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Effect of stimulation in the stress responses in *Hevea brasiliensis*

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Stimulation of latex production by external application of ethephon (2-chloro-ethyl phosphonic acid) on the bark of the trees has been a common commercial practice in rubber plantations for increasing the productivity of the crop. Ethephon application prolongs the latex flow and thereby increases the total latex volume during tapping (Ho and Paardekooper, 1965). It has been reported that ethephon stimulation increased the internal pressure within the latex vessels by changing the water relations. Ethephon stimulation also caused change in the physiological properties governing the flow of latex, the modifications occurring in the latex vessels, which do not plug up so easily (Hanower *et al.*, 1979). Over stimulation with ethylene releasing compounds on the *Hevea* bark reported to induce formation of metabolic disorders like abnormal thickening of the bark, spongy appearance of necrotic areas, small local cracks and appearance of non-productive zones along the tapping cut etc. (Paranjothy *et al.*, 1979).

Ethephon application induces several compounds, which can make metabolic changes in the laticiferous tissue. It has been shown that ethephon stimulation of trees increases the general latex metabolism, including over-expression of some genes in the laticiferous tissue (Pujade-Renaud *et al.*, 1997). Some of the effects of stimulant application on the physiological response of laticiferous tissue have been studied earlier (Coupe and Chrestin, 1989; Yang and Fan, 1995; d'Auzac *et al.*, 1997). Chrestin (1989) has suggested a possible oxidative stress in lutoid cells in the latex due to etheral application. Several studies were made on the physiological effect of stimulation and exploitation intensities in relation to tapping panel dryness (Vijayakumar *et al*., 1991; Xu *et* al., 1994; Nair *et al.*, 2004). Apart from these, no studies were made on ethephon-induced stress response in *Hevea.* The present study was taken up to closely observe if a single dose of ethephon application can lead to stress responses in the tissue.

Twenty year old trees of *Hevea brasiliensis* (clone RRII 105) which were never stimulated before were selected for this experiment. The trees were in the sixth year of tapping under the alternate daily (½S d/2 6d/7) tapping system in the B-panel. The trees were divided into two groups of ten trees each with uniform growth. One group was stimulated with ethephon and the other kept as untreated control. The tapping panel of all the trees were scraped well (2.5 cm below the tapping cut) to remove the dead tissues and rubber particles before ethephon was applied. Commercially available ethephon (2-chloro-ethyl phosphonic acid) was diluted to 2.5 % with coconut oil and applied on the bark of the experimental trees and the control trees were similarly treated with coconut oil.

Latex C-serum and bark tissues from both the experimental and control trees were used for the following analysis. Samples were collected two times before stimulation at 10 days intervals (pre-treatment A and B) and 3rd and 10th day after stimulation (post-treatment C and D). Total latex yield in each tree was recorded by measuring the latex volume after each tapping. Determination of thiol levels in the latex was made using the method of Boyne and Ellman (1972). Proline content in the C-serum of the latex was analysed with acid ninhydrin reagent using proline as standard (Bates *et al.*, 1973). Ethanol (80 %) extract of the C-serum was used for determination of the phenol content (Swain and Hillis, 1959). Peroxidase enzyme activity in

the C-serum was determined after the method of Guilbault (1976). Wound induced ethylene produced in *Hevea* bark tissue was quantified through gas chromatography (Krishnakumar *et al.*, 2006).

The total latex volume was comparable between the experiment and control groups of trees before the ethephon application. As expected the latex volume increased in the experimental trees after the stimulant application (Fig.1). A significant increase in turgur pressure, initial flow rate and decrease in plugging index are the factors leading to prolonged latex flow and increase in the latex volume due to ethephon treatment (Abraham *et al.*, 1971; Thomas *et al.*, 1999).

It was observed that the total phenol content in the C-serum increased significantly after the stimulant application and the level was maintained high till the end of the experiment (Fig. 2). In untreated control trees, phenol level in the latex C-serum was small and remained almost steady. Phenols are generally formed and accumulated in plant tissues when they are in a state of stress (Archer, 1963), including when the plants experience biotic stresses caused by pests and diseases (Gupta *et al.*, 1995). The accumulation of phenols in the latex of experimental trees indicates that stimulation led to internal stress in the laticiferous tissues, which is a general adaptation reaction noticed in *Hevea* trees due to stimulation.

The experimental trees had a high peroxidase enzyme activity in the latex C-serum immediately after the stimulant application (Fig. 3). Peroxidases often increase in plant tissues as a response to stresses and this has vital role in the cells protecting them from

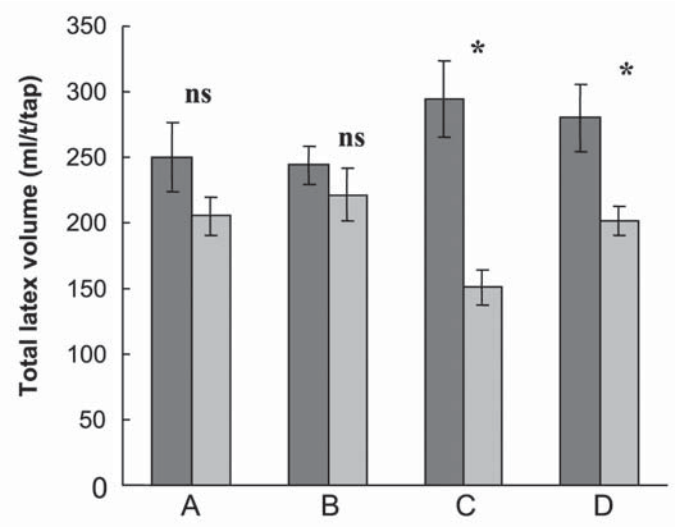

Fig. 1

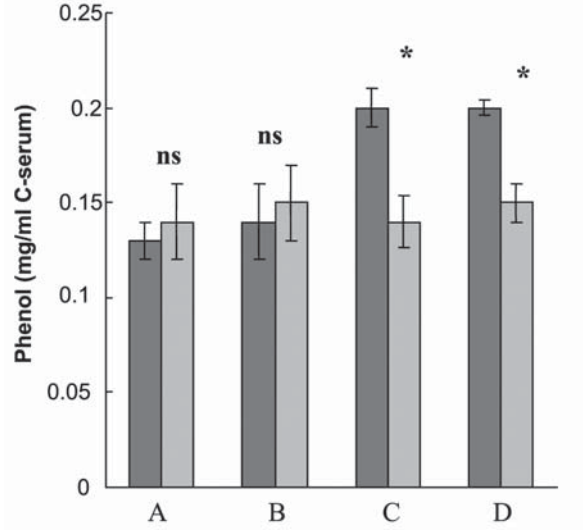

Fig. 2

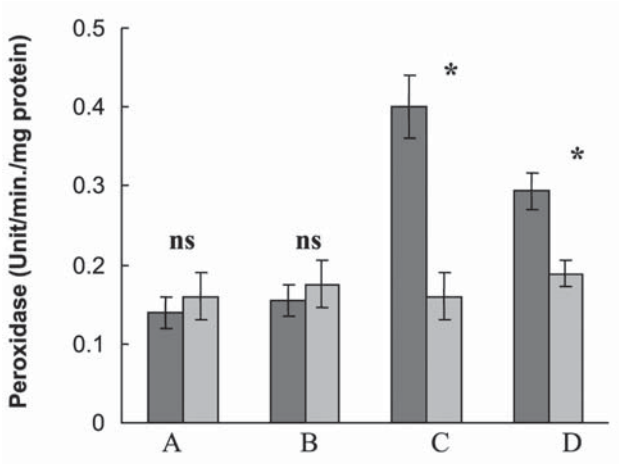

Fig. 3

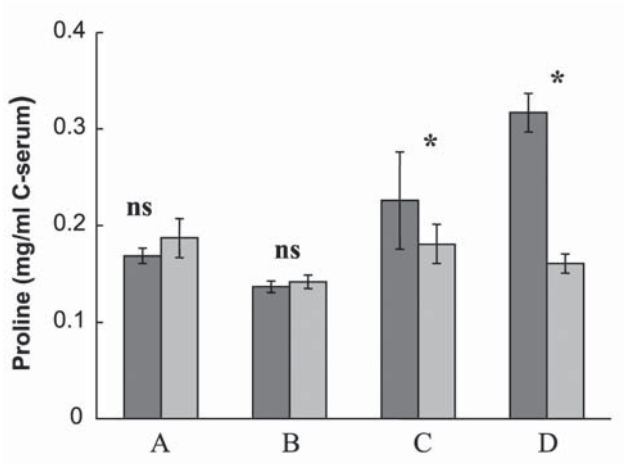

Fig. 4

Fig.1. Total Latex volume, Fig.2. Phenol content in the C-serum, Fig.3. Peroxidase enzyme activity in the C-serum, Fig.4. Proline content in the C-serum during pre-treatment (A& B) and post-treatment (C & D) periods in the experiment (■) and control (□) groups of *Hevea brasiliensis* (n = ± SE; ns = not significant; * P ≤ 0.01)

oxidative damage (Siegel, 1993). Peroxidases not only remove the toxic peroxide radical but also immobilize the toxic phenolic-ligin precursors in plant tissues (Pauls and Thompson, 1984). In short, the high phenol levels and peroxidase enzyme activity noticed in the stimulated *Hevea* trees are indications of intense stress response by the laticiferous tissues due to ethephon application. Proline content also increased with the application of ethephon (Fig. 4). Proline has been known to accumulate in plant tissues as a common reaction to various forms of physiological stresses. It has been demonstrated earlier that intensive tapping along with ethrel stimulation aggravated the localized water deficit in the tree, and that the corresponding rise in proline level was an indication of the general adaptation reaction for all physiological response of the tree to stress (Yang and Fan, 1995).

A significant decrease in the thiol content was noticed in the latex of the stimulated trees within three days after the stimulant application. However, the thiol level attained almost normal level by the 10th day after the treatment (Fig.5). The low thiol content in the latex of the ethephon treated trees indicates that they were experiencing internal stress as a result of ethephon application.

Wound-induced ethylene produced by the bark tissue of both stimulated experimental and unstimulated control trees was more or less in the same level before stimulation. But after three days of stimulation this was remarkably high in the ethephon treated trees than the untreated controls (Fig. 6). Exogenous ethylene is known to autocatalytically enhance the production of endogenous ethylene (Kevin *et al*., 2002). This may be responsible for the observed enhanced ethylene production by the bark tissues even 10 days after ethephon application. There are reports that excess levels of ethylene in plant tissue can cause oxidative stress and thereby damage the tissues and organelles by changing the normal physiological and biochemical activities leading to irreversible damage and senescence (Siegel, 1993). The high ethylene level generated in the bark tissues of the ethephon treated trees may eventually result in oxidative stress leading to oxidative damage of the laticiferous tissues.

Our earlier results indicated that wound induced ethylene production was related to oxidative stress and tapping panel dryness (TPD) (Krishnakumar and Jacob, 2003). Several studies have shown that over-stimulation with ethephon will lead to TPD (Vijayakumar *et al*., 1991). In the present study, even a single application of ethephon led to significant stress responses in the bark and latex, a general adaptation mechanism, which when exceeds the metabolic capacity of the system can result in adverse physiological processes including TPD.

In the *Hevea* clone RRII 105, stimulation with 2.5 % ethephon resulted in an abrupt stress response. Only a single dose of stimulant application was required to trigger stress responses in the tissue. More investigations are required to understand the stress response of the tree when the stimulation frequency is increased. Assessing and characterizing these responses are important in careful and judicious use of ethephon for yield stimulation.

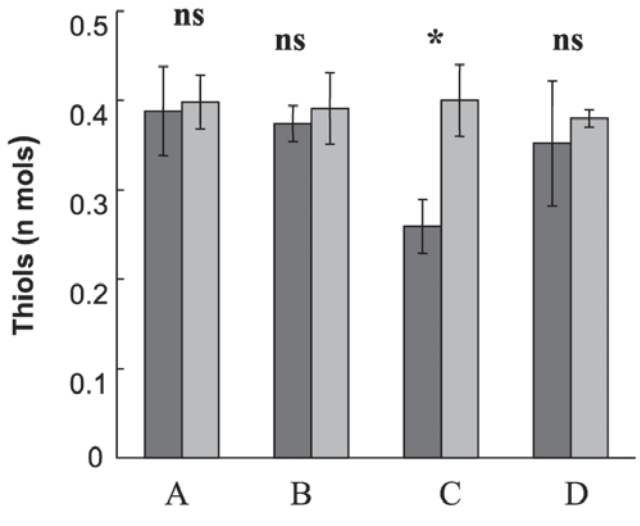

Fig. 5

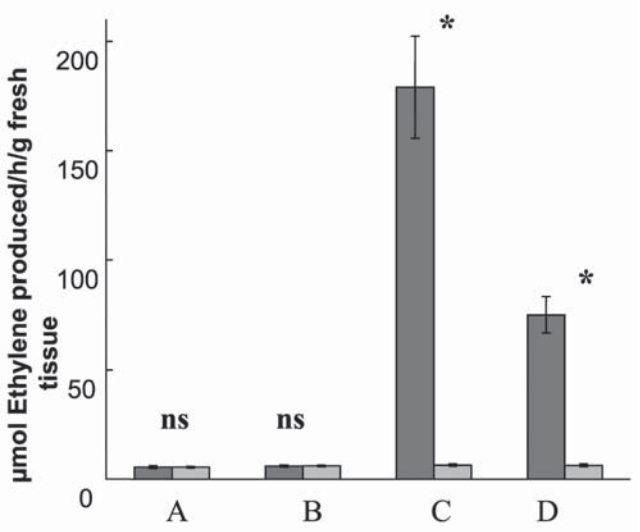

Fig. 6

Fig.5. Thiol content in the latex, Fig.6. Wound induced ethylene levels in the bark tissues during pre-treatment (A & B) and post-treatment (C & D) periods in the experiment (▩) and control (□) groups of *Hevea brasiliensis* (n = ± SE; ns = not significant; * P ≤ 0.01)

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Rubber Research Institute of India,
Kottayam-686 009,
Kerala, India

R. Krishnakumar,
P.K Ambily,
James Jacob