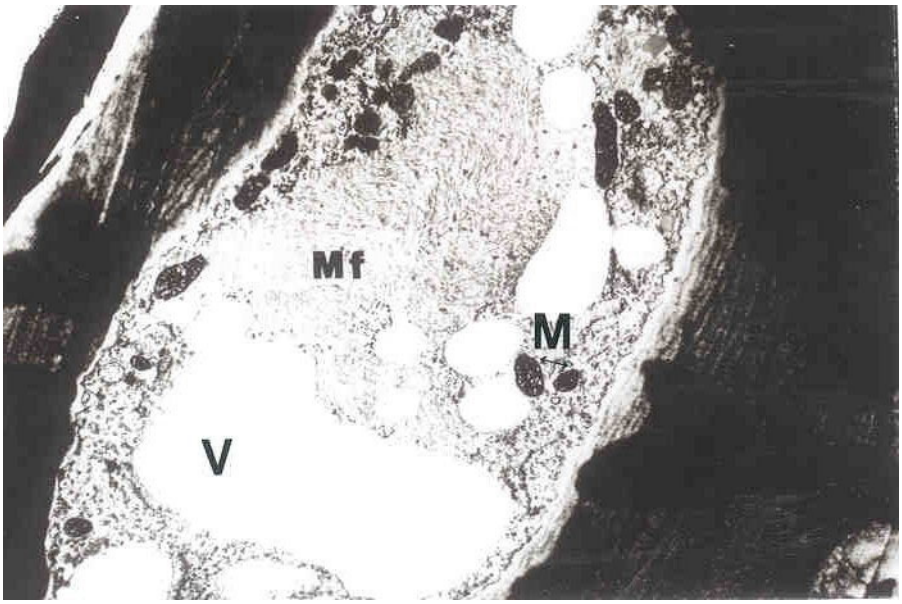

Plate 3. Electron micrograph of an **exocarp** cell of the boll wall at 14 days after anthesis showing thin, continuous strands of microfibrils (Mf) in the cytoplasm. (X10,000). V=vacuole, M=mitochondria.

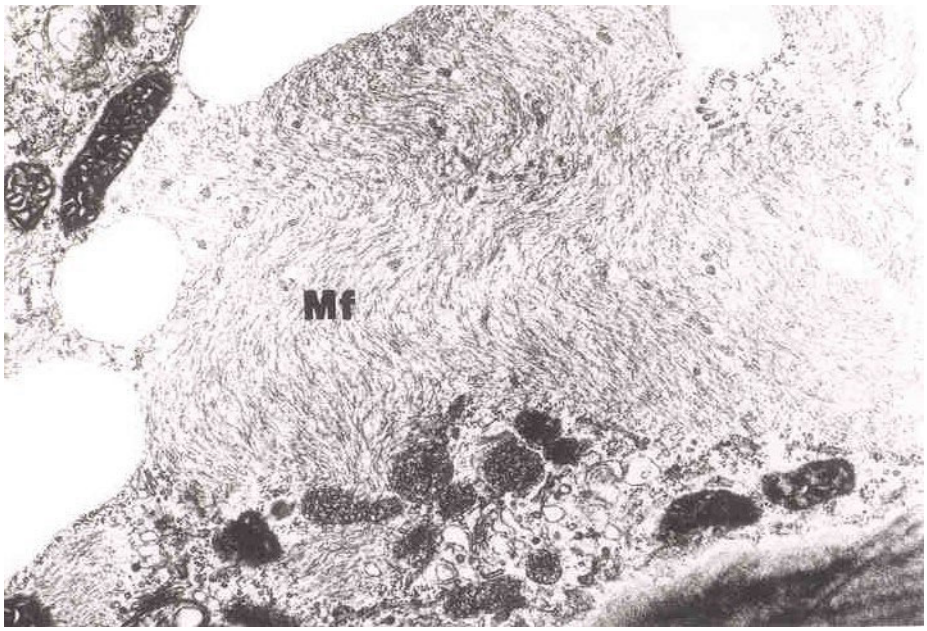

Plate 4. (Higher magnification of Plate 3) showing an **exocarp** cell at 14 days after anthesis showing microfibrils (Mf). (X20,000).

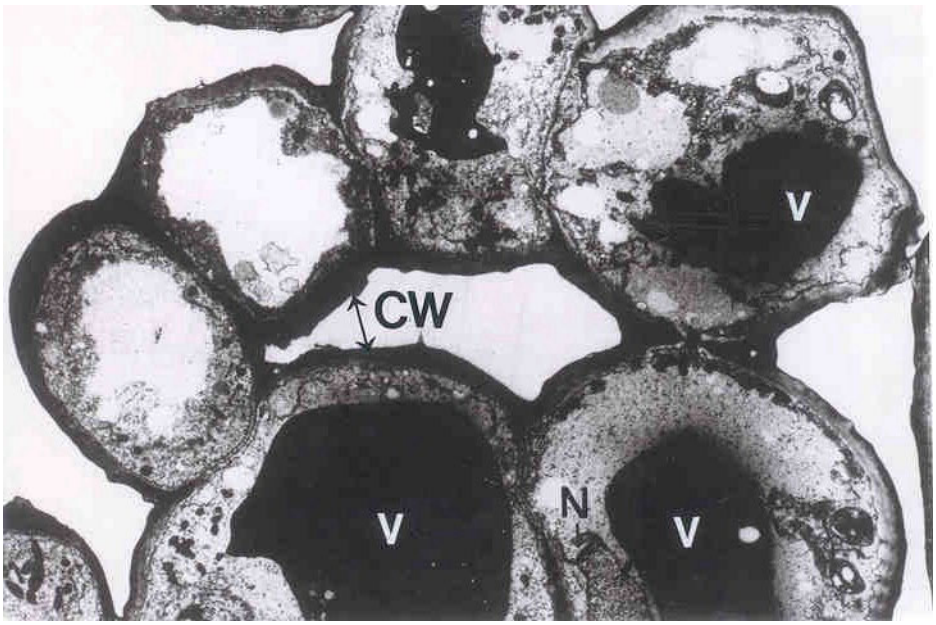

Plate 5. Mesocarp cells of the boll wall at 7 days (x4,000) N = nucleus, CW = cell wall.

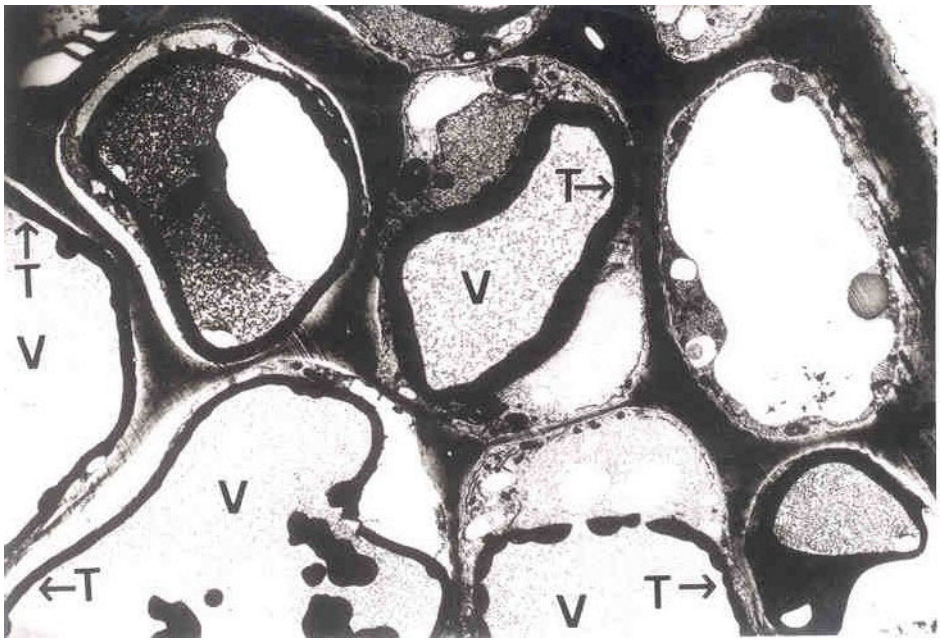

Plate 6. Mesocarp cells at 14 days showing electron-dense ring-shaped tannin-filled tonoplast (T) of vacuoles (V) (x3,2000).

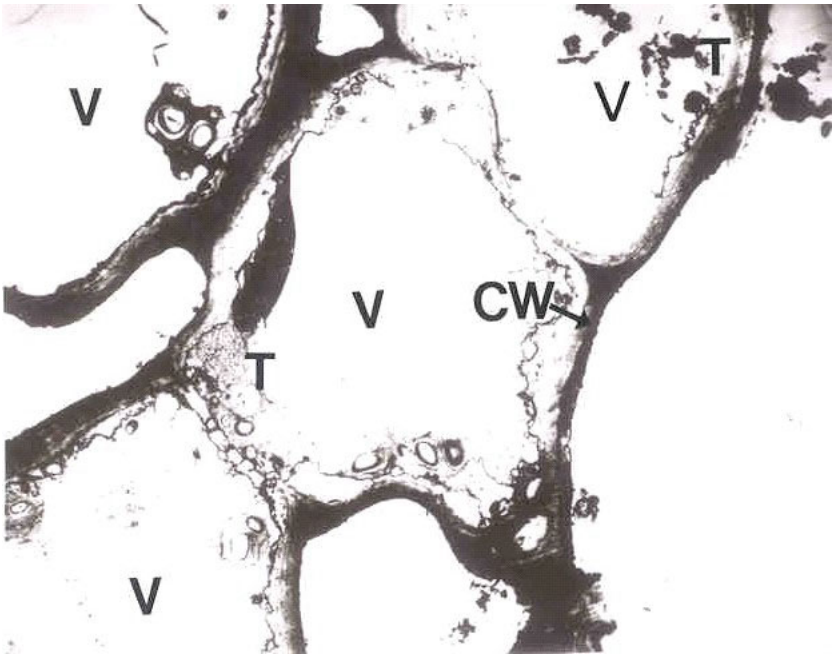

Plate 7. Mesocarp cells of the boll wall at 21 days after anthesis. (x3,2000).

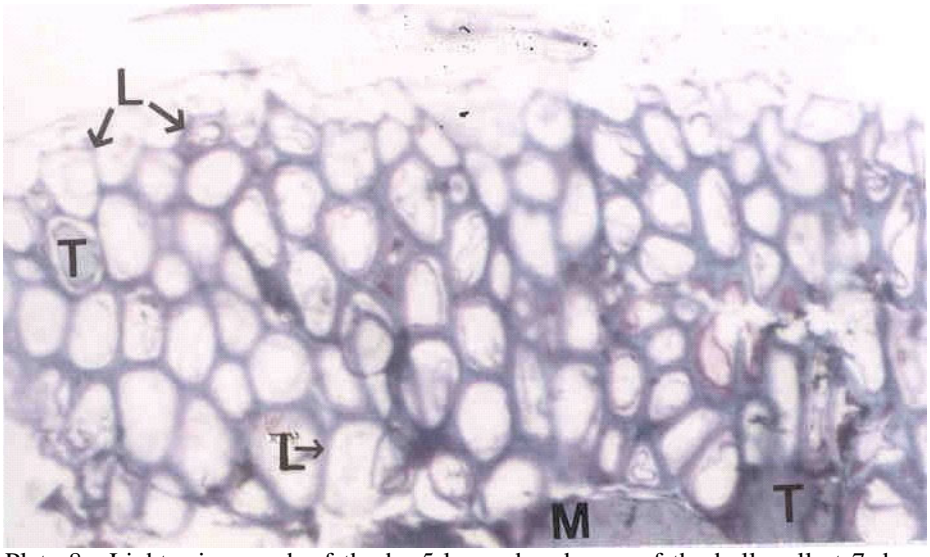

Plate 8. Light micrograph of the he 5-layered endocarp of the boll wall at 7 days (x100). M = mesocarop cell, CW = cell wall.

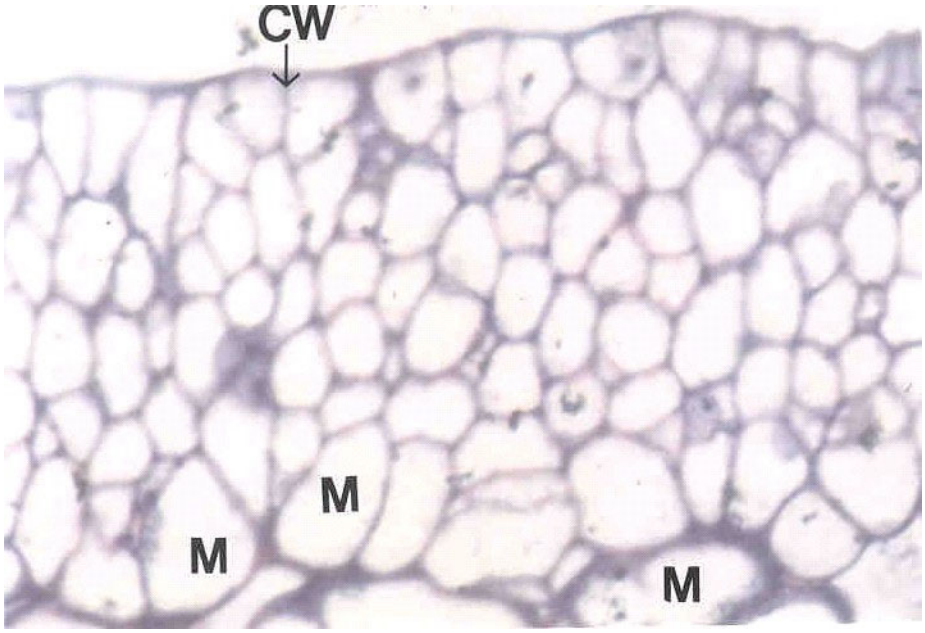

Plate 9. Endocarp cells at 14 days. (x100) CW = cell wall, M = mesocarop cell, L = lignified cell walls, T = tannin material.

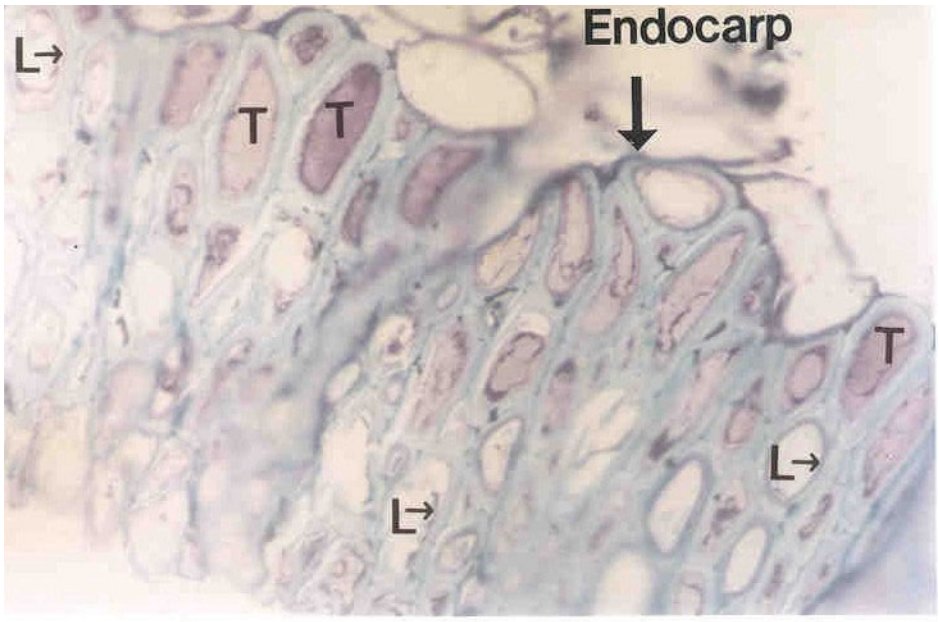

Plate 10. Endocarp cells at 21 days. (x100) CW = cell wall, M = mesocarop cell, L = lignified cell walls, T = tannin.

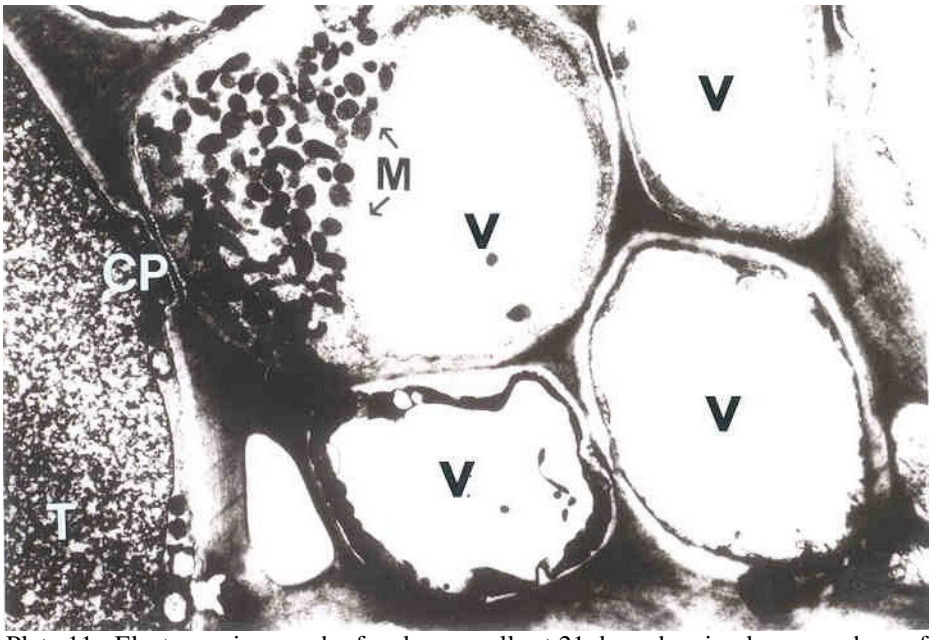

Plate 11. Electron micrograph of endocarp cells at 21 days showing large numbers of mitochondria (M) in the cytoplasm. (X6,600). V = vacuole, CP = compound plasmadesmata.

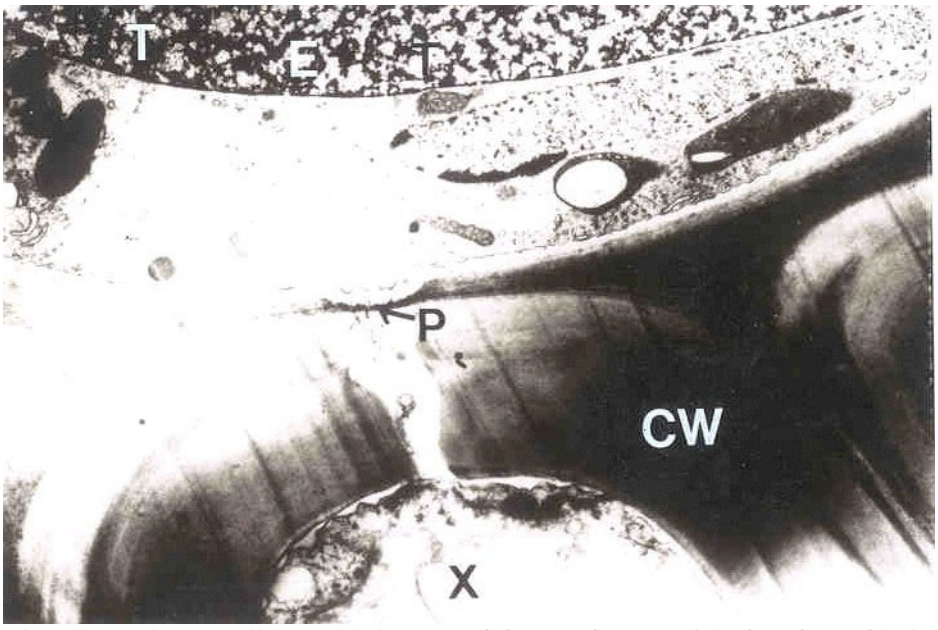

Plate 12. Endocarp cell (E) at 21 days containing tannin (T) and the junction with the xylem with a compound plasmadesmata (P) between the massive cell wall (CW) of the xylem. (X10,000).

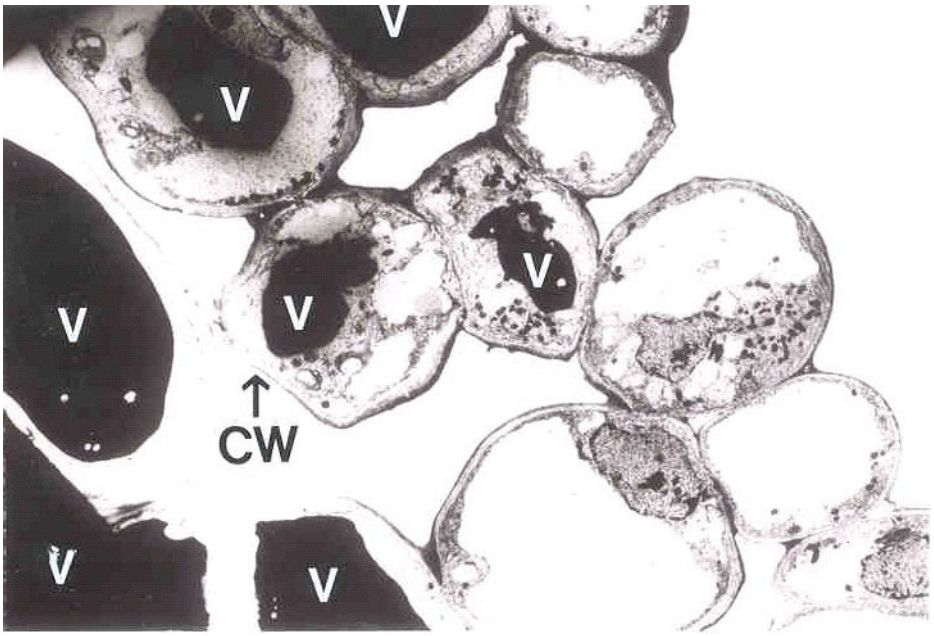

Plate 13. Endocarp cells at 7 days. (X3,200). V = vacuole, CW = cell wall.