VITALITY OF WOOD PARENCHYMA

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ABSTRACT

Vitality of wood parenchyma has been studied in three tropical trees with the help of vital dyes like neutral red, Evan's blue and Janus green B. Cells accumulating neutral red and excluding Evan's blue are considered to have intact plasmalemma and cells stained with both of these vital dyes are presumed to be dead. The intactness of plasma membrane has been considered *in situ* with neutral red and Evan's blue. The wood of *Bauhinia purpurea* with a diameter of 30 cm has parenchyma cells presumably with intact plasma membrane from outer secondary xylem to inner secondary xylem as there is no heartwood. In *Callistemon citrinus* and *Manilkara hexandra* plasmalemma was found to be intact in parenchyma cells only up to sapwood-heartwood boundary beyond which the conduction of dye did not occur in the heartwood suggesting that these cells were dead. The death of some of the cells at the sapwood-heartwood boundary is probably caused by deposition of phenolic compounds in the lumen. Fibre cells are dead from outer sapwood inwards. Based on Janus green B staining, the terminal oxidase system of mitochondria is considered functional in all the cells of the sapwood and some cells of the sapwood-heartwood boundary. It is not functional in the cells containing phenolic compounds.

INTRODUCTION

VITAL dyes are employed extensively for various types of cytological studies including investigations in the uptake and accumulation of molecules and intracellular transport of substances[1]. Uptake and accumulation of neutral red by leaf epidermal tissue and its effect on guard cells have been studied[2,3]. At low concentrations toxic dyes such as neutral red and Janus green B have little effect upon cell viability for a number of hours[4]. Intactness of membrane system and capacity of the cells to transport dye molecules and accumulate them in vacuole, or actively exclude dye molecules from the cells are indicated by the use of vital dyes.

Neutral red is used to indicate the capacity of living cells to actively uptake and accumulate the dye molecules in the vacuole, whereas Evan's blue is excluded by viable cells[2]. The exclusion of Evan's blue depends on the semipermeable properties of plasmalemma, whereas neutral red uptake depends on the semipermeable properties of the tonoplast[5]. Hence Evan's blue, unlike neutral red, is applicable to nonvacuolate as well as to vacuolate cells[6]. In some cases destruction of cytoplasm has no effect on the semipermeable properties of tonoplast[5]. In such cases, observations based only on neutral red may lead to misinterpretation of the viability of cell being studied. A combined observation of stainability with neutral red and Evan's blue can be reliable to indicate the vitality of the cells. Janus green B interacts chemically with a system in a living cell. On entering the living cell reduction of the dye to its colourless form occurs. Reoxidation of the dye to its coloured form by the terminal oxidase system in the mitochondria will give the cell a bluish tinge at the site of oxidation[7]. Thus a dead cell will be completely blue, while a living cell will show bluish loci only at the site of mitochondria[2].

Vital dyes have not been employed in studies dealing with wood physiology, where cell senescence and death, either due to ageing or heartwood formation are a regular phenomenon.

The present investigation was carried out to study the vitality of parenchyma cells across the wood tissue in three tropical trees.

MATERIALS AND METHODS

Aqueous solutions of neutral red (0.5%) and Evan's blue (0.5%) were prepared in distilled water. Janus green B was prepared according to the method described by Lillie[8]. All these solutions were prepared fresh and filtered before use. Normal and healthy trees were selected in the University Botanical Garden. Bores of 0.5 cm diameter were made on the side branches of *Bauhinia purpurea* (Linn.), *Callistemon citrinus* (Curtis) (Stapf.) and *Manilkara hexandra* (Dub.) with the help of a driller up to the region of inner secondary xylem/heartwood. There is no heart-

wood in *B. purpurea* but in the other two species, the wood is distinguished into sapwood and heartwood. The bores were made oblique at an angle so that the dye solution could be filled into the cavity.

Immediately after making a bore the lumen was filled either with a solution of neutral red or Evan's blue. The openings were kept filled continuously for 2 hr by pouring more dye. Bores were made on different sides of branches to reduce the influence of one dye on the other. The branches were then cut into discs. Blocks of 1 cm^3 were made radially across the bore and 10–15 μm thick radial longitudinal sections were cut with a sledge microtome. Minimum amount of water was used during sectioning to reduce leaching of dye. Sections were mounted in glycerine and observed under the microscope. Photomicrographs were taken on a Carl Zeiss photomicroscope.

Janus green B was stained by incubating the fresh sections in the stain for 15 min. The sections were observed after mounting in glycerine. As the dye colour fades within 2 hr only temporary preparations were made.

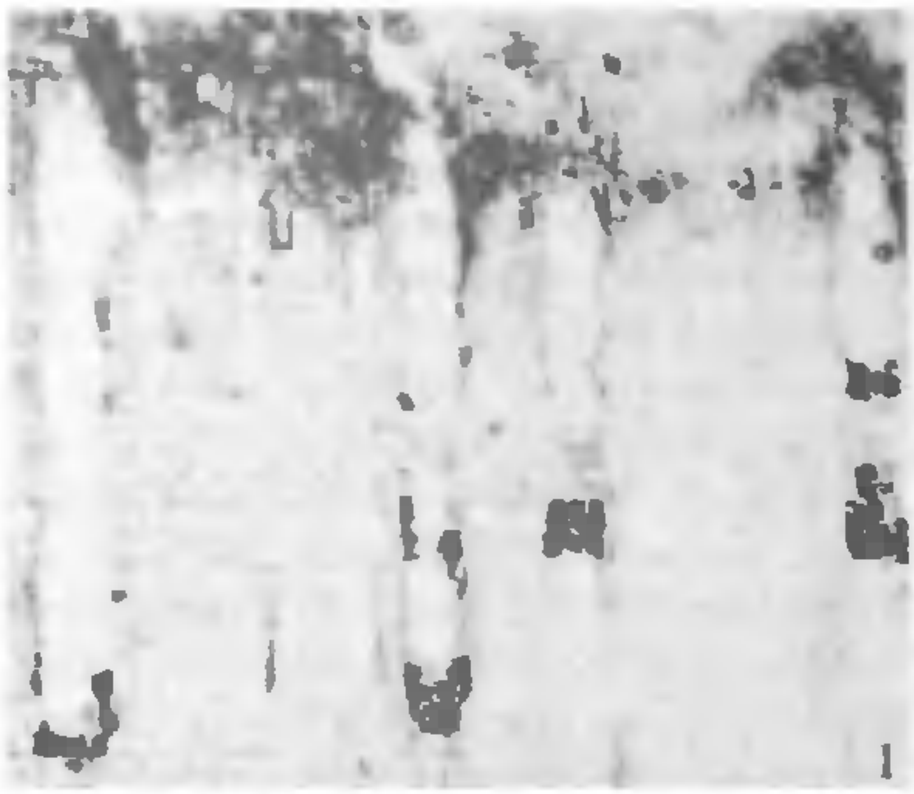
RESULTS AND DISCUSSION

When the sections of fresh wood were incubated in a solution of neutral red, it stained cell wall and nucleus of parenchyma cells (pH range 3.0–6.5). Globule formation was observed in these cells. This globule formation is considered to be due to the presence of tannins, flavone glycosides or acid carbohydrates[3]. The controversy over the chemical nature of these globules is still unresolved. This technique does not help in concluding about the intactness or rupture of the plasmalemma of parenchyma cells, and in deciding whether the transport of dye molecule across the cell is possible. The failure might be due to some artefacts while sectioning. The technique adopted by us eliminates these artefacts, as the cells were not subjected to any treatment before the process of accumulation of dye in the vacuole and its subsequent transport across the cell. All the wood elements are ruptured in the immediate vicinity of the bore, but the tissue situated away from the wound appears normal (figure 1). The dye poured into the bore is carried through the ruptured vessels in both upward and downward direction in the sapwood region in all the three species. Continued feeding for 2 hr is sufficient to build up a supply for accumulation of dye in parenchyma cells and its radial transport. Neutral red collects in the contact parenchyma cells which are contiguous with the vessel/s of sapwood region (figures 2, 4 & 5). The dye is supplied to the contact cell through the large pits between the vessel and parenchyma cell. Radial transport of the dye from the contact cell to a non-contact cell was also observed (figure 5). Cells situated away from the vessels were stained lightly while those in contact with vessels were deeply stained. A gradient of colour intensity developed from the contact cell towards the non-contact cell (figure 3). Evan's blue when fed through the bore did not accumulate in any of the contact parenchyma cells of sapwood or heartwood. When the fresh sections were stained with Janus green B, all parenchyma cells up to the inner secondary xylem/sapwood heartwood boundary showed blue coloration at the site of mitochondria where reoxidation of Janus green B was brought about[7].

Neutral red staining of contact and non-contact ray and axial parenchyma cells was found from outer secondary xylem to inner secondary xylem, in the case of *Bauhinia purpurea* (tree without heartwood). In *Callistemon citrinus* and *Manilkara hexandra* staining was noted from outer sapwood to sapwood-heartwood boundary.

According to Gahan[1] accumulation of neutral red and exclusion of Evan's blue are features characteristic of cells having an intact membrane system. A dead cell will take up both the stains. On this basis it may be presumed that parenchyma cells in the secondary wood of *Bauhinia purpurea* (30 cm in diameter) have an intact membrane system from outer xylem to inner xylem. Also these cells are capable of radial transport. Cells in the wood of *Callistemon citrinus* and *Manilkara hexandra* have intact plasmalemma up to sapwood-heartwood boundary beyond which no conduction of dye occurs. This may be due to the deposition of phenolic compounds/extractives in the cells and vessels. Absence of dye in the cells with phenolic contents in the sapwood-heartwood boundary supports this assumption.

The terminal oxidase system of mitochondria appears to be intact in parenchyma cells as they give blue colouration only at some loci in which mitochondrial reoxidation of Janus green B takes place. Some axial parenchyma cells of the middle sapwood and inner sapwood in *Callistemon citrinus* were seen to accumulate Evan's blue. These cells and cells near sapwood-heartwood boundary appear completely blue when stained with Janus green B. It might be due to rupturing of these cells near the bore or due to the loss of semipermeable properties of plasma membrane in the sapwood region or due to deposition of phenolic compounds in the sapwood-heartwood boundary. Neutral red and Evan's blue accumulate in the xylem



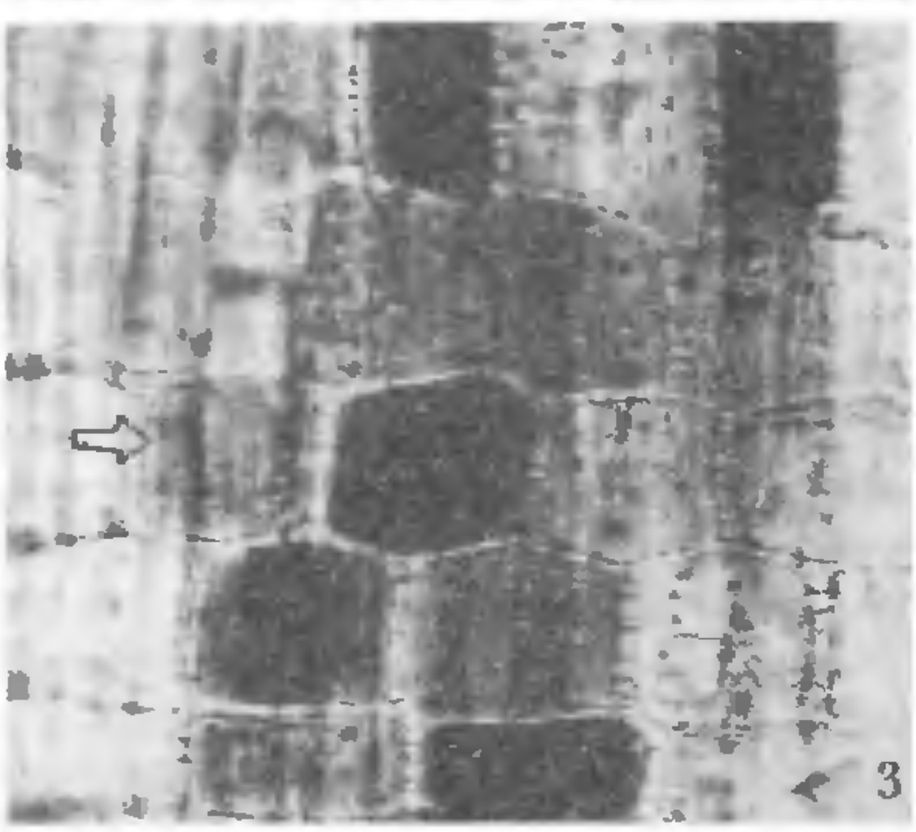
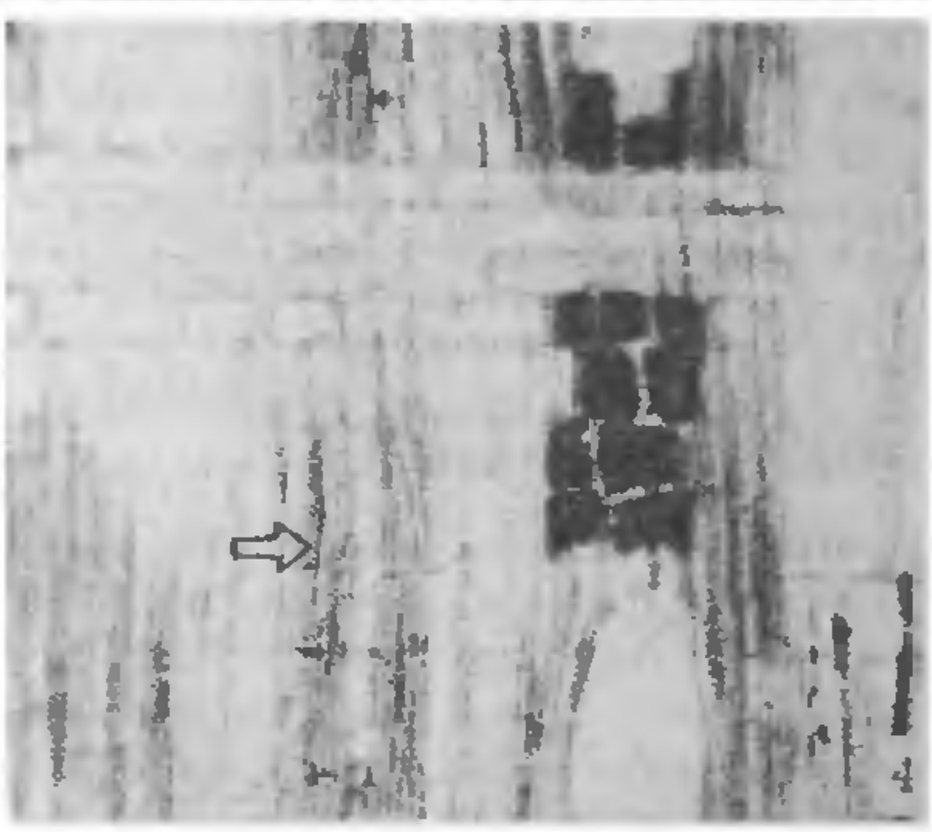
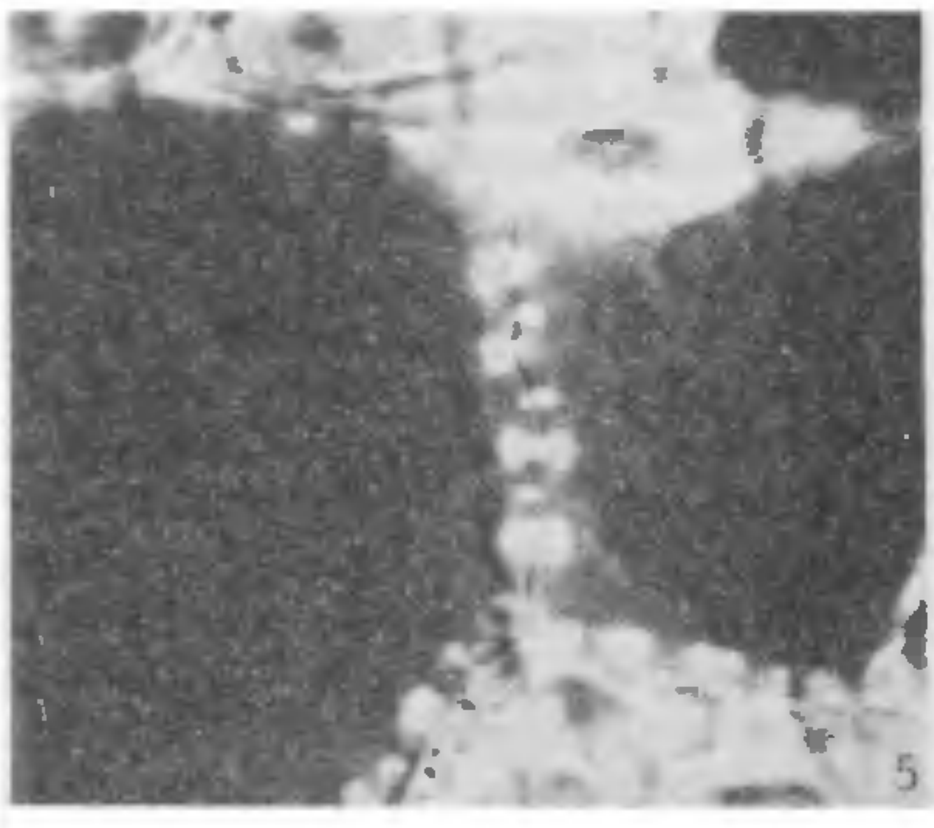
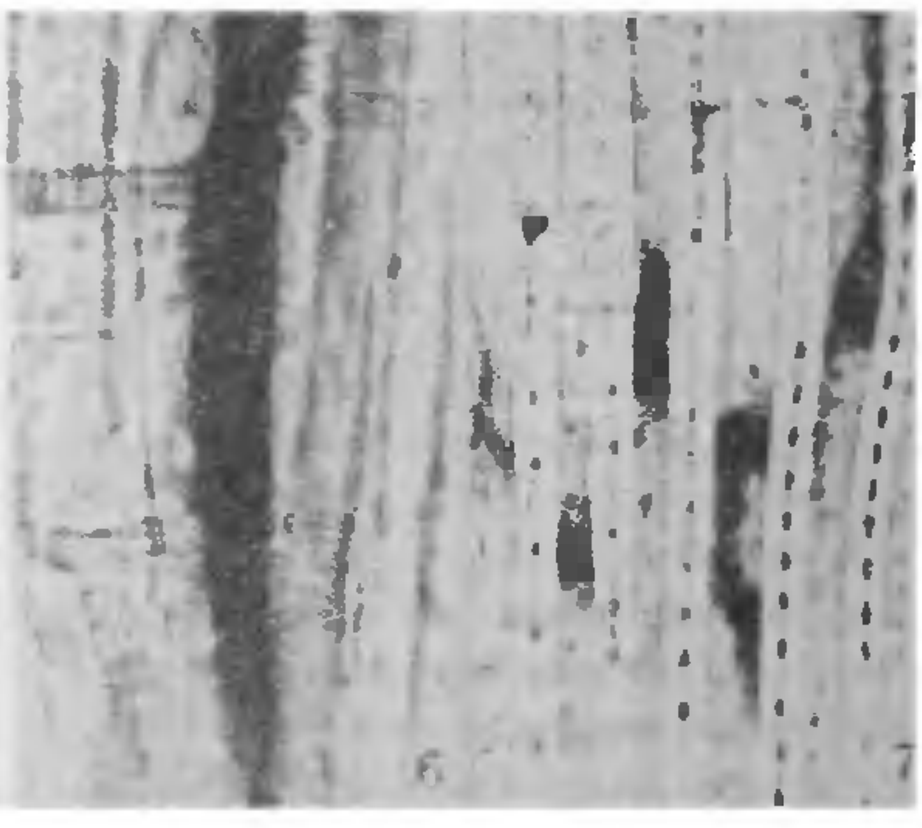





Figure 1–6. Neutral red staining *in situ*. **Figure 7.** Evan's blue staining *in situ*. **1.** Ruptured wood elements in the immediate vicinity of the bore (arrow) (× 60). **2.** Neutral red accumulation in the contact parenchyma cells (× 592). **3.** A gradient of colour intensity from contact cell to non-contact cell (arrow) (× 370). **4.** Accumulation of neutral red in contact cells and fibres (arrow) (× 135). **5.** Cell lumen and pit cavities filled with dye (× 1140). **6.** Fibres with neutral red (× 592). **7.** Evan's blue in fibres (× 592).

fibres near the bore possibly because there is no control on the entry of dye since plasmalemma of fibres is disorganised during the course of its maturation. Hence dead fibres accumulate both the dyes *i.e.* neutral red and Evan's blue in their lumen (figures 6, 7).

Cell walls of ruptured wood elements near the bore were stained by neutral red; also the walls of vessels in which vertical translocation of dye took place were stained. The cell in which the dye accumulates does not show staining of its wall (figure 5). No wall staining was reported by Evan's blue either in the ruptured or in the intact tissue. It can be said that cell wall staining with neutral red is possible only when the dye comes in direct contact with it.

To conclude, it may be stated that most of the parenchyma cells of sapwood in all the three species studied are living and can transport dye molecules across the pits in radial direction. The death of the parenchyma cell primarily takes place at the sapwood-heartwood boundary in plants having a heartwood and most probably it is due to deposition of phenolic compounds in the pits and lumen of the cells causing physiological isolation of a cell and its ultimate death.

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1. Gahan, P. B., In: *Growth regulators in plant senescence*, British Plant Growth Regulator Group, 1982, p. 47.
2. Willmer, M. W., *Protoplasma*, 1976, **87**, 253.
3. Lekhak, H. D. and Sen, D. N., *Biol. Plant (Prague)*, 1982, **24**, 101.
4. Gahan, P. B., In: *Cell death in biology and pathology*, Chapman and Hall, London, 1981, p. 145.
5. Gaff, D. F. and Okango'O-Ogala, O., *J. Exp. Bot.*, 1971, **22**, 756.
6. Levitt, J., *Introduction to plant physiology*, Mosby, 1969, p. 13.
7. Lazarow, A. and Cooperstein, S. J., *J. Histochem. Cytochem.*, 1953, **1**, 234.
8. Lillie, R. D. and Fullmer, H. M., In: *Histopathologic technique* and *practical histochemistry*, McGraw Hill, New York, 1976, p. 327.

ANNOUNCEMENT

ENZYMES FOR GENETIC RESEARCH

For the first time enzymes used for genetic engineering research will now be available for research institutions in the country. This will go a long way to save foreign exchange and boost the country's biotechnology research programme.

The first three enzymes and other products which constitute a basic tool in genetic engineering research was released by Prof. M. G. K. Menon, Member Planning Commission at a simple function held at New Delhi.

Enzymes and other products are jointly produced by the Council of Scientific and Industrial Research (CSIR) Centre for Biochemicals—Indian Institute of Chemical Biology, Calcutta, National Chemical Laboratory, Pune, Department of Environmental Sciences of Jawaharlal Nehru University and School of Biological Sciences, Madurai Kamaraj University, Madurai.

Speaking on the occassion Dr S. Varadarajan, Secretary, Department of Science and Technology said the government would provide all facilities to the scientists engaged in genetic engineering research.

Dr G. S. Sidhu, Director General CSIR said enzymes would be used to bring about cutting of DNA chains at an appropriate site.