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# *Changes in Some Physiological Latex Parameters in Relation to Over-exploitation and the Onset of Induced Tapping Panel Dryness*

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*As tapping panel dryness of the rubber tree developed in response to various stress treatments, significant changes in invertase and polyphenol oxidase activities, total sugars and total solids in the latex were found to be associated with the intensive tapping and ethephon treatments These changes appeared to be related more to the exhaustion of the laticifer system (over-exploitation) than to the basic causes of dryness They were unreliable predictors of dryness in situations where the dryness was not accompanied by increased yield output, as exemplified by the pin-pricking stress treatment In the early stages of the stress treatments, latex proline and copper content increased with all the experimental treatments attempted, irrespective of whether yield output was increased These results suggest that an increase in latex proline or latex copper might serve as a warning indicative of incipient or impending dryness*

Even from the early days of rubber planting, tapping panel dryness, or its manifestation as brown bast in the later stages, has been recognised to be more prevalent when the tree is over-intensively exploited[1-3]. Hence, the disorder is commonly associated with physiological stress imposed upon the tree by excessive withdrawal of latex Rubber is the main component of latex (other than water) and it needs to be regenerated to replace the loss from tapping. There could therefore be a relationship between tapping panel dryness and the capacity of the tree to regenerate rubber Photosynthate in the form of latex sugars (mainly sucrose) are the basic substrate for rubber biosynthesis. The conversion of sucrose into reducing sugars by invertase is thought to be a rate-limiting step in the rubber biosynthetic pathway because of the very low invertase activity in latex[4]. Consistent with this hypothesis, invertase activity increases markedly when yield output is enhanced by stimulation with auxin or ethephon[5,6]. Therefore, the availability of sugars in the latex and the invertase activity in latex could be indicative of the capacity of the tree to replace the latex loss from tapping

The accumulation of proline in plant tissue is a common reaction to various forms of physiological stress, such as that induced by drought, cold or salt[7,8]. Whether this is an adaptive response is a subject of debate, but proline nevertheless serves as a convenient marker for physiological stress. As water is the main constituent of latex, it is not inconceivable

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that excessive withdrawal of latex would lead to a localised water deficit in the region of the tapping panel.

A decrease in polyphenol oxidase activity in the latex resulting from intensive tapping and subsequent onset of dryness has been reported[9]. The content of copper in latex has also been reported to be related to dryness[10,11] although its mechanism is less well understood.

The onset of tapping panel dryness has often been associated with latex instability within the latex vessels. This is believed to result in coagulation *in situ* leading to blockage of the latex vessels and the cessation of flow when the tree is tapped[12]. The effect of latex stability on the onset of dryness in this study will be reported separately.

In this study, changes in various physiological parameters are investigated in relation to the onset of dryness. To assess rubber biosynthesis and regeneration, latex sugars and invertase levels and the total solids (comprising about 94% rubber) in latex were monitored. Other stress indicators studied were proline and polyphenol oxidase. Changes in latex copper was also studied. Dryness was induced by two methods, *viz.*

(a) Intensive tapping and stimulation with ethephon to increase latex withdrawal and

(b) Insertion of pins into the panel[13] which induces dryness without increasing the rate of latex withdrawal.

## MATERIALS AND METHODS

### Experimental Design

The experiment was carried out on RRIM 605 tapped on *Panel B0-2*. Eight trees, comprising two blocks of four trees per treatment, were subjected to each of the following treatments aimed at inducing dryness of the tapping cut:

- Untreated control (tapped alternate daily, 1/2S d/2)
- Intensive tapping (tapped twice daily, 1/2S 2d/1)
- Intensive tapping + ethephon stimulation
- Tapped 1/2S d/2 with pin pricking (see below)
- Tapping and pin pricking + ethephon stimulation.

At the initiation of the pin pricking treatment, four evenly spaced steel pins were inserted into the bark (to tapping depth) just below the tapping cut. Another four pins were similarly inserted in a second row 0.5 m below the tapping cut and a further four pins were inserted 1 m below the tapping cut. One new pin was inserted into the bark below each of the above pins on every tapping day.

Ethephon stimulation was carried out with 10% *a.i.* ethephon applied to the tapping groove monthly.

Latex sample collections were made at approximately weekly intervals. On a designated latex collection day, collections were made from individual trees of one block of each treatment. Collections were made from trees in the second block the following week. Yield was determined as the dry weight of rubber per tree per week while dryness of the tapping cut is the length of dry cut expressed as percent of the total length of the tapping cut.

**Analyses of Physiological and Biochemical Parameters**

After tapping the tree, the first 3 ml of exuded latex was discarded and about 50 ml of the subsequent flow was collected from individual trees into ice-chilled containers. The latex and sera derived from it were kept chilled thereafter for the various laboratory analyses. Latex centrifugation was carried out at 19 000 r.p.m. (44 000 g) for 1 h in a Sorvall RC 5C centrifuge after which the serum phase (C-serum) was recovered. Latex pH was estimated from the C-serum pH within three hours of tapping. Following this, invertase activity in the C-serum was assayed by the rate of sucrose hydrolysis at its native pH (*i.e.* unbufferred) by incubating a mixture of 1 ml C-serum, 0.1 ml 1.62*M* sucrose and 0.66*M* sodium fluoride at 30°C for 30 min. Other details of the assay have been described previously[14].

Total latex sugars was determined by the anthrone method based on Dische[15]. Latex was coagulated by adding 10% v/v of 5% trichloroacetic acid. A 2 ml aliquot of the aqueous extract was reacted with 10 ml 0.2% anthrone.

To determine polyphenol oxidase (PPO) activity, a portion of each latex sample was added to 0.125% Triton X-100 (1:4) before centrifugation. The detergent Triton X-100 ruptured latex organelles such as the Frey-Wyssling particles from which polyphenol oxidase was derived[16]. The serum phase of the centrifuged latex was extracted and PPO activity in this extract was determined as the rate of change of photometric absorbance at 400 nm using catechol as the substrate[9].

Determination of latex proline was based on the method of Bates *et al.*[17] A 0.25 ml aliquot of latex was added to 5 ml 3% sulphosalicylic acid. The mixture was stirred to coagulate the rubber and the latex serum recovered by centrifugation. A 2 ml aliquot of the serum was added to 2 ml acid ninhydrin and 2 ml glacial acetic acid. The mixture was boiled for 1 h and the reaction then terminated in an ice bath. The mixture was extracted with 4 ml toluene and the absorbance read at 520 nm.

To determine the latex copper content, latex was filmed and dried in the oven. The copper content in the film was assayed by atomic absorption spectrometry.

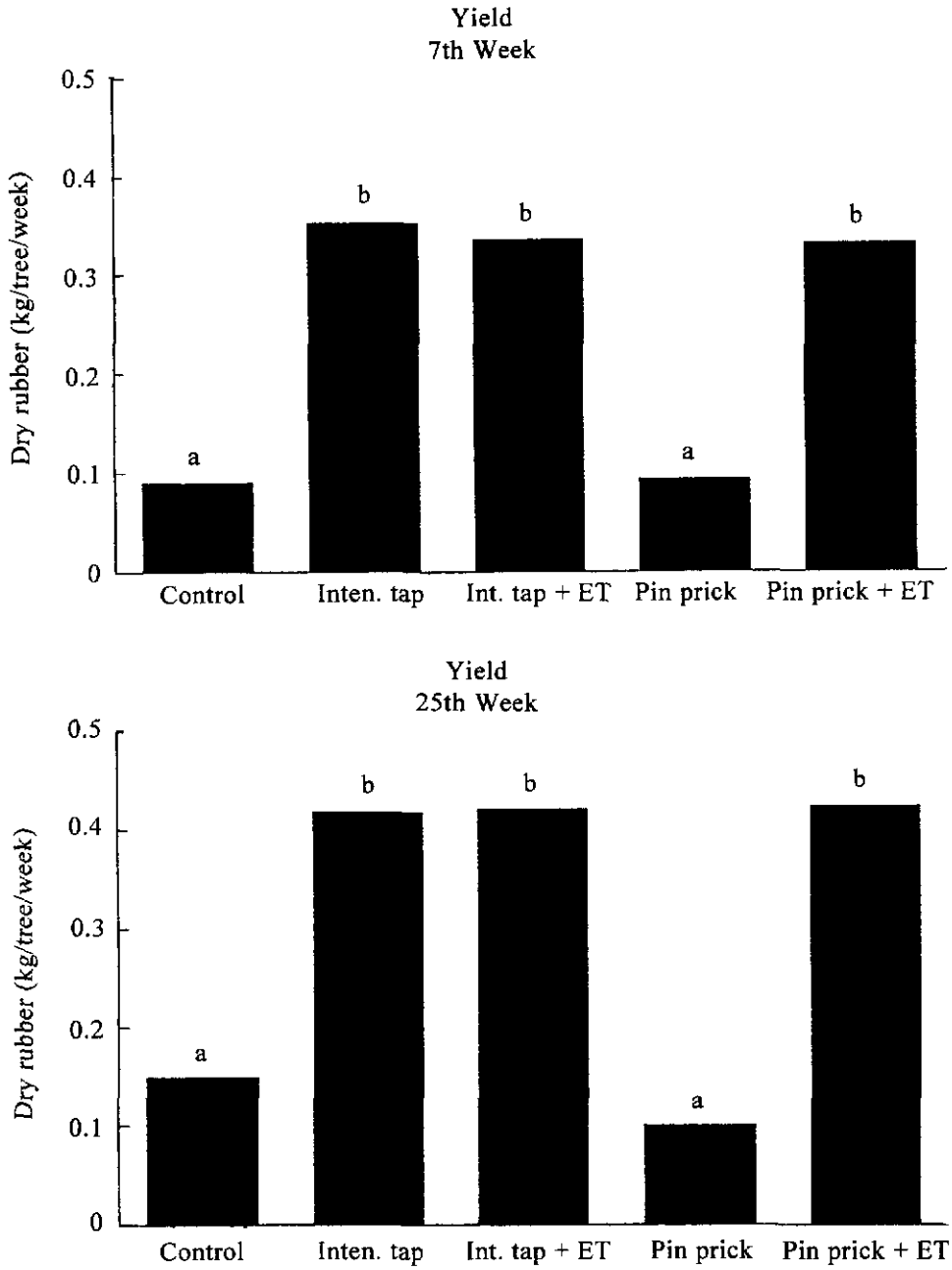
**Data Analyses**

The experimental data was analysed with the aim of revealing the trends in the physiological parameters during the short term and longer term from the time the stress treatments commenced. Readings representing the short term effects were taken at about the *7th week* after commencement of the experimental treatments (two sets of readings taken from the 6th to 9th weeks). Readings representing the longer term effects were taken at about the *25th week* (two sets of readings from the 24th to the 27th week). The exception was in the case of total carbohydrates where one set of readings were taken after 7 and 25 weeks. Analyses of variance were then carried out on the data for each period separately. The least significant difference between two treatments was computed at $P<0.05$.

## RESULTS

The means of each treatment for yield, dryness and the various physiological parameters studied are given in *Figures 1* to *9*.

The yield of dry rubber from the different treatments is shown in *Figure 1*. There was little difference in the yield trends for the




Inten. tap = Intensive tapping; ET = Ethephon application

*Figure 1. Effect of stress-inducing treatments on yield. Values bearing the same letter are not significantly different at $P<0.05$.*

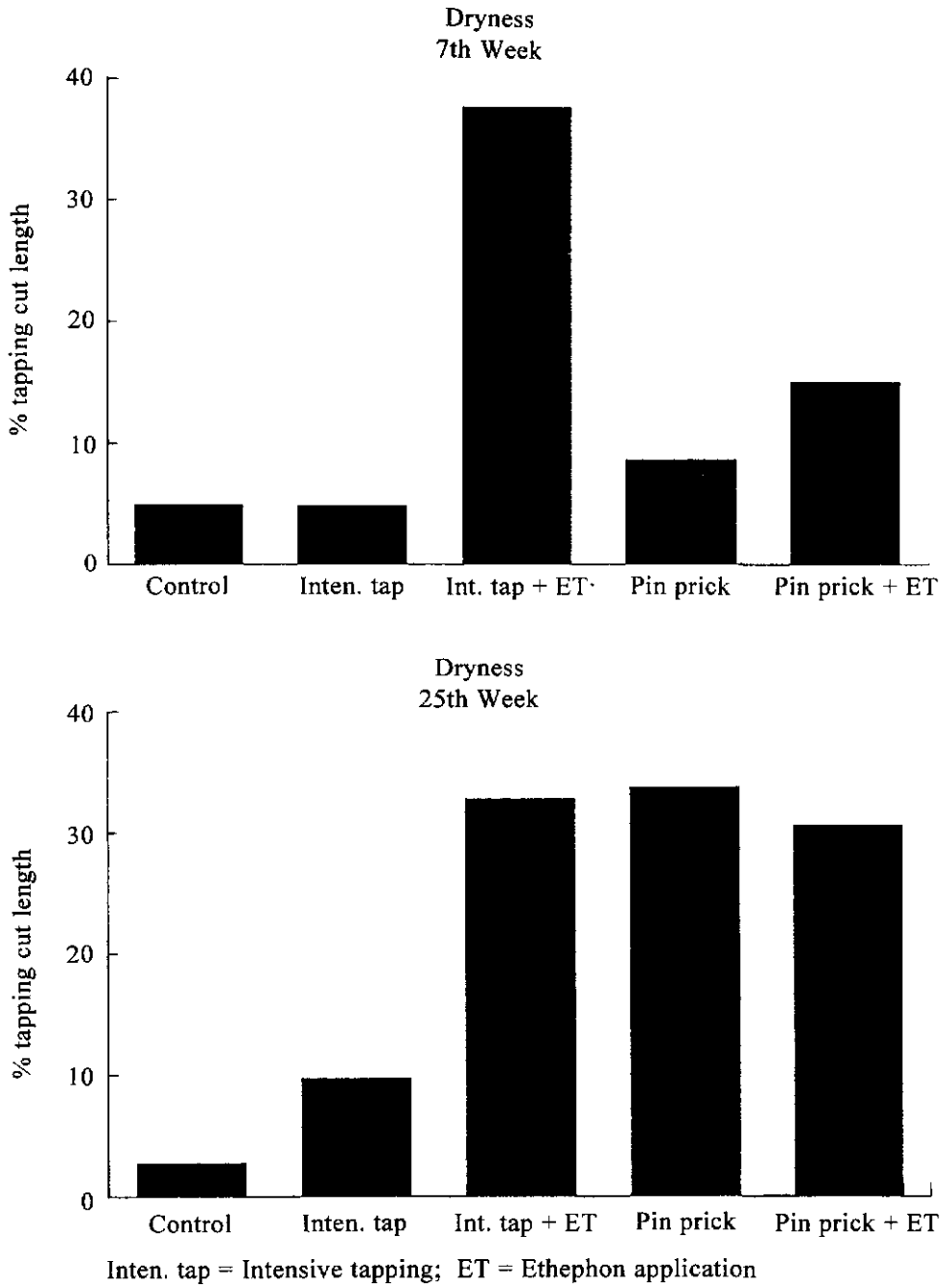
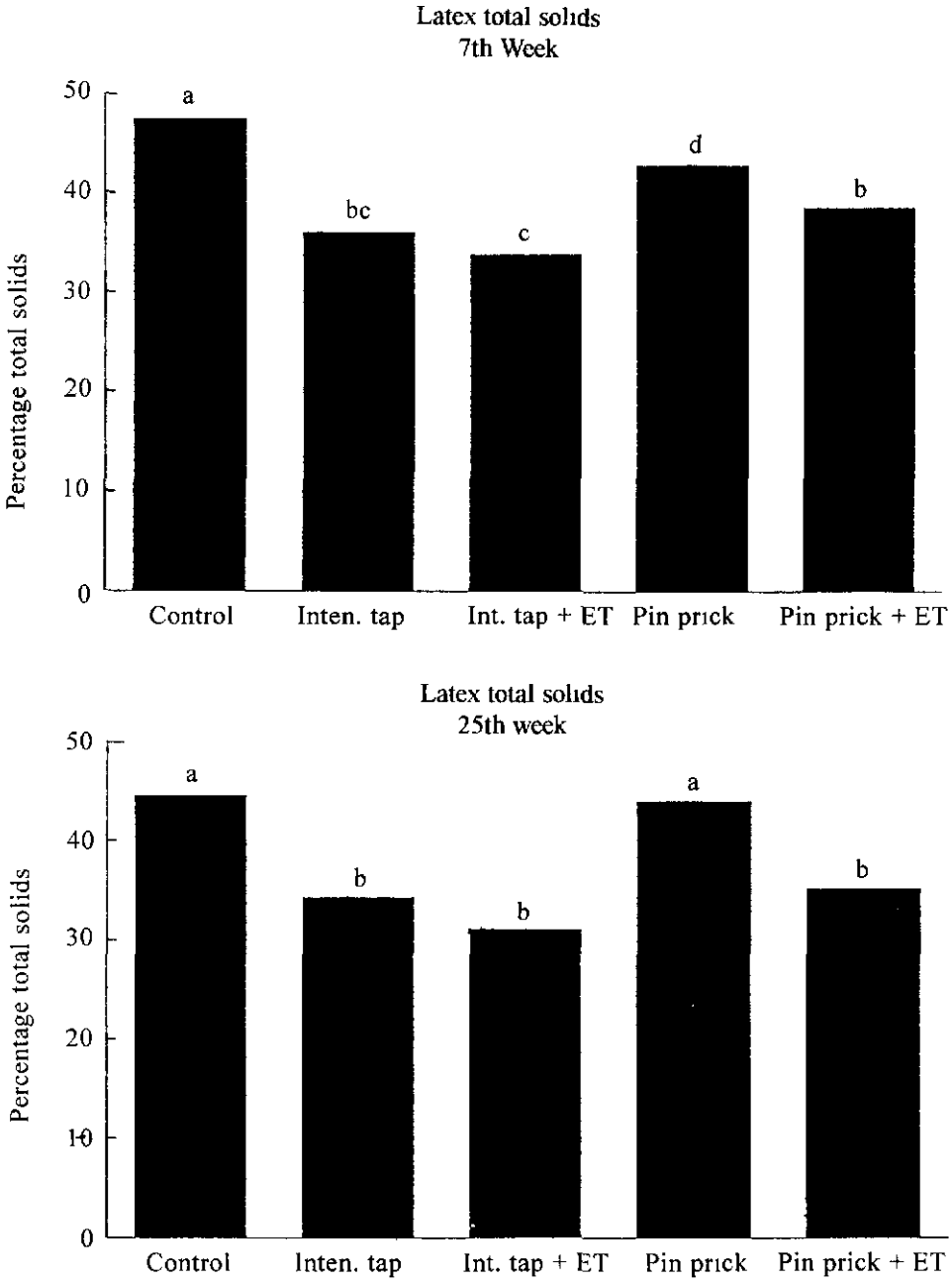



*Figure 2. Effect of stress-inducing treatments on dryness of the tapping cut.*




Inten tap = Intensive tapping; ET = Ethephon application

*Figure 3 Effect of stress-inducing treatments on latex total solids Values bearing the same letter are not significantly different at P<0 05*

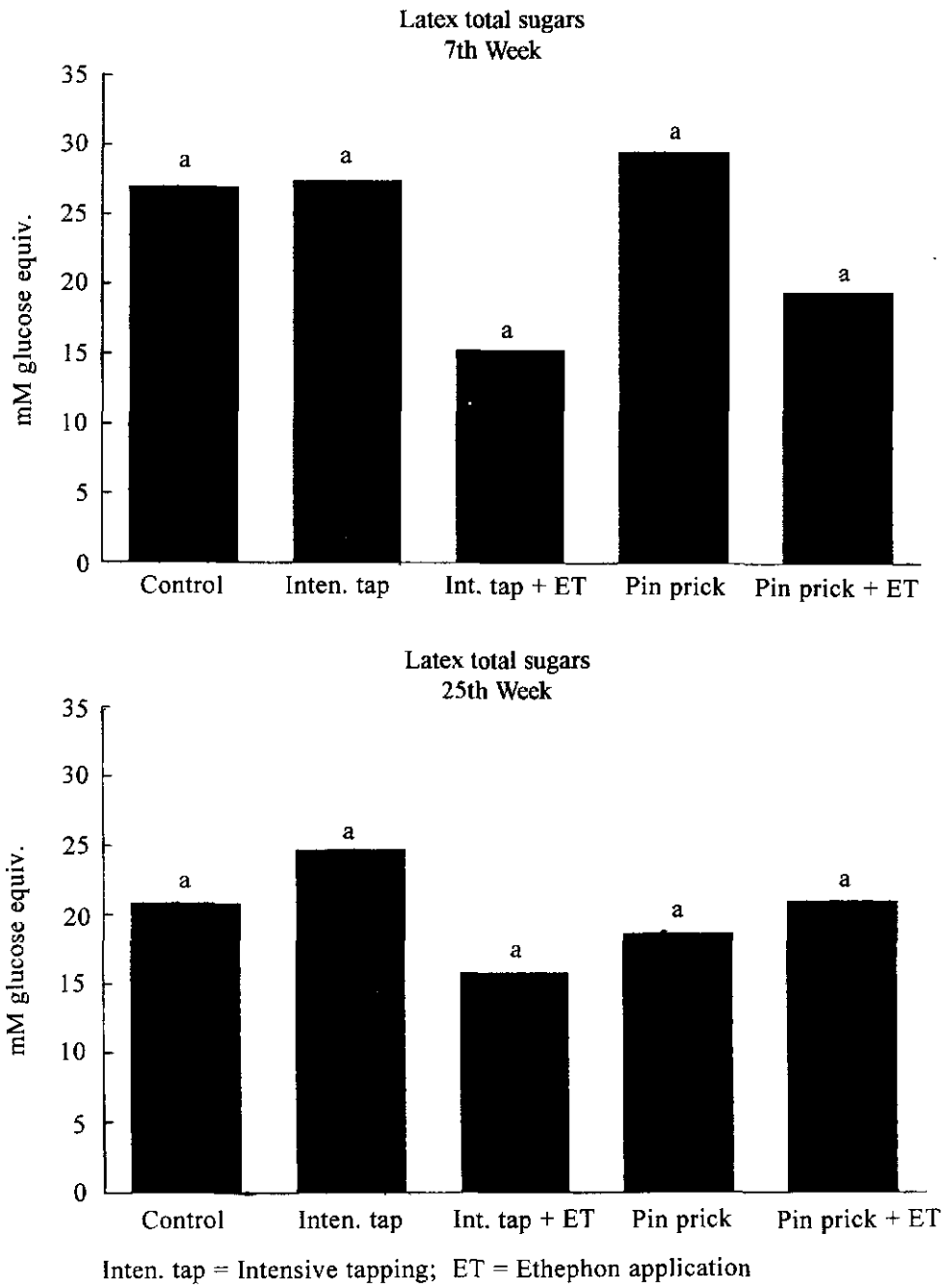
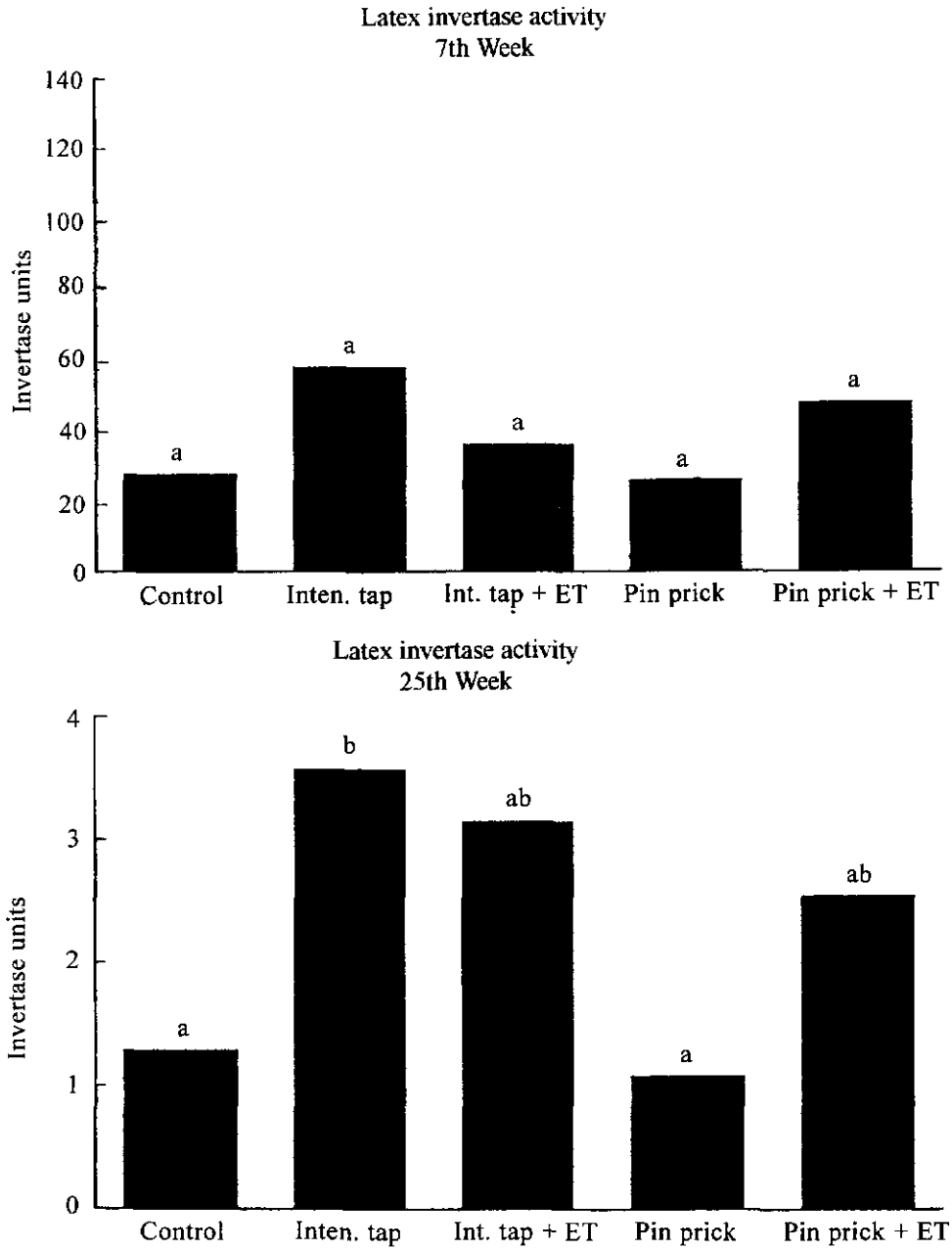
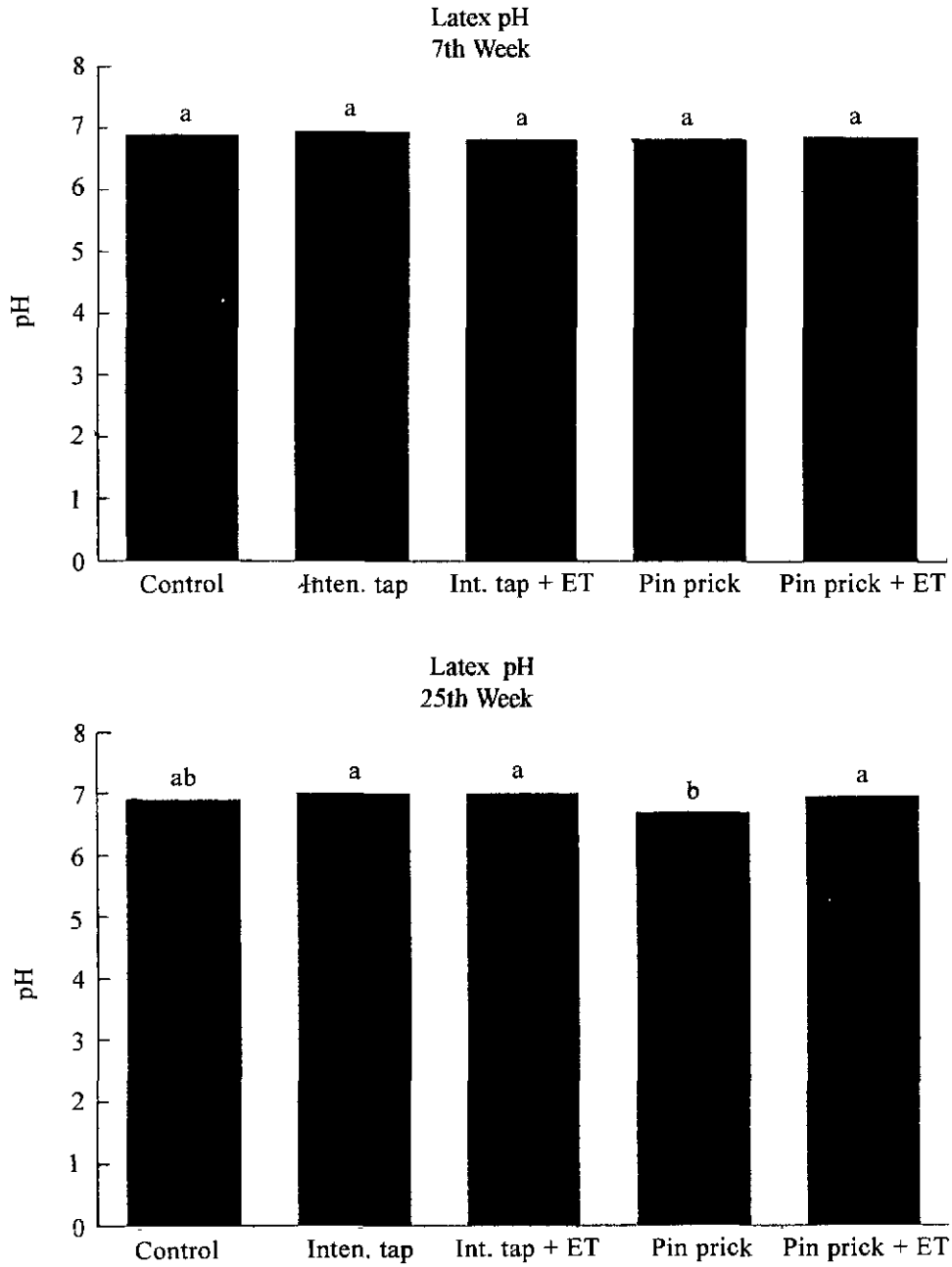



*Figure 4. Effect of stress-inducing treatments on latex total sugars. Values bearing the same letter are not significantly different at $P<0.05$.*



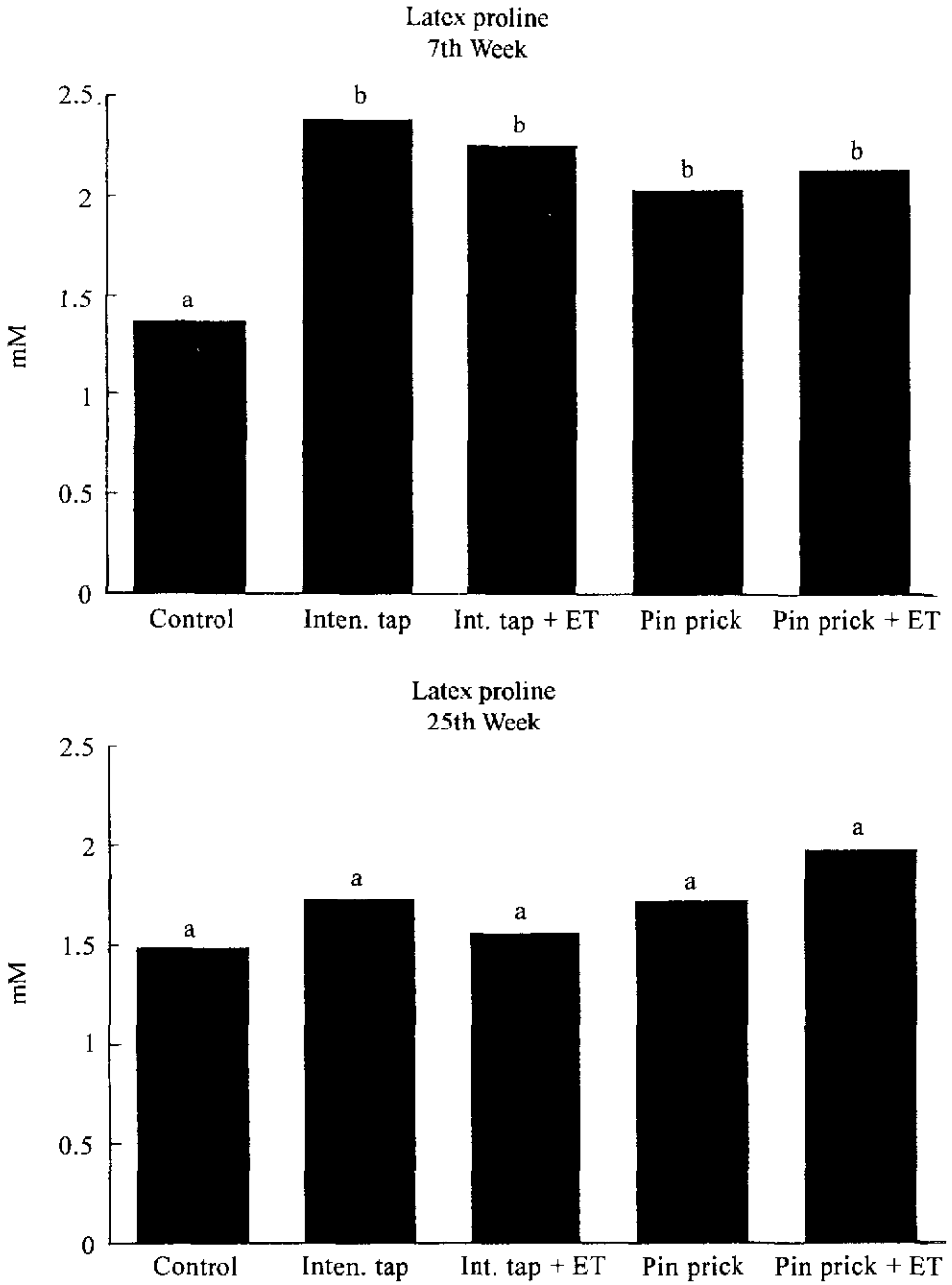

Inten. tap = Intensive tapping; ET = Ethephon application

*Figure 5. Effect of stress-inducing treatments on latex C-serum invertase activity. Values bearing the same letter are not significantly different at $P<0.05$.*



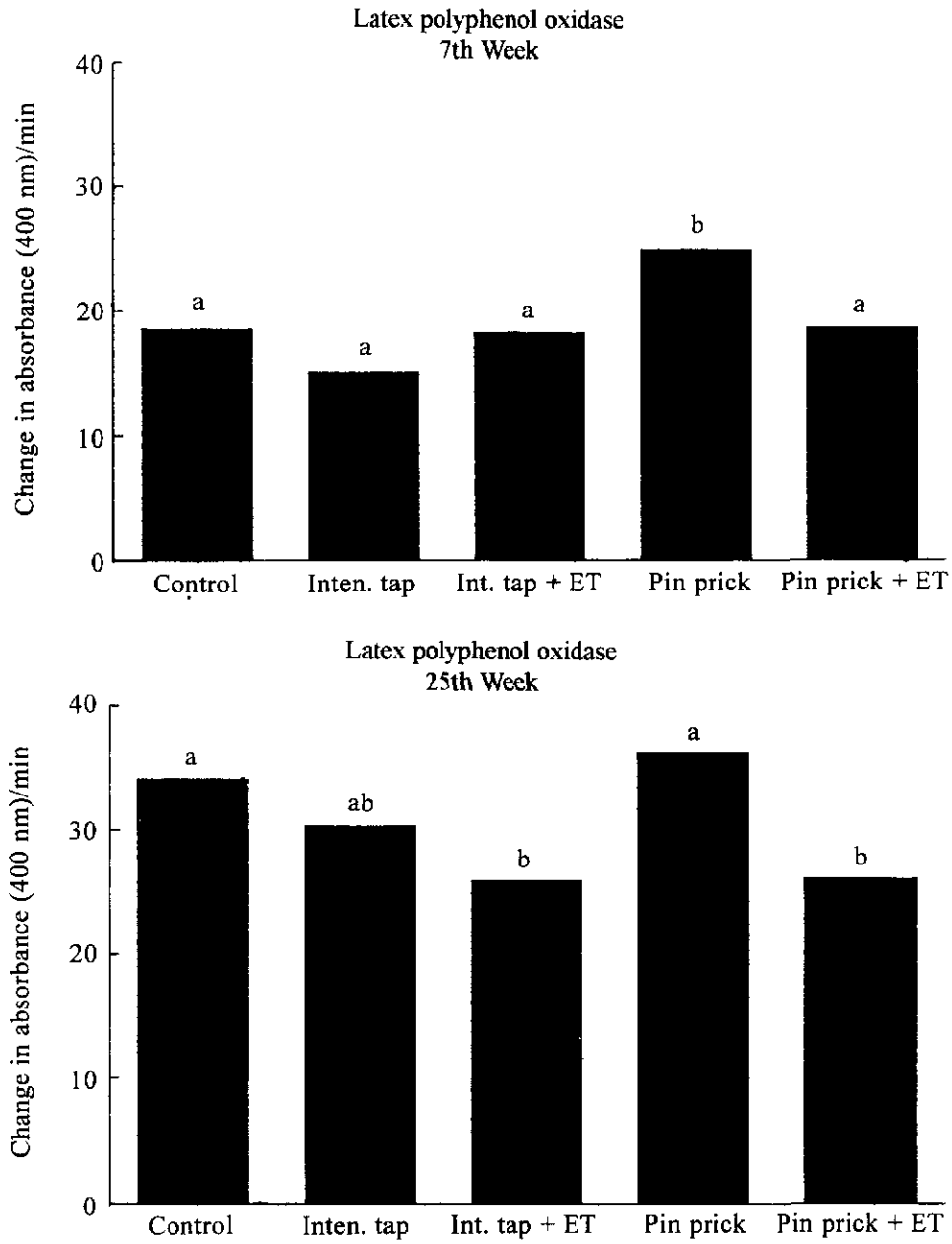

Inten. tap = Intensive tapping; ET = Ethephon application

*Figure 6. Effect of stress-inducing treatments on latex (C-serum) pH. Values bearing the same letter are not significantly different at $P<0.05$.*



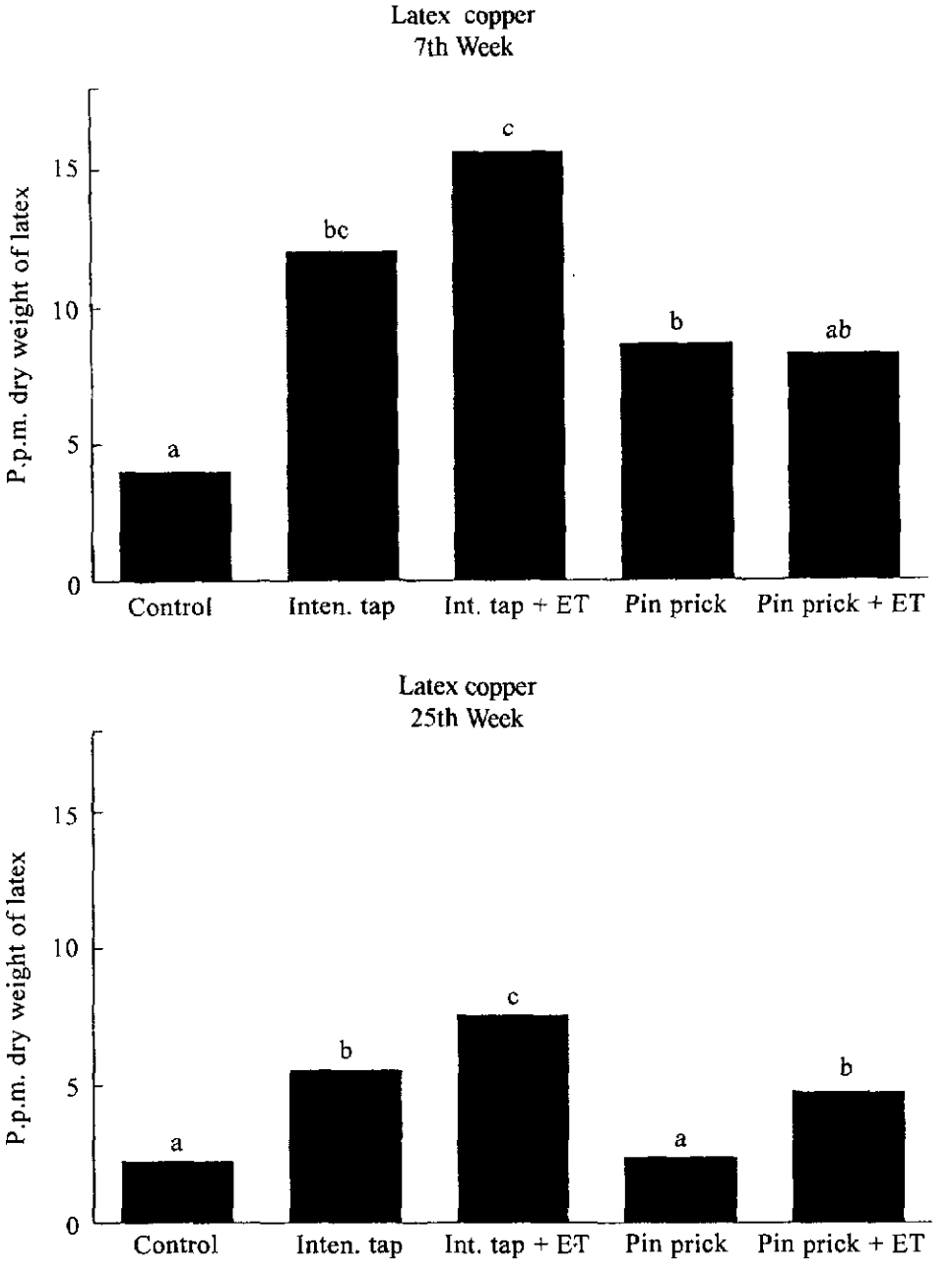

Inten. tap = Intensive tapping; ET = Ethephon application

*Figure 7. Effect of stress-inducing treatments on latex proline.*
*Values bearing the same letter are not significantly different at $P<0.05$.*




Inten. tap = Intensive tapping; ET = Ethephon application

*Figure 8. Effect of stress-inducing treatments on latex polyphenol oxidase activity. Values bearing the same letter are not significantly different at $P<0.05$.*




Inten. tap = Intensive tapping; ET = Ethephon application

*Figure 9. Effect of stress-inducing treatments on latex copper content. Values bearing the same letter are not significantly different at $P<0.05$.*

different treatments between the 7th and 25th weeks with treatments involving intensive tapping or stimulation giving significantly higher yields than control (approximately 3-fold calculated on a weekly basis). Intensively tapped trees did not increase further in yield when they were additionally stimulated with ethephon although the ethephon effect on yield was very evident in the case of the pin-prick treatment.

Although ethephon did not increase the yield output of intensively tapped trees, it increased the incidence of dryness markedly from as early as the 7th week when 37.6% dryness was recorded *(Figure 2)*. Intensive tapping alone did not induce much dryness at the 7th week and even the onset of dryness at the 25th week was relatively low at 9.7%. (Control trees had an average of 4% – 5% dryness.) The observation that intensive tapping did not result in massive panel dryness has been reported previously[9], even though physiological stress was evident from the low d.r.c. latex produced (see below).

The pin-prick treatments, with or without ethephon stimulation, gave rise to increased dryness at the 7th week, this condition developing further by the 25th week when over 30% dryness was recorded. Although ethephon increased dryness induced by pin-pricking earlier in the experiment, it had no additive effect by the 25th week *(Figure 2)*.

Commensurate with the rubber yield output, the intensive tapping and ethephon treatments showed significantly depressed total solids from the outset of the experiment *(Figure 3)*. In this study, the total solids were unusually high, being in the region of 44% for the control trees. Even with stimulation, the mean total solids did not drop below 30%.

Results from previous studies have shown that ethephon stimulation increased latex sugars initially[5,6], although latex sugars tended to fall below control levels on prolonged ethephon treatment[18]. In the present study, total sugars of latex from the ethephon treatments (in conjunction with intensive tapping and pin-pricking) had fallen below control by the 7th week *(Figure 4)*.

While the increase in latex invertase activity in response to yield stimulation is known[5,6], the current experimental results showed that intensive tapping also had the same effect *(Figure 5)*. Generally, therefore, treatments that increased latex output were associated with increased latex invertase activity. This effect was more prominent in the later part of the experiment *(Figure 5)*. At this time, intensive tapping induced a 2.5-fold increase in latex invertase activity. The enhancement of latex invertase by stimulation treatment has been reported to decline even below control when stimulation was prolonged[19]. This decline had not yet set in by the 25th week.

The pH of latex normally fluctuates within a very narrow range. While the increase in latex pH with ethephon stimulation that has been reported previously was observed[6], the increment was not significant when compared with the pH of latex obtained from control trees *(Figure 6)*. It was only in the pin-prick treatment (without ethephon) at the 25th week that showed significantly reduced pH compared with all the other treatments. Even in this instance, the mean pH of 6.7 for latex obtained from trees subjected to the pin-prick treatment was not significantly lower than that of control trees (pH = 6.9).

The effects of the various stress treatments on latex proline was very distinct at the 7th week of the experiment when latex proline increased significantly (by about 60%) for all treatments compared with control *(Figure 7)*. By the 25th week, however, the discrepancies in latex proline of these treatments from control

were considerably reduced and no longer statistically significant.

PPO activity in latex decreased with intensive tapping, in agreement with previous observations[9] *(Figure 8)*. However, the difference from control was not statistically significant. Ethephon treatment did not affect latex PPO initially, but it depressed the enzyme activity to a greater extent than intensive tapping alone by the 25th week. Ethephon applied to trees that were subjected to the pin-prick treatment gave rise to similarly depressed latex PPO activity. The diminution of PPO activity with ethephon stimulation has been noted previously[20,21].

All the stress treatments – intensive tapping and pin-pricking with or without ethephon – induced an initial upsurge of latex copper content *(Figure 9)*. The increase in latex copper content induced by intensive tapping[11,22] or by (auxin-based) yield stimulation[23,24] has been previously observed. In the present study, the effect was especially marked where ethephon application was combined with intensive tapping. By the 25th week, however, this response was no longer evident in the pin-pricked trees without ethephon application.

## DISCUSSION

While ethephon stimulation can stress the tree physiologically through exhaustion when the yield output is increased greatly, the stimulant can also induce other stress effects such as by increasing the production of toxic oxygen from lutoidic NAD(P)H oxidase activity[25]. Hence, the stress effects arising from exhaustion may not be entirely responsible for the occurrence of the irreversible panel dryness commonly called 'brown bast'. Similarly, tapping panel dryness has been induced by various forms of intensive tapping, up to 4 or 16 times normal intensity[12]. While such treatments undoubtedly did result in physiological stress to the tree, the excessive drainage of latex that was far greater than in normal tapping made it difficult to separate the effects of rapid exhaustion (over-exploitation) from the type of sustained stress that the tree may be subjected to under a less intensive tapping regime.

Of the four treatments adopted to induce tapping panel dryness in the present study, three of them involved increased latex withdrawal (intensive tapping or ethephon stimulation). However, the pin pricking treatment that has been shown to induce dryness effectively[13] does not incur an increase in latex output. As such, this combination of experimental treatments affords a good opportunity to view the onset of dryness in the presence or absence of the latex exhaustion effect. As the results of the study indicates, a comparison can be made between dryness associated with increased yield and the same degree of dryness (about 32%) without increase yield output. In this connection, the earlier readings at the 7th week might be of greater significance as it is more likely that these are associated with the onset of dryness and its cause. On the other hand, it is possible that some of the observations from the later readings could stem from the secondary effects of the dryness that had already set in.

Three of the physiological parameters studied were associated with latex regeneration and rubber biosynthesis. These are latex pH and invertase activity, latex total sugars and latex total solids (basically the rubber content). Changes in these parameters were seen only with the intensive tapping and ethephon treatments whereas trees subjected to the pin-prick treatment that did not cause increased latex drainage were not similarly affected. While changes in the latex serum pH occurred

within a very narrow range, it could be seen in the 25th week that treatments giving the lowest latex pH (control and pin pricking, *Figure 6*) also had the lowest latex invertase activity. Hence, there was evidence of possible pH control of latex (C-serum) invertase[6,26] activity. The trends in latex invertase activity appeared also to be related to the trends in latex total solids. Generally, latices with low total solids had high invertase activities. This implies that invertase activity increased in response to the need to replace lost rubber, but this increment was insufficient to compensate for the rate of rubber loss in the intensive exploitation treatments. Intensive exploitation involving yield stimulants also resulted in a decrease of latex sugars. This was not observed with intensive tapping alone (without stimulants).

The fact that the changes in latex invertase, sugars and total solids are prominent only when latex withdrawal is increased suggests that these changes are likely to be related more to over-exploitation leading to exhaustion of the laticifer system rather than be related to the basic causes of tapping panel dryness. The same trends can be said of latex PPO activity which is thought to be related to latex stability and hence to latex vessel plugging and the duration of flow. These physiological parameters that appear to respond more to latex output than specifically to the onset of dryness may have a limited utility as indicators of incipient or impending dryness. However, they could be unreliable where the occurrence of dryness is not accompanied by increased yield output, as exemplified in the case of dryness induced by pin-pricking. In commercial tapping, changes in such physiological indicators might not be well correlated with dryness in susceptible clones subjected only to moderate exploitation.

Two other parameters, latex proline and latex copper, might be more closely linked to the onset of dryness, at least in the stages of incipient dryness. Proline and copper contents in the latex increased in all the stress treatments at the 7th week, irrespective of whether yield output was increased. In the case of proline, the increase was not sustained into the 25th week *(Figure 7)*. With latex copper, the increase above control was observed up to the 25th week with the treatments that increase latex outflow, but not the pin-prick treatment where latex output was not enhanced. These results suggest that an increase in latex proline or latex copper might serve as a warning indicative of incipient or impending dryness. More work, especially with different clones, is required to verify the usefulness of these two parameters in indicating the state of physiological stress that would lead to dryness. Knowledge of the physiological states of the laticiferous system will determine the necessity for reducing exploitation intensity or even resting trees from exploitation to avert dryness with its deleterious consequences on yield productivity.

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