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# *Physiological and Nutritional Aspects in Relation to the Spontaneous Development of Tapping Panel Dryness in Clone PB 260*

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*The changes in several physiological and nutritional parameters were investigated in relation to the spontaneous development of tapping panel dryness in clone PB 260 These parameters were monitored at regular intervals on sixty core trees over a period of twenty-three months Dryness developed in several trees irrespective of their initial latex yield Among the latex physiological parameters studied, significant differences between dry and normal trees were recorded for four parameters* viz *bottom fraction volume, inorganic phosphorus, proline and free magnesium However, detailed examination of these parameters preceding onset of partial dryness on the cut in affected trees did not show any distinct pattern or trend that could serve as a consistent and reliable early warning of the impending onset of dryness Changes in the concentration of macro- and micro-nutrients in both latex and bark tissue did not show marked differences between normal and dry trees, but a significant increase in tylosis was recorded in latex vessels of dry trees*

**Key words:** tapping panel dryness, PB 260, bottom fraction, inorganic phosphorus, latex, magnesium, macro-nutrients, micro-nutrients, proline, tylosis, physiological parameters

Tapping panel dryness has long been recognised as a serious malady afflicting rubber trees, with consequent economic implications With the recent introduction of several precocious high yielding clones in rubber growing countries, tapping panel dryness has gained greater prominence due to the above-average susceptibility of these clones to this malady[1,2] In view of this, the International Rubber Research and Development Board (IRRDB) has initiated several joint research efforts in member countries to address the problem of high incidence of dryness in modern clones

Tapping panel dryness is seen largely as a physiological disorder Several researchers have reported that its development was linked to changes occurring in certain latex physiological and biochemical parameters in the latex vessels[3,4] It was decided among IRRDB researchers that one of the studies on

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this problem would be to monitor in network, field experiments pre-selected common latex physiological/biochemical parameters in trees, from the commencement of tapping, and to subsequently correlate changes in these parameters with the onset of natural dryness, if and when this occurs. This paper reports on data obtained from these studies in a field experiment on clone PB 260 carried out in Malaysia to examine the possible relationships of various physiological and biochemical parameters in relation to the onset of tapping panel dryness.

## MATERIALS AND METHODS

### Experimental Design

Six-year-old trees of the clone PB 260 planted in a commercial estate, and opened for tapping on 1/2S d/3 system in August 1994, were chosen for the IRRDB-network field experiment. Upon opening the trees for tapping, the first twelve recordings were used to classify the trees into three groups *viz.* low, medium and high yielders, with twenty trees per group. These sixty trees were the core trees for monitoring physiological, biochemical, nutritional and anatomical parameters. Field sampling and laboratory analyses for the physiological parameters was carried out weekly on each of the sixty core trees, while readings for latex superoxide dismutase (SOD) and the Bursting Index of lutoids were taken from 48 core trees (16 per yield group) at monthly intervals. Latex and bark samples for determination of macro- and micro-nutrient levels were sampled from all sixty core trees at biweekly intervals. Bark shavings for measurement of tylosis in latex vessels were sampled from eight trees at monthly intervals.

### Determination of Latex Physiological Parameters

Partial dryness of the tapping cut was measured as the length of the dry portion of the cut as percent of the total cut length.

To determine dry rubber content (D.R.C.), about 10 g of latex was accurately weighed in an aluminium coagulating dish. The latex was coagulated with acetic acid and heated over a steam bath until a clear serum was obtained before it was rolled to a thickness of 2 mm. The coagulum was thoroughly washed, and oven-dried at 70°C, for about 16 h.

The bottom fraction volume was measured by drawing fresh latex into haematocrit tubes, sealing the tubes and then centrifuging at 15 000 r.p.m. for 15 min using a micro-haematocrit centrifuge. The bottom fraction volume was determined as the length of the haematocrit tube occupied by the bottom fraction as percent of the total tube length.

To determine the Aerosol OT stability index[5], 8.5 mL of latex was equilibrated at 30°C for 15 min in a water bath. Aerosol OT (0.5%, 1.5 mL) was then added, shaken and kept in a water bath for 15 min. The mixture was filtered through muslin cloth to remove coagulated rubber and the filtrate was collected and measured.

Thiol measurements based on the method of Boyne and Ellman[6] were carried out by adding 1 mL of latex to 9 mL of 3% sulphosalicylic acid chilled in ice. After 4 h the extract was removed and stored. Three mL of the extract were added to 200 microlitres of DTNB (dithio-*bis*-nitrobenzoic acid) and 1.6 mL of Tris buffer (pH 9.7). The mixture was mixed and read in the spectrophotometer at 412 nm.

For proline measurements[7], 2 mL of 3% sulphosalicylic acid extract of latex was added to 2 mL of acid ninhydrin and 2 mL of glacial acetic acid. The mixture was boiled for 1 h and cooled in ice. The optical density was read at 520 nm.

Sucrose[8] was measured by adding 5mL of latex to 45 mL of 5% trichloroacetic acid. The extract was removed after 1 day and diluted 10 times. Two mL of the diluted extract was added to 10 mL of 0.2% anthrone in 95% sulphuric acid. The test tube covered with parafilm and cooled in ice. After boiling for 15 min, the reaction was terminated in ice. The optical density was read at 620 nm.

Latex was treated with 5% trichloroacetic acid to extract free potassium, magnesium, calcium and phosphorus (the last being commonly referred to in literature as inorganic phosphorus). Phosphorus in the supernatant after the rubber had coagulated was read directly by the molybdenum blue method using an autoanalyser. The rest of the extract was diluted and potassium was measured using the flame photometer, while magnesium and calcium were determined simultaneously using the atomic absorption spectrometer.

Measurement of the Bursting Index of lutoids was carried out according to the protocol described in the IRRDB manual on biochemical and physiological tests[9].

Latex superoxide dismutase (SOD) activity was determined using a procedure based on Polle and co-workers[10]. One part of latex (collected chilled) was added to four parts of extraction buffer, consisting of 0.125% sodium dodecyl sulphate, 0.5% Triton X-100® and 1% soluble polyvinyl pyrolidone in 100 mM phosphate buffer pH 7.8. The lutoids in the latex were ruptured in this preparation and the lutoid contents released. After centrifugation of the mixture at 44 000 g on a Sorvall RC5C centrifuge for 1 h, the serum phase ('latex extract') consisting of a mixture of *B-serum* and *C-serum* was recovered. Kinetic assays were carried out with the reaction mixture: 1780 µL buffer (62.5 mM $Na_2CO_3$, pH 10.2); 40 µL catalase (1.3 units $mL^{-1}$); 8 µL epinephrine (5.5 mg $mL^{-1}$ 100 mM HCL); 100 µL latex extract. Control reactions were carried out at the same time in the absence of the latex extract. Reactions were monitored at 480 nm over 1 min and the rate of reaction was estimated from a 30 s linear segment of the reaction rate curve SOD activity was determined as:

$$\frac{V \text{ control}}{V \text{ extract}} - 1 \text{ units} \qquad \ldots 1$$

where $V$ was the slope of the change in absorbance in the presence or absence of the latex extract. Assays were carried out in triplicate. In determining the specific SOD activity (SOD units per mg protein), total protein of the latex extract was assayed as described by Yeang and co-workers[11].

**Latex and Bark Analyses for Macro- and Micro-nutrients**

Analysis of elements in field latex was conducted on the raw total solid film prepared by spreading a thin film of latex on a flat surface and allowing it to dry. Bark samples collected from the same experiment were dried at 80°C in an oven. The dried samples were ground to pass through 0.5 mm sieve.

Nitrogen was determined by a semi-micro kjeldahl procedure. Rubber (0.1 g) was oxidised by heating with a catalyst mixture (15

parts of $K_2SO_4$ 2 parts of $C_uSO_4$ $5H_2O$ and 1 part of Se powder) and concentrated $H_2SO_4$ Nitrogen in the bark sample was acid digested About 50 g of oven-dried bark was digested with 2 g of sodium sulphate – selenium mixture and 2 mL of concentrated $H_2SO_4$ in a digestion block for 2 h Nitrogen was determined using an auto-analyser that employed the reaction of ammonium ion obtained after oxidation with alkaline reaction of sodium salicylate and sodium nitroprusside to form a blue complex The nitrogen concentration was read directly from the Alpkem computer

To measure phosphorus, potassium, magnesium, calcium, copper and boron in latex, 9 g of rubber was ashed at 500°C After ashing 10 mL of concentrated $HNO_3$ was added and re-ashed at 500°C for 30 min complete digestion For bark analyses, two grams bark samples for element determination was dry ashed in a muffle furnace at 550°C Fifteen mL of 1 6% $HNO_3$ were added and made up to 25 mL in a volumetric flask The digests were used for the determination of P and other elements Phosphorus was determined using an auto-analyser by the molybdenum blue method An aliquot of the digest was examined simultaneously for K, Mg, Ca, Cu and B using the Inductively Coupled Plasma Emission Spectrometer (ICP-ES)

**Quantification of Tylosis in Latex Vessels**

Bark shavings from normal tapping were macerated overnight in sodium hypochlorite solution (clorox) Glass beads were further used to help in the maceration This was followed by washing in 20% ethanol and staining in Sudan III for 2–3 minutes Washing in 50% ethanol was carried out twice before mounting in glycerine Tyloses were counted in at least 100 macerated latex vessels from three different regions of the tapping cut, with a total of 300 vessels examined per sample

## RESULTS

**Yield Performance and Incidence of Dryness**

The mean yield in g per tree per tapping (g/t/t) of normal trees (with no traces of partial dryness) are compared with that of trees with varying degrees of partial dryness, among the high, medium and low yielding trees (*Figure 1*) The yield in normal high yielding trees, with the exception of the decline noted during the wintering months, fluctuated during the four 6 months periods within a range of 80 g/t/t to 100 g/t/t, while in normal medium and low yielding trees, with the exception of similar depressions during wintering months, ranged between 40 g/t/t to 70 g/t/t and 30 g/t/t to 60 g/t/t, respectively

The trees developed varying degrees of partial dryness on the tapping cut Some trees recovered, while in others there was a progressive and consistent increase in length of dry cut over the period of 24 months Data given in *Figure 2* show the number of partially dry trees and the extent of dryness in the high, medium and low yielding trees The mean yield of all the five dry trees among the high yielding group were below those of normal trees In fact, the yield had progressively declined from a mean g/t/t of 60 in the first six monthly period to a low of 20 g/t/t in the third six monthly period Similarly in both the medium and low yielding groups, with the exception of the first 6 months, the yields were depressed to, below that of corresponding normal trees throughout the rest of the three six monthly periods

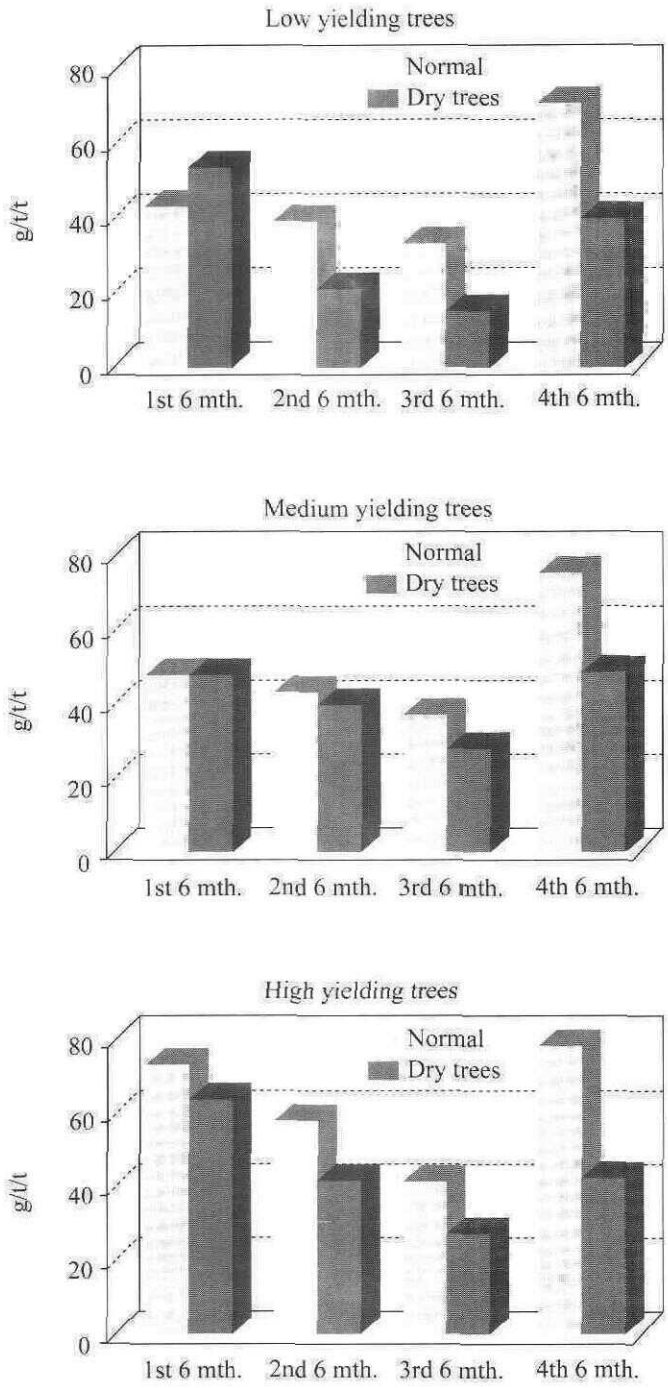



*Figure 1. Yield trend in normal and partially dry trees over a period of 24 months.*

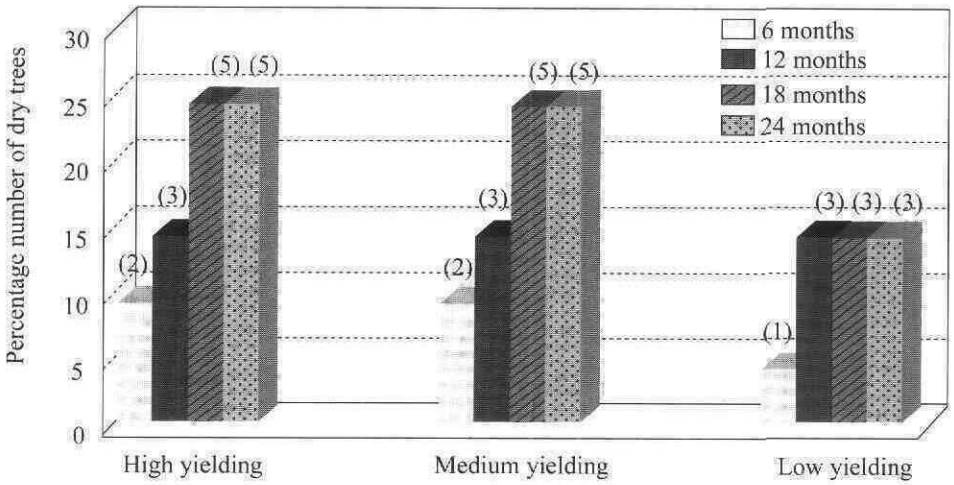



*Figure 2. Number of dry trees from high, medium and low yielding trees on clone PB 260.*

**Changes in Some Latex Physiological Parameters with the Onset of Dryness**

Data obtained over 24 months for selected physiological parameters *viz.* latex bottom fraction volume, D.R.C., Aerosol OT Stability Index, sucrose, thiols, proline and inorganic phosphorus, free magnesium, free potassium and free calcium were compared between partially dry trees and normal trees (*Table 1*). There were no significant differences in D.R.C. recorded between normal and partially dry trees. Neither were there differences between normal and partially dry trees for readings of Aerosol OT Stability Index, sucrose, thiols and free potassium.

The values for bottom fraction volume and levels of inorganic phosphorus were very significantly lower in partially dry trees when compared to values recorded for normal trees. Proline and free magnesium were significantly lower in partially dry trees, relative to values recorded for normal trees; while for free calcium, it was significantly higher in partially dry trees.

Four parameters with significant differences between partially dry trees and normal trees, namely bottom fraction volume, inorganic phosphorus, proline and free magnesium were further examined in detail to determine if there were marked or abnormal changes in these parameters prior to the onset of dryness on the tapping cut in affected trees. In *Figures 3* to *6*, readings from two selected partially dry trees were compared with the mean reading from the group of normal trees. The values for percentage bottom fraction compared between the normal trees and the two dry trees over 23 months show that the percentage of bottom fraction in both the dry trees were consistently lower than that of the normal trees after the onset of dryness (*Figure 3*). However, a marked decline in percentage of bottom fraction was recorded just prior to occurrence of partial dryness on the tapping cut in both the affected trees.

TABLE 1. MEASUREMENTS OF VARIOUS PHYSIOLOGICAL PARAMETERS IN NORMAL AND PARTIALLY DRY TREES

| Physiological parameters | Normal trees | Partially dry trees | T value |
|---|---|---|---|
| Aerosol OT Stability Index | 2.0 | 2.3 | -1.391* |
| Bottom fraction size (%) | 12.3 | 10.5 | 6.59*** |
| Dry rubber content (%) | 38.2 | 37.1 | 1.56 NS |
| Sucrose (mM) | 22.6 | 22.8 | - 0.343 NS |
| Thiols (μM) | 405 | 368 | 1.249 NS |
| Proline (μM) | 633 | 482 | 2.263* |
| Inorganic phosphorus (p.p.m.) | 720 | 653 | 3.874*** |
| Free magnesium (p.p.m.) | 159 | 137 | 2.813*** |
| Free calcium (p.p.m.) | 33 | 38 | -2.142* |
| Free potassium (%) | 0.192 | 0.188 | 0.972 NS |

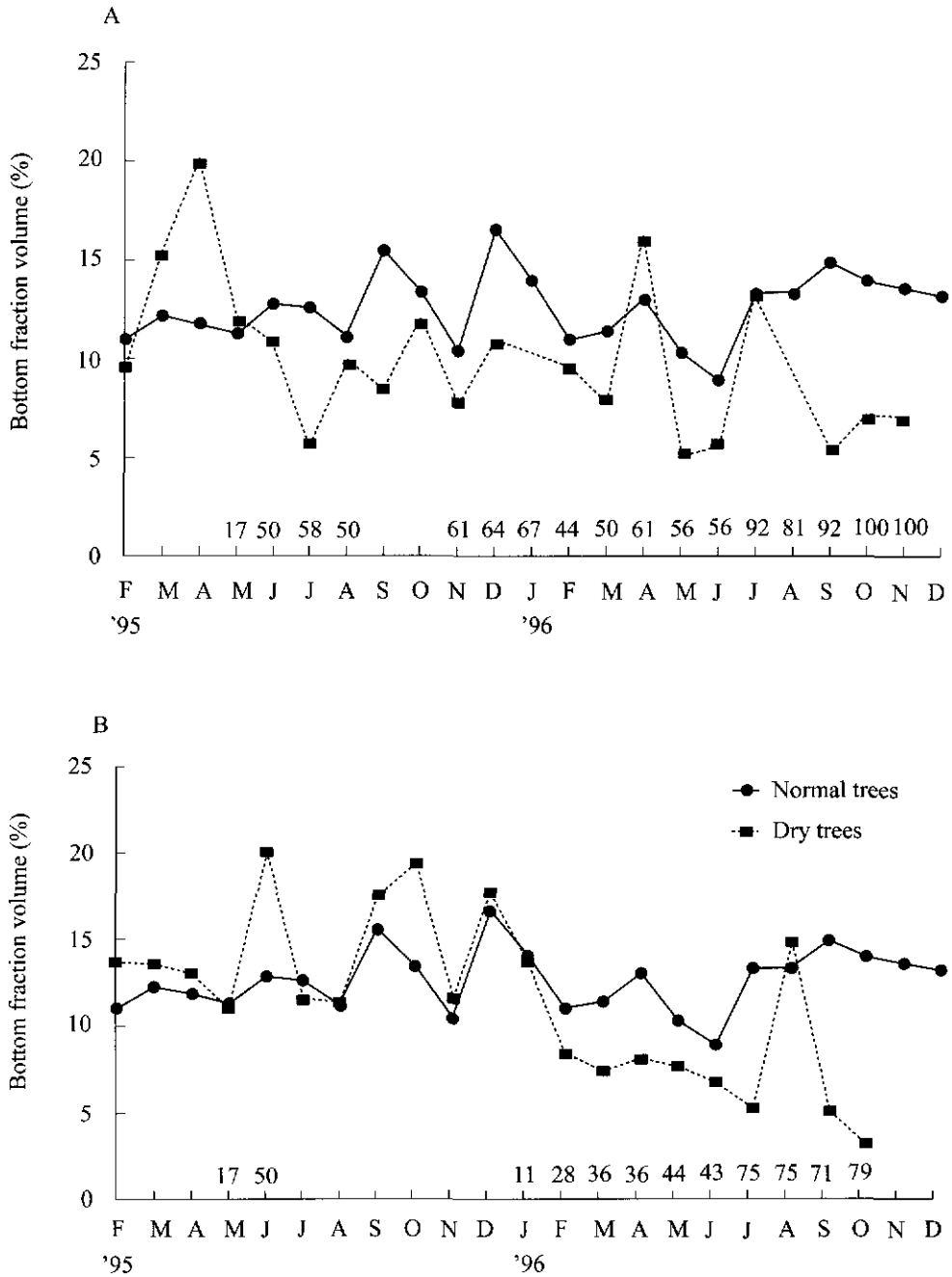
The t value denote statistical significance in the differences in readings between normal and partially dry trees. * $P<0.05$; *** $P<0.001$; NS = Not significant

The comparison of inorganic phosphorus levels between normal trees and two partially dry trees over 23 months in *Figure 4* shows no consistent pattern prior to the onset of partial dryness on the tapping cut. The levels of inorganic phosphorus in both the dry trees remained below that of normal trees after the onset of dryness on the tapping cut. A comparison of proline levels between normal trees and two partially dry trees over 23 months (*Figure 5*) shows that in both the partially dry trees, there was a decline, just prior to the onset of partial dryness on the cut. The level in both the dry trees remained below that of normal trees as dryness progressively developed on the tapping cut. The levels of free magnesium compared between normal and two partially dry trees over 23 months (*Figure 6*) shows no consistent pattern prior to the onset of partial dryness on the tapping cut.

In addition to the above mentioned parameters, two other latex physiological parameters, namely latex superoxide dismutase (SOD) activity and the bursting index of lutoids (BI), were recorded at less frequent intervals. The values obtained for these two parameters in both normal trees and two selected partially dry trees have been compared similarly (*Figures 7* and *8*). It is evident that for both these parameters, there was no consistent association seen between the two parameters and the development of dryness.

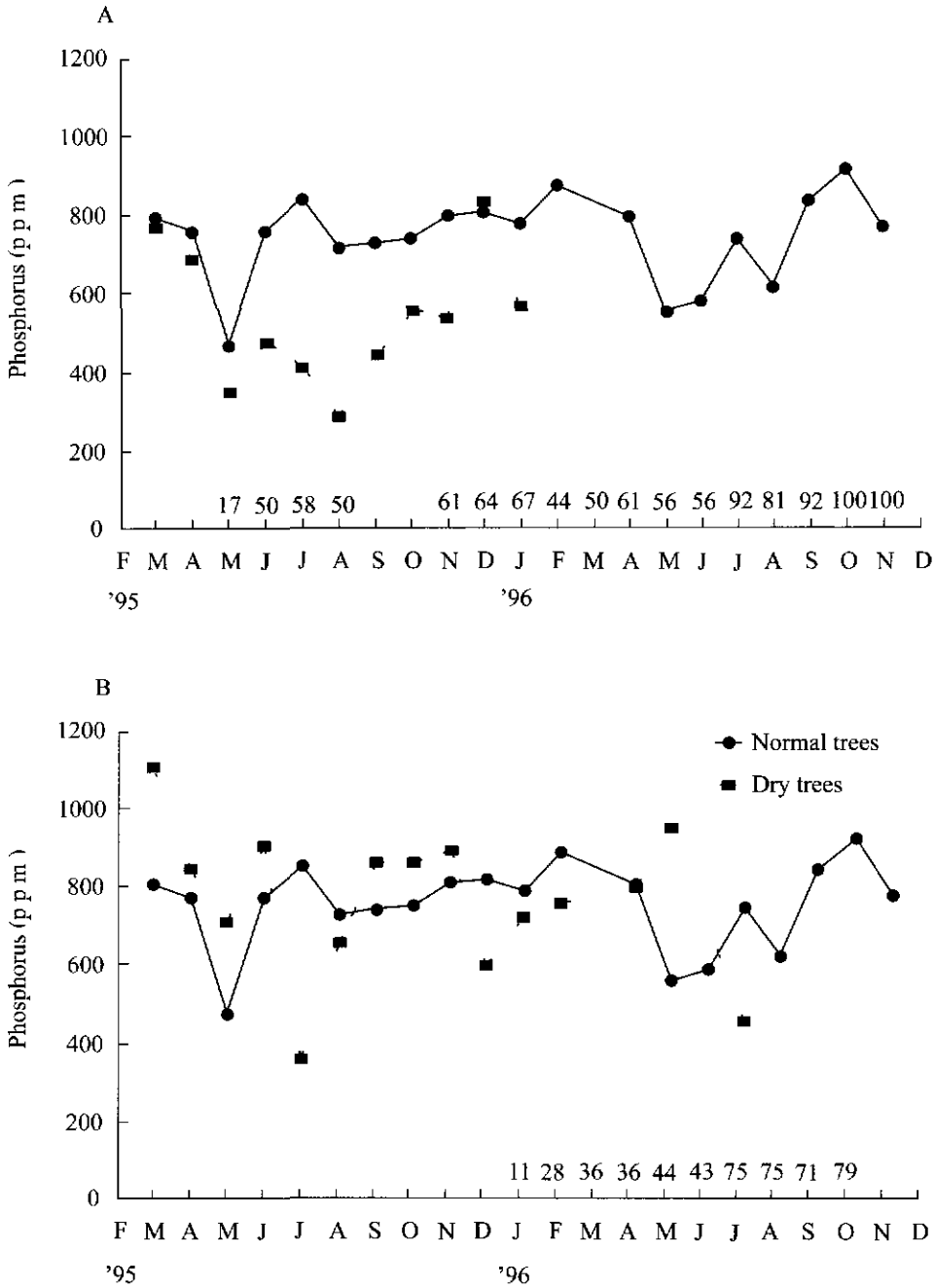
## Comparative Assessment of Macro- and Micro-nutrients in Latex and Bark Tissue of Normal and Dry Trees

The mean values of macro- and micro-nutrients in latex and bark tissues are compared between normal and dry trees in *Table 2*. Unlike for free latex mineral content reported above, total latex minerals were examined in this aspect of the study. It is apparent that for latex of dry trees, the levels of the macro-nutrients *viz.* nitrogen, phosphorus, potassium and calcium were all lower than that recorded for latex of normal trees, with the exception of magnesium which



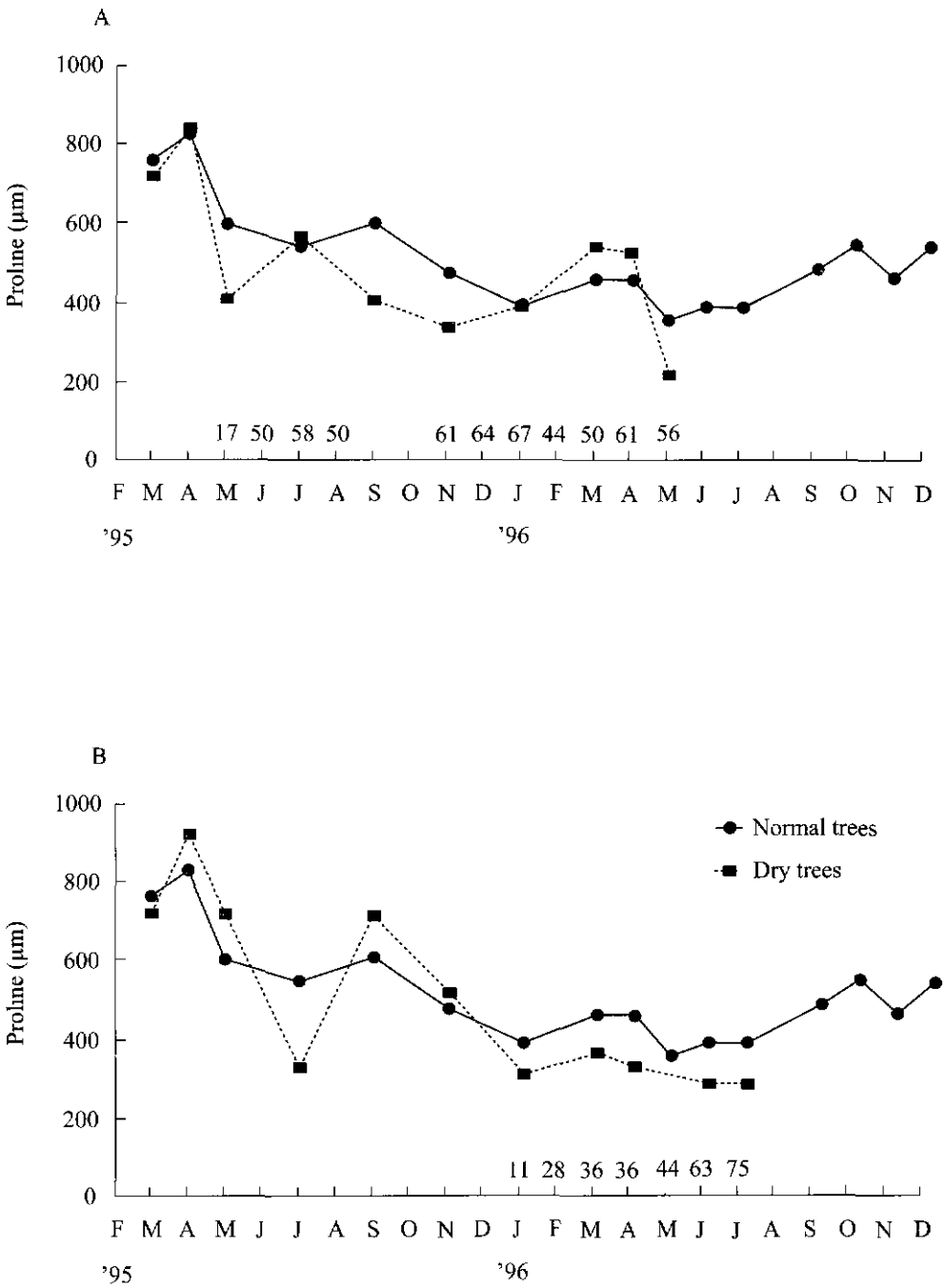

(Figures above the X-axis denote tapping cut % dryness of the partially dry tree.)

*Figure 3. Latex bottom fraction volume compared between the mean for normal trees and two partially dry trees (A and B).*



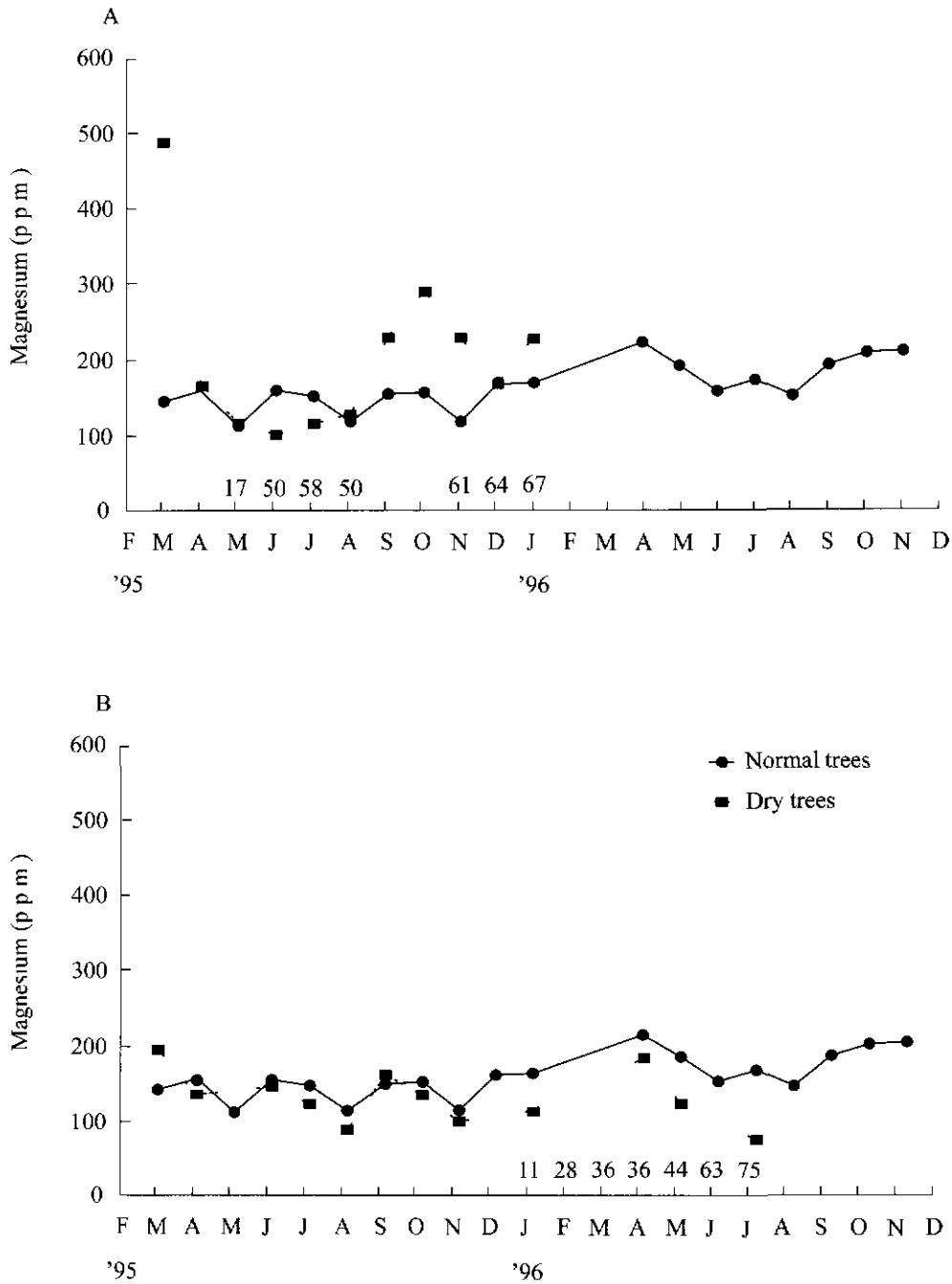

(Figures above the X-axis denote tapping cut % dryness of the partially dry tree )

*Figure 4 Latex inorganic phosphorus level compared between the mean for normal trees and two partially dry trees (A and B)*



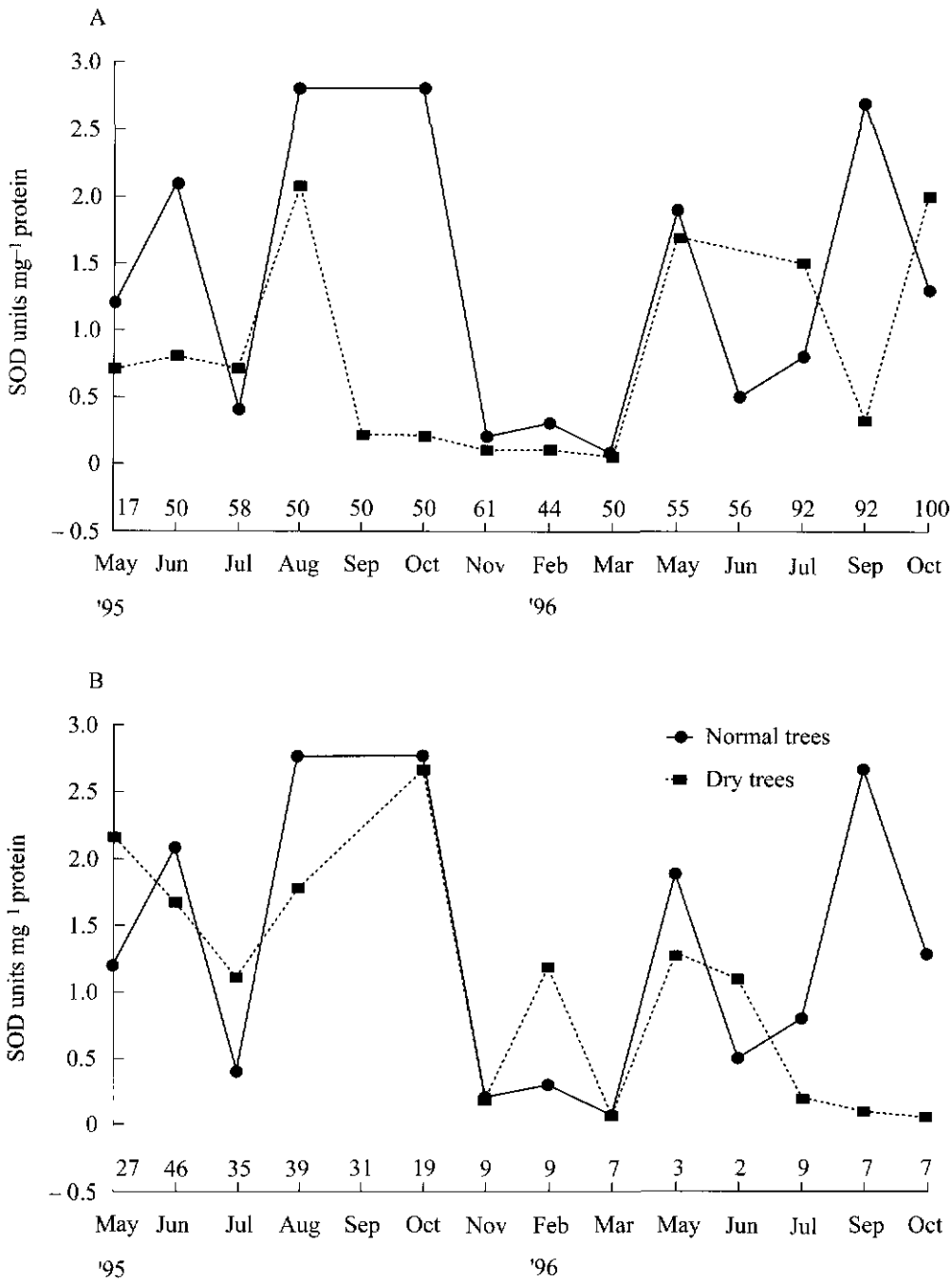

(Figures above the X-axis denote tapping cut % dryness of the partially dry tree.)

*Figure 5. Latex proline compared between the mean for normal trees and two partially dry trees (A and B)*



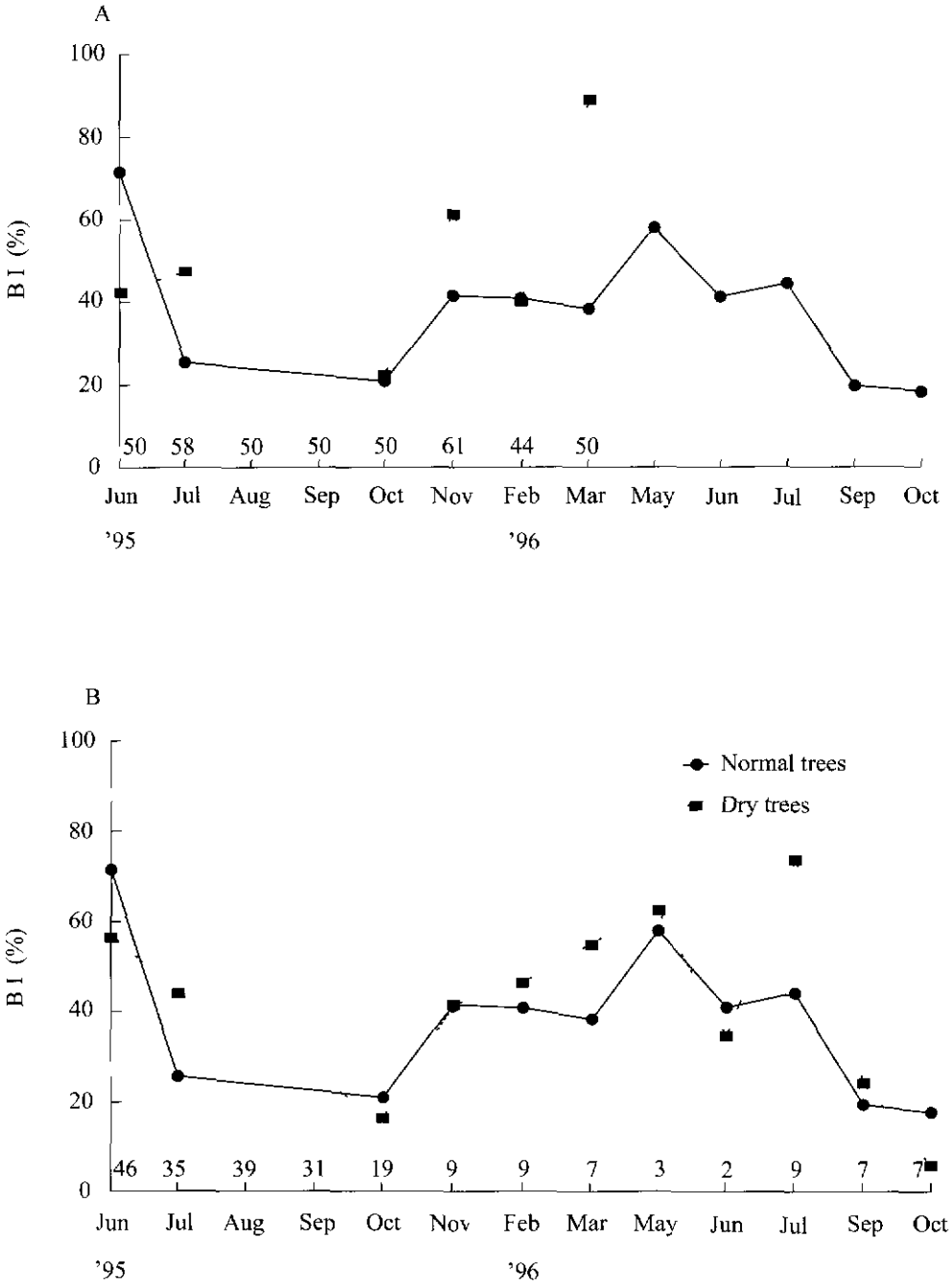

(Figures above the X-axis denote tapping cut % dryness of the partially dry tree )

*Figure 6 Latex free magnesium compared between the mean for normal trees and two partially dry trees (A and B)*




(Figures above the X-axis denote tapping cut % dryness of the partially dry tree.)

*Figure 7. Comparison of latex superoxide dismutase activity in latex between the mean for normal trees and two partially dry trees (A and B).*




(Figures above the X axis denote tapping cut % dryness of the partially dry tree )

*Figure 8 Comparison of bursting index of lutoids between the mean for normal trees and two partially dry trees (A and B)*

TABLE 2. COMPARATIVE ASSESSMENT OF MACRO- AND MICRO-NUTRIENTS IN LATEX AND BARK TISSUE OF NORMAL AND DRY TREES

| Macro- and micro-nutrients | Latex | | Bark | |
|---|---|---|---|---|
| | Dry trees | Normal trees | Dry trees | Normal trees |
| N (%) | 0.490 | 0.550 | 0.690 | 0.700 |
| P (%) | 0.220 | 0.260 | 0.760 | 0.810 |
| K (%) | 0.500 | 0.670 | 0.750 | 0.780 |
| Mg (%) | 0.041 | 0.037 | 0.134 | 0.140 |
| Ca (p.p.m.) | 12.830 | 18.120 | 2.305 | 2.378 |
| Cu (p.p.m.) | 4.040 | 5.070 | 6.900 | 7.030 |
| B (p.p.m.) | 3.280 | 4.090 | 16.410 | 17.410 |
| Zn (p.p.m.) | 4.580 | 6.160 | 31.650 | 43.750 |
| Fe (p.p.m.) | 4.030 | 5.020 | 59.930 | 90.800 |
| Al (p.p.m.) | 6.530 | 5.950 | 48.370 | 72.640 |

Values given for dry trees are the mean values of all determination carried out over the duration of study. Values given for normal trees are the mean values of determinations carried out over the duration of study.

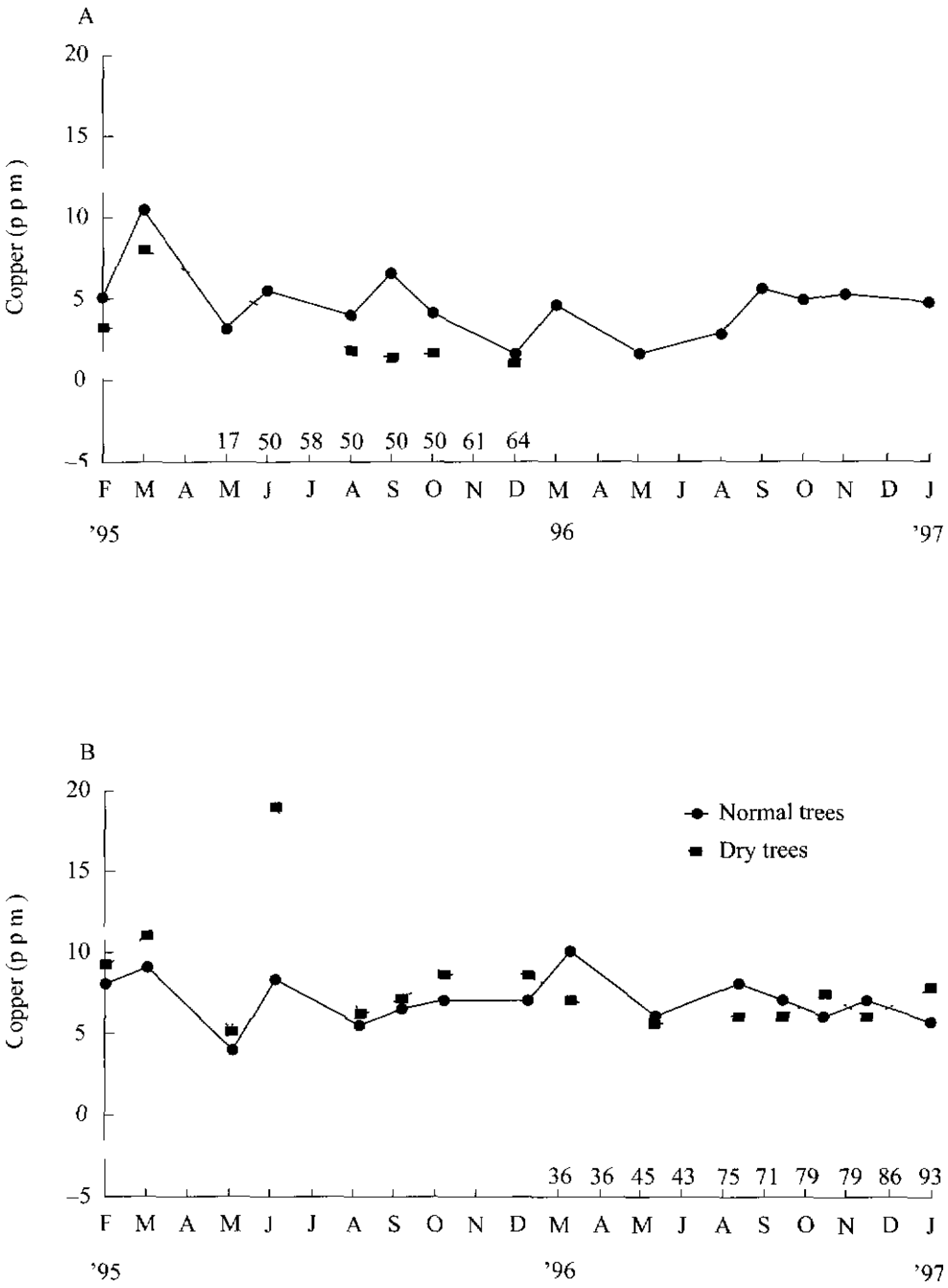
showed a reverse trend. Similarly for the micro-nutrients, namely copper, boron, zinc and iron, the levels in latex of dry trees were lower than that of normal trees, with the exception of aluminium which showed a higher concentration in dry trees.

In bark tissue, there were no marked differences in levels of all the macro-nutrients between normal and dry trees. For the micro-nutrients, with the exception of copper, which was comparable, the levels of all the other micro-elements were lower in the dry trees, relative to that recorded for normal trees. A marked reduction in dry trees was particularly noted for iron.

Since it has previously been reported by other researchers on tapping panel dryness that changes in copper and boron were prominent in dry or stressed trees, these two micro-elements were further examined in detail to establish if there were marked or abnormal changes in concentrations of these elements in latex and bark during the months preceding the onset of dryness on the cut. Boron in latex and bark tissue of the two dry trees was not markedly different from that of normal trees, during the months preceding onset of dryness on the tapping cut of the affected trees (*Figures 10* and *12*). Copper in the bark tissue of the two dry trees was also generally comparable to that of normal trees, with the exception of one dry tree, which had a marked increase in copper levels six months prior to the onset of partial dryness on the tapping cut (*Figure 11*). The levels of latex copper were initially high prior to the development of dryness but thereafter declined progressively with the onset of dryness to be either comparable or lower than the levels recorded in normal trees (*Figure 9*).

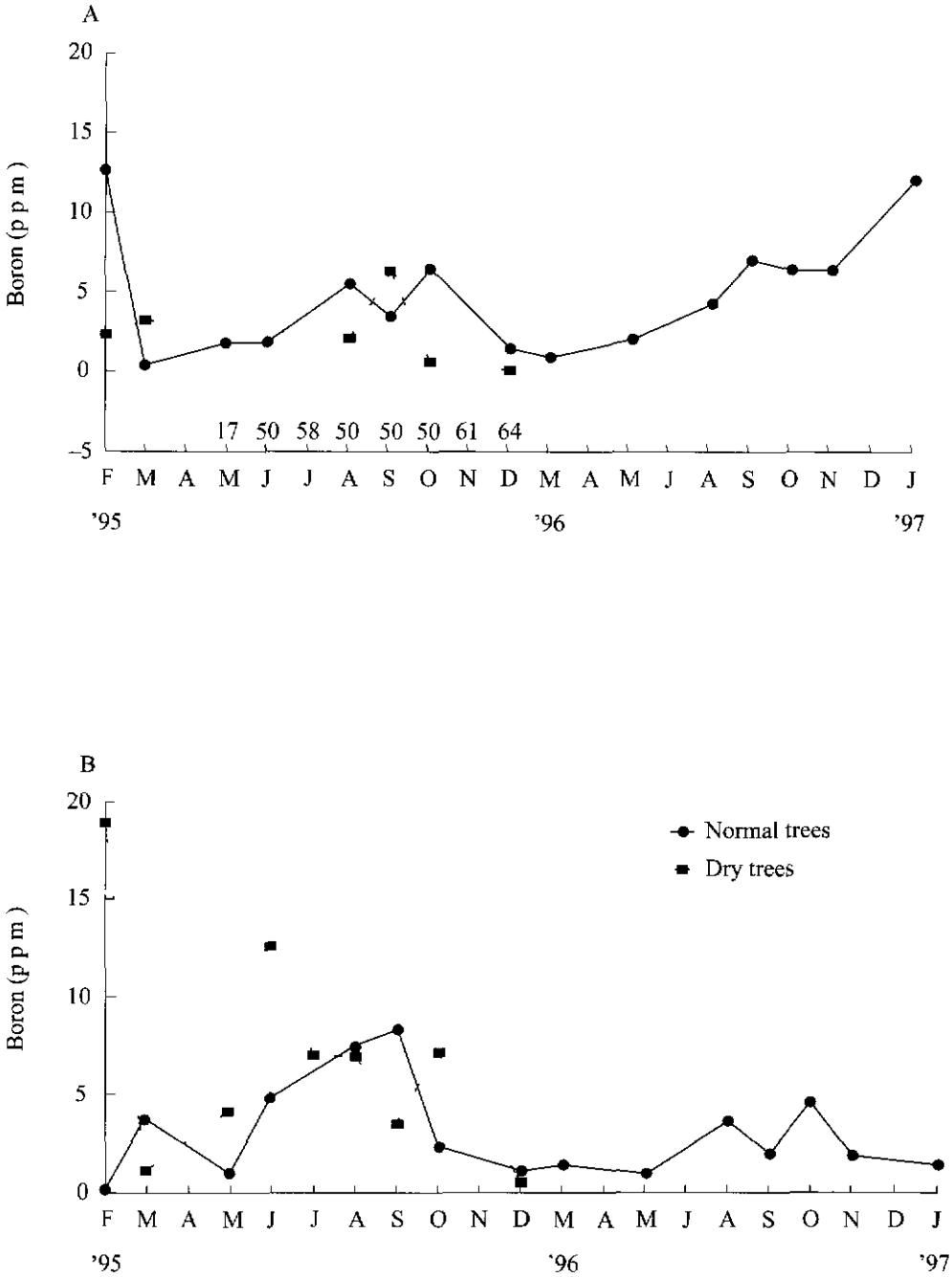
## Tylosis in Latex Vessels of Normal and Partially Dry Bark

Tylosis in latex vessels was already observed in normal trees sampled at the



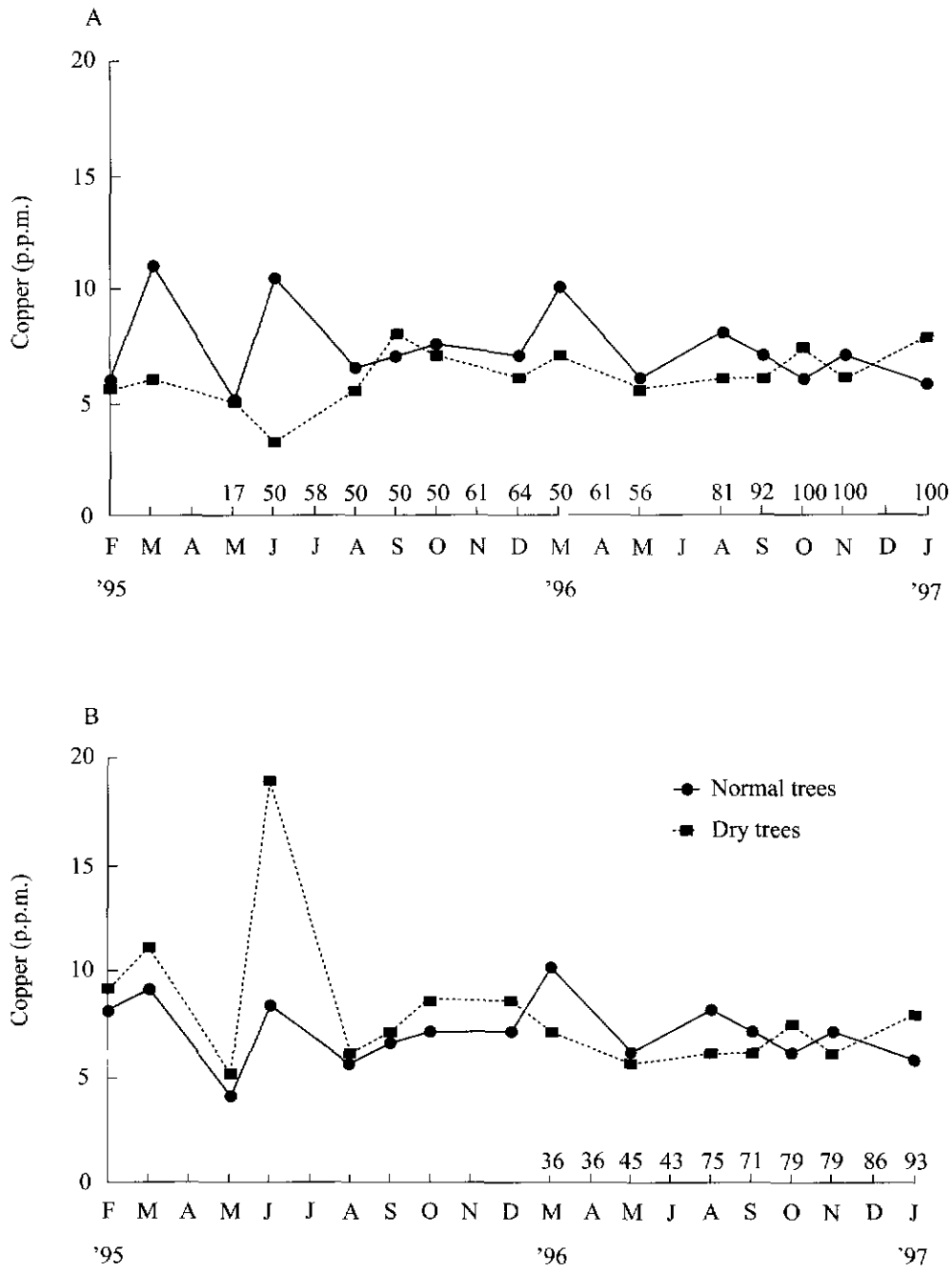

(Figures above the X-axis denote tapping cut % dryness of the partially dry tree )

*Figure 9 Comparative study of latex copper between the mean for normal trees and two partially dry trees (A and B)*



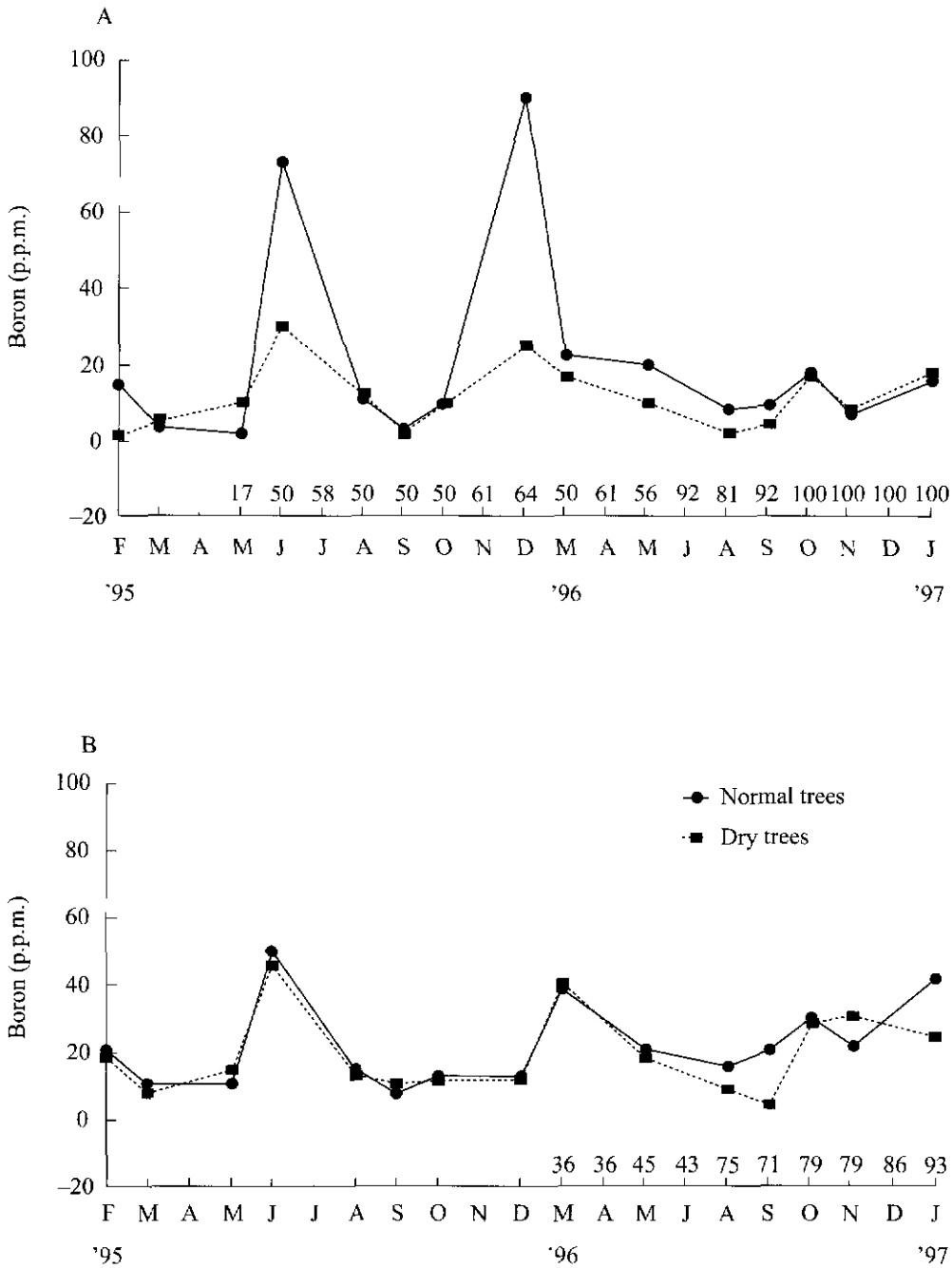

(Figures above the X-axis denote tapping cut % dryness of the partially dry tree )

*Figure 10 Comparative study of latex boron between the mean for normal trees and two partially dry trees (A and B)*




(Figures above the X-axis denote tapping cut % dryness of the partially dry tree.)

*Figure 11. Comparative study of copper in bark tissues between the mean for normal trees and two partially dry trees (A and B).*




(Figures above the X-axis denote tapping cut % dryness of the partially dry tree.)

*Figure 12. Comparative study of boron on bark tissues between the mean for normal trees and two partially dry trees (A and B).*

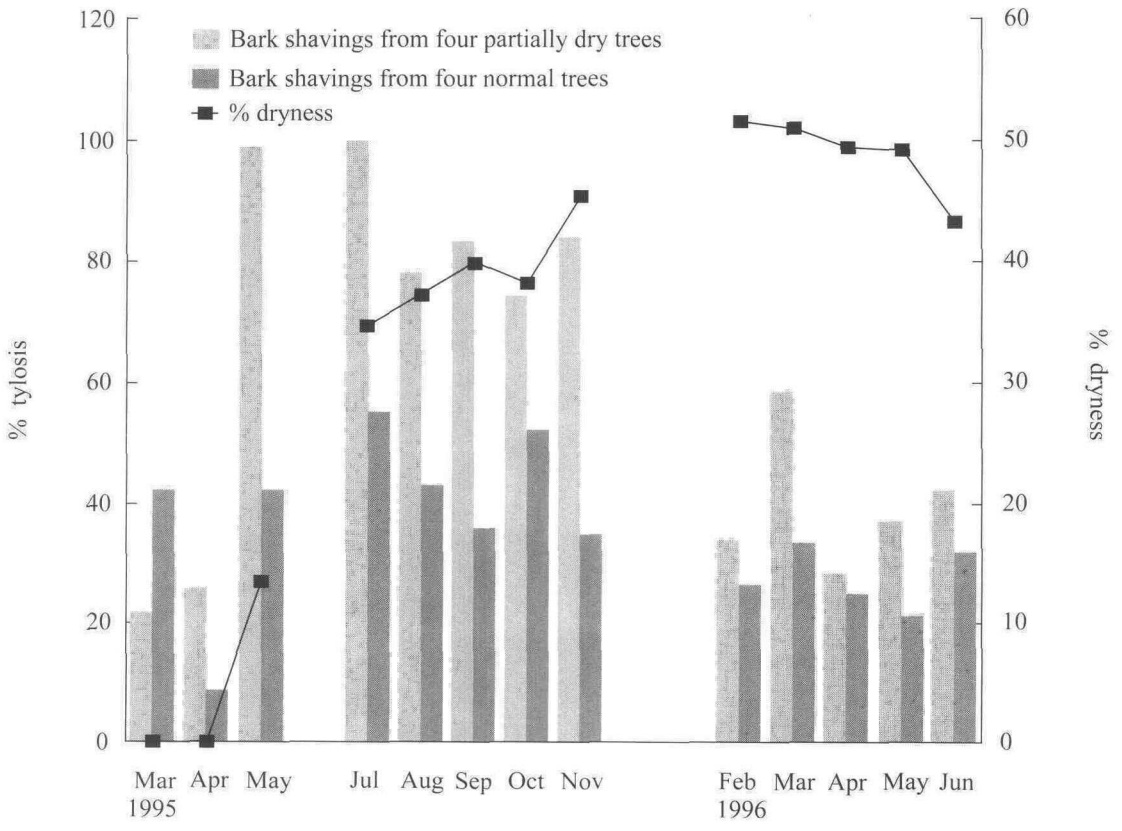
commencement of the experiment (*Figure 13*). However in trees which subsequently developed dryness, there was a significant increase in tylosis content. With the exception of the first month when dryness had not set in yet, the average tylosis content in dry trees was significantly higher than in normal trees during the duration of the study.

## DISCUSSION

Tapping panel dryness that developed in this study was not the transient type caused primarily by over-exploitation of latex, although some trees showed varying degrees of recovery with continued tapping. Dryness that was observed, was mainly the progressive form that occurred spontaneously in trees tapped on moderate intensity. The propensity for natural development of dryness was apparent in all trees irrespective of inherent initial latex yield at commencement of tapping. The latex dry rubber content values were not markedly different between normal and trees which developed partial dryness. A number of physiological parameters which were routinely monitored in this study did not show significant differences between normal and dry trees, although these parameters were chosen for the study based on previously reported findings on dryness by several researchers.[12–14] This could be attributed to the fact that most of the earlier investigations were on trees which were induced dry with either intensive tapping or stimulation, while in studies reported here, it was on trees which developed dryness naturally when subjected to normal tapping system without stimulation. Regular periodic readings in this study were aimed at observing physiological changes that took place just prior to the onset of dryness, since these could be causal factors for tapping panel dryness.

There were significant differences between normal and dry trees for four latex physiological parameters namely percentage bottom fraction size, inorganic phosphorus, proline and magnesium as in reported studies, which have associated these parameters with either dryness development or stress[15]. Thus the reduction in bottom fraction and magnesium levels have been linked to increased instability of latex, while inorganic phosphorus has been associated with increased rates of metabolism in stressed trees. Proline has been known to accumulate in plant tissue as a common reaction to various forms of physiological stress, although in this case, proline levels declined as dryness set in. The values of these parameters relative to normal trees, changed after the occurrence of dryness on the tapping cut. There were no consistent abnormal or marked changes in these four parameters during the months *preceding* onset of dryness development on the cut, when compared to normal trees. The lack of association between these parameters and early stages of dryness development prior to expression of symptoms on tapping cut would therefore preclude the possibility of using these parameters as early warning indicators or predictors of tapping panel dryness. Moreover, the differences tended to be small in most cases, even if statistical significance between the differences could be demonstrated. It is possible that the frequency of sampling carried out in this study may not have been sensitive enough to pick up rapid changes in latex physiological parameters before the onset of dryness.

The above notwithstanding, the lack of significant changes in other measurements of latex stability carried out in this study, namely the bursting index of lutoids (BI) and the Aerosol OT Stabilty Index, needs to be rationalised and explained. In this connection, it should be noted that latex from partially dry




(Figures above the X-axis denote tapping cut % dryness of the partially dry tree.)

*Figure 13. Comparative study of tylosis in latex vessels of normal and partially dry trees.*

trees were derived from latex vessels that were still yielding, and this would include latex vessels that were physiologically normal and free even from incipient dryness.

Latex SOD, located in the lutoids, has an antioxidant function and serves to nullify toxic oxygen that could damage lutoid membranes leading to latex instability and dryness. There was, however, no consistent pattern with regard to changes to latex SOD activity in relation to the onset of dryness. Differences in the levels of macro-nutrients between both latex and bark tissues of normal and dry trees were also unremarkable, though further detailed examination of the data may be necessary to establish distinctive trends. This was also largely true for the micro-nutrients.

The anatomy of the bark revealed changes that could have a bearing on dryness. The presence of tyloses in latex vessels could

impede the flow of latex. The increased tylosis in dry trees might therefore have contributed to the development of tapping panel dryness.

These studies have served a purpose in assessing the role of various latex physiological parameters concerned with dryness, and in screening out those parameters that may not be implicated in its development. It is obvious that further detailed statistical analysis of the data available from this study is necessary for establishing correlations between dryness incidence and the numerous physiological parameters that have been investigated.

## CONCLUSION

Significant correlations existed between dryness and four latex physiological parameters, namely bottom fraction volume, inorganic phosphorus, proline and magnesium. However, detailed examination of these parameters preceding onset of partial dryness on the cut in affected trees did not show any pattern or trend that could serve as a consistent and reliable early warning of impending onset of dryness. The significant increase in tylosis in the latex vessels of dry trees needs to be further examined to establish if it might have a predictive value with regard to impending dryness.

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