

# Cyclic patterns of growth and rubber deposition in guayule *Parthenium argentatum*. Suggestions for a management programme

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As the natural pattern of development and rubber synthesis in guayule (*Parthenium argentatum*) is cyclic any treatments to improve growth and rubber production should be synchronized with the normal sequence of events pertaining to growth and metabolism. Starting with 25-week-old seedlings an attempt has been made to acquire information about rubber deposition, growth and defoliation. Observations were made in the field as well as at the light and electron microscope level. Rubber is deposited mainly in the bark during winter. The highest deposits were found in the secondary stem tissue.

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Daar die natuurlike ontwikkelingspatroon en rubbersintese in guayule (*Parthenium argentatum*) siklies is moet enige suksesvolle behandeling ten einde groei en rubberproduksie te bevorder gesinkroniseer word met die normale gebeure ten opsigte van groei en metabolisme. Beginnende met 25-week-oue saailinge is pogings aangewend om bruikbare inligting te bekom omtrent rubberdeposisie, groei en blaarval. Waarnemings is gemaak in die veld sowel as op lig- en elektronmikroskopiese vlak. Rubber is hoofsaaklik in die bas gedurende die winter neergelê. Die grootste hoeveelhede is in die sekondêre stingelweefsel gevind.

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## Introduction

Research with respect to establishing guayule as a commercial source of natural rubber in South Africa was started in 1979. As the crop is expensive to process, efforts are currently being made to increase the rubber yield. One method of approach is the application of so-called bioregulators pioneered by Yokoyama, Hayman, Hsu & Poling (1977). These compounds are mainly derivatives of tri-ethylamine and are claimed to stimulate rubber biosynthesis in young plants as much as six-fold. Guayule does not have laticifers that can be tapped as is the case with *Hevea brasiliensis*. Rubber is stored in the parenchyma cells of the stem and root, which obviously have a limited storage capacity. Attempts are presently being made to increase the number of these parenchyma cells by the use of growth-promoting substances. A third channel of research relates to removing the leaves with ethephon before harvesting (Gilliland & Van Staden, 1985). The leaves contain very small amounts of rubber and they complicate processing.

For the above procedure to be carried out effectively, it is necessary to have detailed information about patterns of growth and rubber deposition taking place throughout the year. The growth pattern of the plants is known to be cyclic (Bonner, 1943; Bonner & Galston, 1947) and treatments to improve rubber synthesis should be synchronized with the natural sequence of events.

This study was started with 25-week-old seedlings and their development monitored over a period of 12 months to collect information about the occurrence, extent, and location of rubber deposition, and the ultrastructural and anatomical changes taking place. These findings are intended for the purpose of planning a management programme to optimize rubber production.

## Materials and Methods

Uniform 25-week-old seedlings were selected in January. They were grown out-of-doors in pots in a mixture containing 25% each topsoil, sand, bark chips, and perlite. Plants were fertilized four times during the 12-month experimental period with N:P:K–3:2:2 and lime and watered twice weekly. These plants were used for morphological and anatomical observations. At monthly intervals stem segments (2 mm<sup>3</sup>) were collected 1 and 12 cm from the shoot apex and bark segments from the base of the stem, 3 cm above soil level. These sections were then embedded for light and electron microscopy as described previously (Gilliland & Van Staden, 1985).

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At monthly intervals defoliated plants were dried and the dried material ground to homogeneous powders. These were then analysed for resin and rubber by means of Soxhlet extraction using acetone and petroleum ether respectively. The resin and rubber contents were expressed as a percentage of the dry mass. Leaves were only analysed for these secondary products during January and June.

### Results

The seasonal changes in resin and rubber concentrations as well as the average monthly day and night temperatures recorded are expressed in Table 1. Increases in both rubber and resin occurred from January to September. The sharpest increase in rubber was detected from May to September when the average monthly night temperatures were low. In September spring growth commenced and the rise in rubber content was arrested. The leaves contained about 0,5% rubber during both the summer and winter. The percentage of rubber and resin recorded in the bark alone at the base of the stem at the end of winter was 8,1% and 14,3% respectively.

Morphological observations of the experimental plants over a period of 12 months out-of-doors confirmed that they are semi-deciduous (Lloyd, 1911). Summer leaves are abundant and lobed (Figure 1). By the end of June all but the terminal cluster of leaves had senesced. Some remained attached to the stem but by the end of July most had been mechanically severed (Figure 2). There is no physiological abscission (Addicott, 1945). The terminal cluster of winter leaves which forms the basis of the new season's growth are simple, elongate-ovate, tapering into a petiole. Lobed summer leaves are much larger and much softer than winter leaves. There are only palisade mesophyll cells which are very closely packed and which contain about 60 chloroplasts per cell. The stem becomes covered with a thin-walled phellem which exposes vividly green tissue when stripped (Figure 3). Flowering occurred throughout the summer months from September to April.

**Table 1** Seasonal production of resin and rubber by guayule plants. The experiment was started with 25-day old plants.

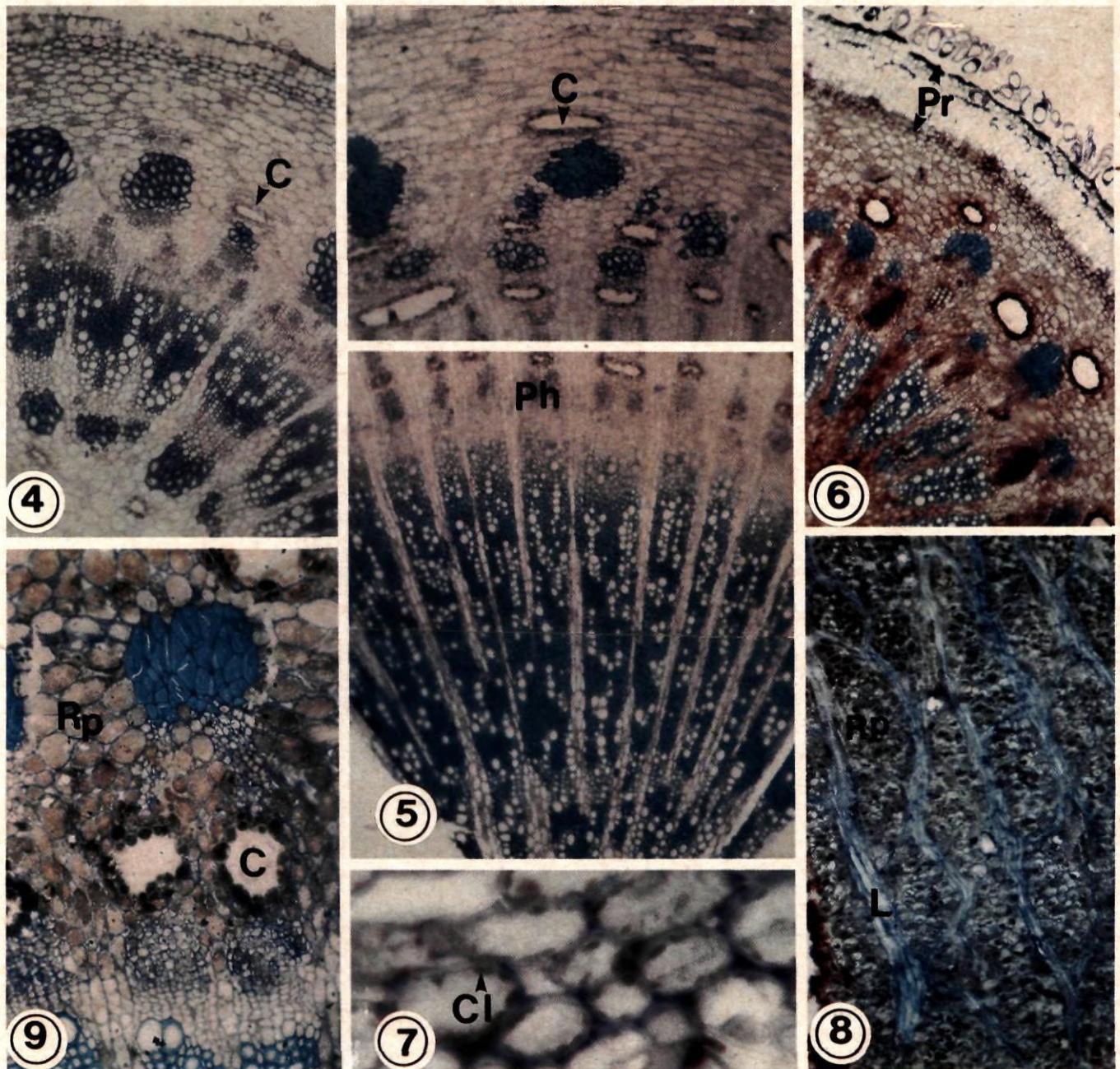
Month	Average day temperature °C	Average night temperature °C	% Resin	% Rubber
<b>Stems</b>				
January	27,4	16,8	6,2	0,6
February	26,4	17,5	7,4	0,7
March	25,4	16,2	7,9	1,1
April	24,4	13,1	9,3	2,5
May	20,9	8,9	9,6	4,5
June	19,9	5,8	9,7	4,6
July	20,3	5,0	9,1	4,7
August	21,8	7,1	9,6	4,8
September	23,1	10,4	10,2	5,8
October	25,1	12,2	9,6	5,1
November	25,8	14,4	8,3	4,7
December	26,8	15,9	7,4	4,5
<b>Leaves</b>				
January	27,4	16,8	7,9	0,6
June	19,4	5,8	9,0	0,5

The anatomy of young guayule stem tissue has been described several times (Lloyd, 1911; Artschwager, 1943; Gilliland & Van Staden, 1983). Very little rubber is deposited in the parenchyma cells of the cortex, pith, and interfascicular rays during the summer months from September to April. What did occur was predominantly found in the epithelial cells of the resin canals (Figure 4). The stem diameter, 1 cm from the shoot apex, was approximately 2 mm. At the same time of year, 12 cm from the shoot apex, it was about 4 mm and the bark width about 1 mm. Considerable secondary tissue had been produced by the activity of the vascular cambium, and several concentric rings of resin canals alternated with strands of phloem, which stained pinkish with toluidine blue and were functional. The interfascicular



**Figures 1–3** Figures 1 and 2 are photographs taken in the Botanic garden in Pietermaritzburg. Figure 3 is a branch from a guayule plant with phellem stripped off to expose the phelloderm. 1. Guayule plant with summer foliage. 2. Guayule plant with winter foliage. 3. Branch of guayule phelloderm (Pd) exposed.





**Figures 4-9** Figures 4 to 9 are light micrographs of semi-thin plastic embedded sections stained with toluidine blue. 4. Transverse section of a young stem in summer 1 cm from the shoot apex with resin canals (C), 55 $\times$ . 5. Transverse section of a young stem in summer 12 cm from the shoot apex showing concentric rings of resin canals (C) and phloem strands (Ph), 55 $\times$ . 6. Transverse section of a young stem in winter showing periderm (Pr), 45 $\times$ . 7. Parenchyma cells from the phelloderm showing chloroplasts (Cl), 400 $\times$ . 8. Tangential section of a mature stem showing lignified phloem (L) and rubber-bearing parenchyma with rubber particles (Rp), 55 $\times$ . 9. Transverse section of part of a young stem in winter showing parenchyma cells filled with rubber particles (Rp) and rubber deposition in epithelial cells of the resin canals (C), 100 $\times$ .

rays were maintained (Figure 5). The rubber content of the whole plant at the end of summer in April was about 2,5%, the highest concentration still being present in the epithelial cells of resin canals. A few rubber particles were also observed in the parenchyma cells in the vicinity of the resin canals (Figure 5). The stem had a periderm consisting of a phellem of very thin-walled, suberized cells, a phellogen, and a phelloderm forming the secondary cortex (Figure 6) which contained about 30 chloroplasts per cell (Figure 7).

The stem diameter at its base, 3 cm from soil level, was about 10 mm, and the width of the bark was about 1,5 mm. During the summer new phloem bundles and resin canals were initiated. The oldest sieve tubes furthest from the cambium stained vividly blue with toluidine blue indicating

they were lignified and hence non-functional (Figure 8). The companion cells had collapsed. The two most recently formed concentric rings of phloem bundles adjacent to the cambium stained pink with toluidine blue and were still functional. The cambium had contributed to the number of cells in the vascular rays and the phellogen had added parenchyma cells to the secondary cortex. All the parenchyma cells at the end of the summer contained a small quantity of rubber, particularly those in the pith and vascular rays. During the winter few new parenchyma cells were added, but by the end of September most of the existing cells contained numerous rubber particles. At this time the total rubber content of the plants was 5,8%. The rubber particles were most abundant in the vascular rays, less so in the



cortex, and fairly sparse in the peripheral cortical layers. These last cells seem to be the only ones which could accommodate more rubber. Even the younger tissue had many cells filled with rubber particles. The resin canals became distended (Figure 9).

In the epithelial cells of the resin canals rubber deposition is always cytoplasmic. Backhaus & Walsh (1983) pointed out that rubber particles in young and primary tissue are mainly cytoplasmic, occurring occasionally in the vacuole (Figure 10). As winter approaches ER vesicles, which have been suggested to contain hydrolytic enzymes (Madhaven & Benedict, 1984), develop in the vacuoles of these cells. They rupture and the cytoplasm is digested leaving isolated rubber particles suspended in the cells (Figure 11). The amount of primary tissue affected in this way is comparatively insignificant after secondary growth has become established.

The secondary parenchyma cells produced by the phellogen and vascular cambium are by far the most significant rubber-producing cells from an economic point of view. Phloem and cortex removed from the base of the stem at the end of winter contains about 8,0% of its dry weight as rubber. This is all vacuolar rubber occurring in large parenchyma cells with a thin peripheral layer of cytoplasm. While rubber particles are in the process of development they are spherical and measure from 0,1  $\mu\text{m}$  to 1,5  $\mu\text{m}$  in diameter. These cells have a very thin peripheral layer of cytoplasm with rubber particles in the vacuole. The particles are surrounded by an osmiophilic layer which is sometimes diffuse (Figure 12). Archer & Audley (1967) established that rubber molecules elongate at the surface of existing rubber particles by the addition of the universal isoprenoid precursor, isopentenyl pyrophosphate (IPP). These rubber particles appear to be enlarging in this way.

By the end of the winter, bark tissue dissected from the base of the stem, and in particular from the interfascicular rays, contained cells where rubber particles were so tightly compressed that they had lost their spherical shape (Figure 13). Some coalesced. New rubber particles are believed to be initiated by a starter molecule dimethylallylpyrophosphate

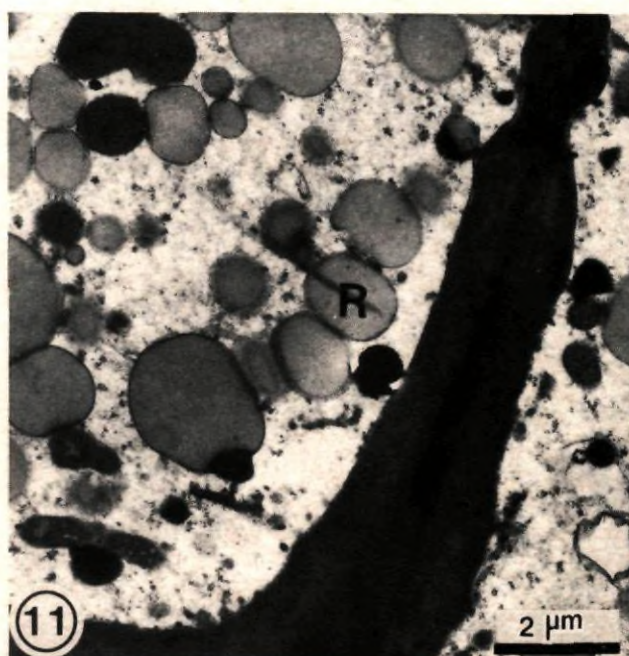
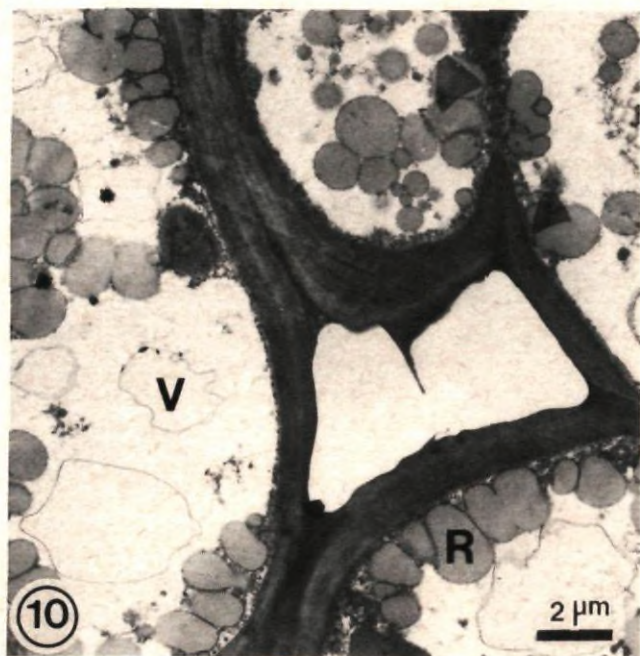
(DMAPP) (Lynen, 1969). Regions were found in the bark parenchyma cells where there were innumerable tiny rubber particles which suggest this phenomenon (Figure 14).

In spite of the large amounts of rubber generated in bark parenchyma cells the majority of these cells appeared to remain viable and active. The tonoplast usually remained intact and the cytoplasm contained a large number of organelles including ribosomes, ER, mitochondria, and a few peroxisomes. Chloroplasts are larger than leaf chloroplasts and occurred mainly in the outer layers of the secondary cortex (Figure 15). Occasional chloroplasts occurred in most of the bark parenchyma cells and were often surrounded with rubber. They rarely contained starch, possibly because reserve carbohydrates in guayule occur as inulin which is insoluble and amorphous (Taub & Slattery, 1947).

### Discussion

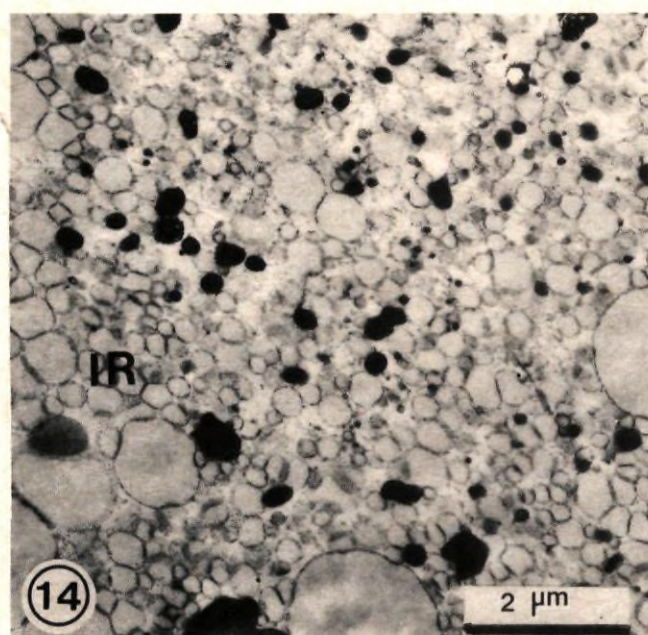
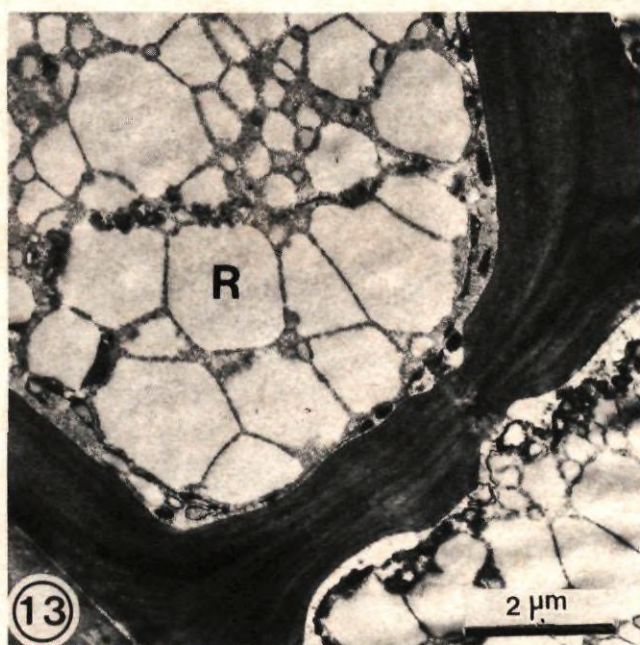
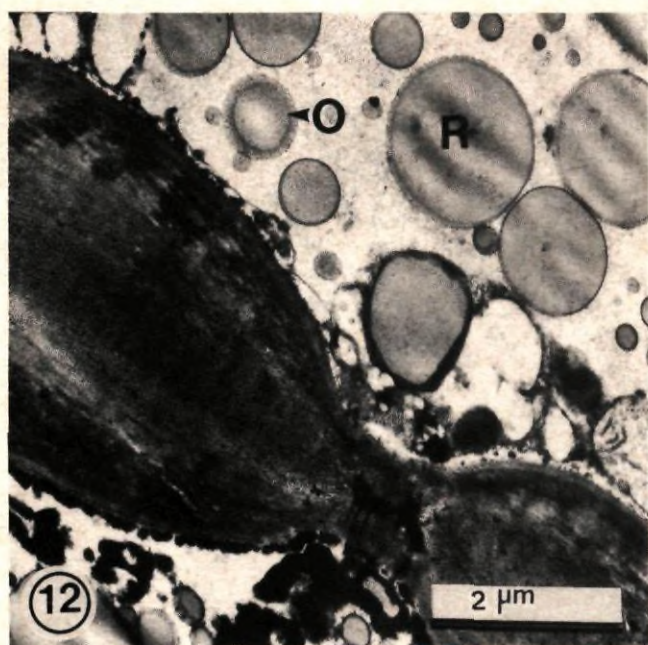
It is obvious that cytoplasmic rubber generated in young tissue of guayule is not present in sufficient quantities to have any commercial significance. If the crop is to be profitable, producers will have to concentrate on increasing the amount of parenchyma tissue of the bark. These cells can accommodate much larger quantities of vacuolar rubber and they remain viable for longer periods. In the early days of guayule research Addicott & Pankhurst (1944/45) stated that the rubber-producing potential of any guayule clone depended on a large amount of bark in proportion to the wood. At that time experiments were conducted to reduce the xylem by subjecting plants to water stress. This did, however, result in a general reduction of biomass.

In 1943 Bonner made the important observation that maximum accumulation of rubber takes place in winter when average night temperatures fall below 7°C. Goss, Benedict, Keithly, Nessler & Stipanovic (1984) conducted well-controlled experiments which confirmed that low non-freezing temperatures stimulate rubber production in guayule. The results in Table 1 substantiate this finding. Under natural conditions rubber biosynthesis therefore is



**Figures 10–11** Figures 10 and 11 are electron micrographs of ultra-thin sections. 10. Cells from the cortex of a young stem showing cytoplasmic rubber (R) and ER vesicles (V). 11. Part of a cell from a young stem showing breakdown of the cytoplasm in winter and accumulation of rubber (R).





**Figures 12–15** Figures 12 to 15 are electron micrographs of ultra-thin sections. 12. Rubber particles (R) with osmiophilic layer (O) in the vacuole of a cell from the bark parenchyma. 13. Closely packed rubber particles (R) from bark parenchyma cells at the end of winter. 14. Initiation of new rubber particles (IR) in a bark parenchyma cell. 15. Chloroplast (Cl) and mitochondria (M) from a cell on the periphery of the secondary cortex. Note osmiophilic layer (O) associated with the rubber particles.

primarily a winter phenomenon. During World War II the United States Department of Agriculture paid considerable attention to the cultivation of guayule as a source of natural rubber. They devoted a lot of attention to carbohydrate resources of the plant and demonstrated that these reach a peak in winter at the same time as rubber (Taub & Slattery, 1947). There is a marked decrease in the carbohydrate reserves at the beginning of the new growing season in spring but as the summer foliage becomes established the level rises. Very little rubber is, however, produced. It seems that in summer the products of photosynthesis are mainly devoted to growth and general metabolism whereas in winter they are used for the biosynthesis of rubber and storage carbohydrates.

The fact that in winter reserve carbohydrates and rubber increase simultaneously suggests that reserve carbohydrate

is not broken down to produce precursors for rubber. The basic precursor for all isoprenoid biosynthesis, including rubber and the constituents of resin, is acetyl-CoA (Arreguin, Bonner & Wood, 1951; Bandurski & Tees, 1958; Goss *et al.*, 1984). Rubber biosynthesis appears to be related directly to photosynthesis. The removal of the leaves in winter produces a marked reduction in rubber production even though the plant may contain large reserves of carbohydrate (Bonner & Galston, 1947). Ultrastructural studies have demonstrated the presence of considerable numbers of chloroplasts in the bark. Experiments with defoliated winter plants have shown that these chloroplasts can fix CO<sub>2</sub> and can contribute about 15% of the products of photosynthesis produced by intact plants. In both intact and defoliated plants the label passed very quickly into rubber (Gilliland, Van Staden & Mitchell, 1985). It therefore appears that the necessary acetyl-CoA



could derive from low molecular weight compounds produced directly from photosynthesis (Givan & Leech, 1971) or that carbohydrates break down almost immediately to form pyruvate and hence acetate.

What effect low night temperatures have on the metabolism leading to rubber biosynthesis is not known. It is possible that they trigger the synthesis of the enzymes involved in the pathway. Benedict, Reibach, Madhaven, Stipanovic, Keithly & Yokoyama (1983) showed that the application of 2-(3,4-dichlorophenoxy)tri-ethylamine promotes rubber biosynthesis in summer. It increases the levels of the four enzymes MVA kinase, IPP isomerase, rubber transferase, and FPP synthetase which are associated with the latter part of the pathway by which rubber or resin is produced. If sufficient acetate is present it seems that this bioregulator can swing the metabolism from the summer to the winter pattern. Yokoyama, Hsu, Hayman & Poling (1984) have pointed out that cells which already contain rubber are not likely to respond to stimulation by bioregulators. If the cambia are active throughout the early part of summer there should be parenchyma cells available for rubber storage by April. This would be a good time to stimulate rubber biosynthesis. There would be sufficient foliage to produce assimilates. In addition the leaf surface area for bioregulator absorption would be adequate.

With respect to the removal of leaves prior to harvesting, it seems that any defoliant should be applied in May before the tough winter leaves become established. Ethephon as defoliant is much more effective on soft, broad summer leaves than on tough narrow winter leaves. In the case of guayule, with a continually changing pattern of behaviour throughout the year, the time at which a treatment is applied is as important as the treatment itself.

### Acknowledgements

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