J. Rubb. Res., **15(4)**, 265–272

Translocation and Accumulation of Phosphorus Applied to Healthy and Brown Bast Affected Rubber (Hevea brasiliensis) *Trees*

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*A study to assess the movement and incorporation of phosphorus from applied source in rubber (*Hevea brasiliensis *Muell Arg.) trees was conducted. Radioactive phosphorus (^{32}P) was applied to mature rubber either through root feeding or by soil injection. The radioactive phosphorus could be traced in the leaves, bark and latex indicating that the applied phosphorus was absorbed immediately by the tree. ^{32}P activity in the different plant parts of three categories of trees viz., normal, partially dry and completely dry were compared. No significant difference between the three groups was recorded in the ^{32}P activity in the leaves on the 15th day of application. However, accumulation to a phenomenal extent was noted in the partially dry and completely dry trees with advancement of time, indicating non translocation to the site of latex biosynthesis and that the phosphorus movement to the sink is demand driven. In the bark on the 30th and 45th day of sampling, no radioactive phosphorus could be traced in the normal and partially dry trees while in the completely dry trees, ^{32}P activity was recorded indicating lack of metabolic utilisation for the synthesis of latex. In the latex, ^{32}P activity was recorded on the 20th day in all the three categories of trees. However, on the 30th and 45th day, no ^{32}P activity was recorded in the completely dry trees indicating non utilisation of phosphorus from applied source for latex biosynthesis and regeneration. The study indicated that in the TPD affected trees, the xylem and phloem transport mechanism is active up to the laticiferous system but the latex biosynthesis and regeneration is affected or impaired.*

Keywords: radioactivity; ^{32}P; tapping panel dryness syndrome; latex biosynthesis; *Hevea brasiliensis*

Natural rubber is composed of isoprene units linked together to form a polymer. In the biosynthesis of rubber, acetyl Co-A is converted to isopentynyl pyrophosphate (IPP) *via* mevalonic acid and then IPP is polymerised to form rubber. Latex contains appreciable quantities of inorganic phosphorus. Inorganic phosphorus content in latex reflects the energy involvement in metabolism and is necessary for the production of nucleic acids required in rubber biosynthesis. Highly significant relation between the availability of labile energy phosphates and production of trees was established and reported. Direct

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correlation between inorganic P (Pi) content and production has been established in many clones[1–3].

Tapping panel dryness (TPD), also known as brown bast can be defined as a spontaneous process of drying of the tapping panel resulting in abnormally low yield or complete cessation of latex production for no apparent reasons and without the death of the tree[4]. Various causes have been postulated for TPD, but there is no conclusive proof for any single cause and hence TPD is generally termed as a metabolic disorder or physiological syndrome. However, there is a general consensus that TPD is a disease or disorder affecting essentially the latex vessels of some high yielding clones/ trees and over exploitation (high frequency, over stimulation, long cuts and deep tapping) can aggravate TPD[5].

The hypothesis of the study is that by incorporating ^{32}P to the plant system, either through soil application or through root injection, the specific activity of applied ^{32}P in the plant parts especially in the bark and latex, can be traced and the pattern of use of applied phosphorus by the rubber tree can be assessed. Similarly, a comparison of the ^{32}P activity in the leaves, bark and latex of normal, partially dry and completely dry (latex from opposite panel) trees will provide insight on the efficiency of phosphorus utilisation between the three groups.

MATERIALS AND METHODS

The study was conducted in a rubber plantation at the Kerala Agricultural University farm, Vellanikkara, Thrissur, Kerala on clone RRII 105, being tapped on BO2 panel with S2 d3 6d/7system of tapping without stimulation. Twelve trees each from three categories *viz*., normal, partially dry and completely dry trees were selected from the same field and marked for conducting the study. Partially dry trees were having latex oozing from portions of the tapping panel. After one month, these trees were revisited at the time of tapping and the stage of TPD reconfirmed. Radioactive phosphorus was applied to individual trees by two methods *viz*., root feeding and soil injection (*Figure 1*). The activity of the ^{32}P was 114.4 m Ci and this was dissolved in two litres of 1000 p.p.m. of potassium phosphate (KH_2PO_4) solution. The activity per ml was 0.055 mCi.

Root Feeding

Six trees from each category were treated with ^{32}P through feeder roots (*Figure 1*). Roots were excavated from the base of the particular tree and two feeder roots were selected. Each of the roots were dipped in 1mCi ^{32}P in 24 mL 1000 p.p.m. phosphorus solution made using KH_2PO_4 placed in a polythene tube, making the total quantity per tree as 2.64 mCi in 48 mL KH_2PO_4 solution. The roots were inserted into the polythene tube and sealed tightly.

Soil Injection

Twelve small PVC tubes of 20 cm in length were inserted in equidistant holes in the root zone at a horizontal distance (HD) of 100-150 cm around the tree and at a depth of 10 cm (*Figure 1*) with 10 cm length of the tubes exposed above ground[6]. Using a dispenser set, 4.0 mL each of radioactive ^{32}P solution was injected through each tube into the soil making the total quantity to be 2.64 m Ci in 48.0 mL KH_2PO_4 solution per tree.

From individual treated trees, leaf and bark samples were collected at 15th, 30th and 45th day of application and latex samples were collected at 20th, 30th and 45th day of application. From each tree, four basal leaves

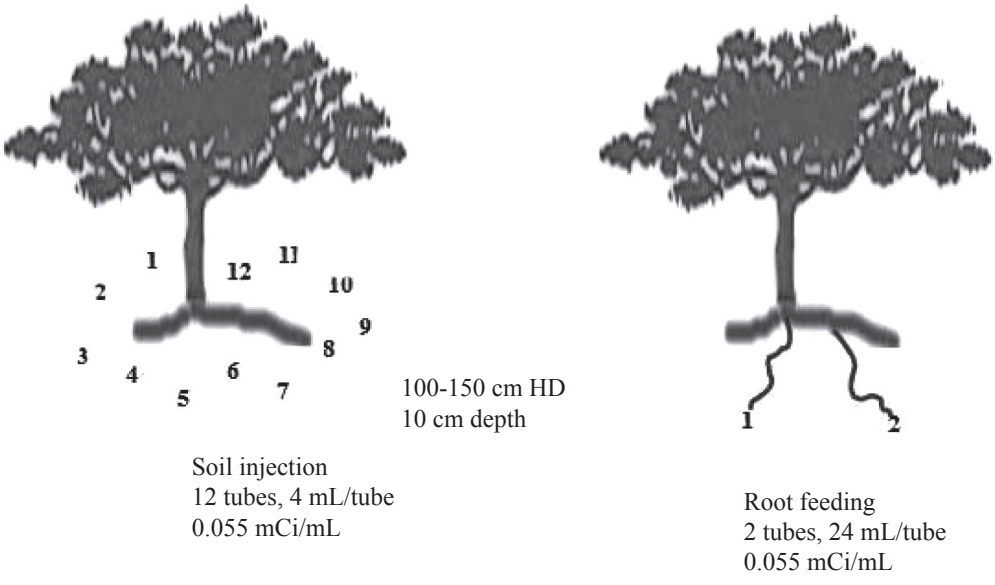

Figure 1. Method of application of radioactive phosphorus.

from the terminal whorl of low branches in shade were collected as per the standard procedure[7]. A square piece of bark sample was collected from the tapping panel 10 cm below the tapping cut from each tree. Leaf and bark samples were dried at 70ºC in a hot air oven and powdered and digested using di-acid mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) at 2:1 ratio. Radioactivity in the digested samples was assayed using a liquid scintillation counter. For processing the latex samples, trichloro acetic acid were added to known weight of latex samples and rubber and serum were separated. Radioactivity of the serum samples was assayed using a liquid scintillation counter[8].

The radioactivity measurement in counts per minute per gram of sample were subjected to square root transformation for accounting the zero counts and statistically analysed for factorial completely randomised design[9].

RESULTS AND DISCUSSION

^{32}P activity measurements recorded in the leaf, bark and latex at periodic intervals are presented (*Tables 1–3*). Radioactivity measurements recorded in the leaf on the 15th day of application is presented in *Table 1*. No significant difference was recorded between the three categories of trees indicating that the root uptake and transporting system were active equally in the three categories of trees. On the 30th day, ^{32}P activity was similar between the three categories of trees but difference was recorded between the two methods of application (*Table 1*). Higher activity was recorded with soil injection. Advantages of ^{32}P soil injection technique for tracer studies in perennial crops were reported[6]. On the 45th day, the absolute values of the ^{32}P activity was high compared to the 15th and 30th day (*Table 1*). In normal trees, the values were 312.0 and 283.7 respectively, for root feeding

and soil injection. However, the values were several times higher for the partially dry and completely dry trees. The mean value was 2196.6 for the partially dry trees and 1646.40 for the completely dry trees indicating that in TPD affected trees, the translocation of phosphorus from leaf to the laticiferous system was arrested because of the lack of utilisation of phosphorus due to stoppage of latex biosynthesis or non-functioning of the laticiferous system.

^{32}P activity in the bark on the 15th day of application is presented in *Table 2*. Difference in activity was statistically significant between the three categories of trees with low values in the completely dry trees (*Table 2*). As in the case of leaves, soil injection recorded higher values compared to root feeding. Rubber is a surface feeder and 55% of the root activity was reported to be confined to the top 10 cm of soil layer[10]. On the 30th day also, significant difference was recorded in the phosphorus movement between the three categories of trees. Partially dry trees recorded highest accumulation of phosphorus (*Table 2*). On the 45th day, significant difference was also recorded between the three categories. Normal and partially dry trees had no ^{32}P activity in the bark on the 45th day (*Table 2*). In completely dry trees, radioactivity was recorded in the bark indicating a low metabolic utilisation and low latex production.

TABLE 1. ^{32}P ACTIVITY IN THE LEAF ON THE 15TH, 30TH AND 45TH DAY OF APPLICATION (CPM/G)

Treatments	Root feeding (M_1)	Soil injection (M_2)	Mean
	15th Day of application		
Normal (T_1)	31.47 (5.31)	35.99 (5.96)	33.73 (5.63)
Partial dry (T_2)	57.43 (7.63)	54.28 (7.09)	55.86 (7.31)
Complete dry (T_3)	65.98 (7.93)	48.98 (6.95)	57.23 (7.43)
Mean	41.14 (6.91)	46.42 (6.67)	48.94 (6.79)
CD(P=0.05)	(T)= NS	(M) =NS	(T × M) = NS
	30th Day of application		
Normal (T_1)	93.93 (9.67)	110.09 (10.39)	102.01 (10.03)
Partial dry (T_2)	35.86 (5.99)	219.30 (14.81)	127.58 (10.40)
Complete dry (T_3)	81.98 (8.89)	196.62 (14.02)	139.30 (11.46)
Mean	70.59 (8.18)	175.24 (13.07)	122.96 (10.63)
CD(P=0.05)	T= NS	M = 1.22	T × M = 2.11
	45th Day of application		
Normal (T_1)	312.0 (17.36)	283.70 (16.75)	297.85 (17.05)
Partial dry (T_2)	1567.0 (39.58)	2825.70 (53.15)	2196.35 (46.37)
Complete dry (T_3)	1544.8 (39.20)	1748.00 (41.74)	1646.40 (40.47)
Mean	1141.3 (32.05)	1619.1 (37.21)	1380.1 (34.63)
CD (P=0.05)	T= 3.34	M= 2.73	T × M = 4.72

Figures in parenthesis are the transformed values.

TABLE 2. ^{32}P ACTIVITY IN THE BARK ON THE 15TH, 30TH AND 45TH DAY OF APPLICATION (CPM/G)

Treatments	Root feeding (M_1)	Soil injection (M_2)	Mean
	15th Day of application		
Normal (T_1)	73.59 (8.56)	262.30 (16.20)	167.95 (12.38)
Partial dry (T_2)	84.01 (9.14)	226.07 (15.02)	155.04 (12.08)
Complete dry (T_3)	64.19 (7.99)	56.28 (7.44)	60.24 (7.72)
Mean	73.93 (8.56)	181.55 (12.88)	127.74 (10.73)
CD(P=0.05)	T = 0.99	M = 0.80	T × M =1.39
	30th Day of application		
Normal (T_1)	100.16 (9.97)	490.40 (22.14)	295.28 (16.05)
Partial dry (T_2)	1441.86 (37.76)	1387.33 (37.18)	1414.60 (37.47)
Complete dry (T_3)	674.30 (25.95)	645.84 (21.82)	660.07 (23.88)
Mean	738.77 (24.55)	841.19 (27.05)	789.99 (25.80)
CD(P=0.05)	T= 3.17	M= NS	T × M= NS
	45th Day of application		
Normal (T_1)	0 (0.71)	0 (0.71)	0 (0.71)
Partial dry (T_2)	0 (0.71)	0 (0.71)	0 (0.71)
Complete dry (T_3)	151.3 (11.97)	159.3 (12.53)	155.3 (12.25)
Mean	(4.46)	(4.65)	(4.57)
CD (P=0.05)	T= 2.11	M= NS	T × M = NS

Figures in parenthesis are the transformed values.

^{32}P activity in the latex on the 20th day of application indicated significant difference between the three categories of trees with completely dry trees recording very low values (*Table 3*). On the 30th day, absolute values were low compared to 20th day and significant difference was observed between the three categories (*Table 3*). Highest activity was recorded in the latex from normal trees indicating proper translocation and metabolic utilisation of phosphorus for latex regeneration. Completely dry trees were tapped on the opposite side and latex collected did not have radioactivity indicating lack of metabolic utilisation of applied phosphorus in the biosynthesis of latex. Latex ATP is reported to be a very important regulator in biosynthesis by its direct effect on metabolic pathways (supplying energy) and indirect effect mediated through lutoid membrane H^+ ATPase activity , which increases the latex pH to favourable levels and activates several pH dependent enzymes in the cytosol[11]. An active metabolism generates sufficient intracellular energy which provides easy flow and thereby increases production. Significant positive correlation between yield and latex ATP, lutoid membrane ATPase and C-serum pH was reported[12–14] . Similarly, on the 45th day of sampling, significant difference was observed between the three categories with the completely dry trees having no radioactivity

TABLE 3. ^{32}P ACTIVITY IN THE LATEX ON THE 20TH, 30TH AND 45TH DAY OF APPLICATION (CPM/G)

Treatments	Root feeding (M_1)	Soil injection (M_2)	Mean
	15th Day of application		
Normal (T_1)	342.44 (18.60)	231.49 (15.12)	286.97 (16.81)
Partial dry (T_2)	241.70 (15.54)	65.20 (8.07)	153.45 (11.80)
Complete dry (T_3)	92.54 (9.60)	61.19 (7.81)	76.87 (8.71)
Mean	225.56 (14.55)	119.29 (10.33)	172.43 (12.44)
CD(P=0.05)	T= 1.22	M= 1.00	T × M = 1.73
	30th Day of application		
Normal (T_1)	105.74 (10.26)	129.18 (11.25)	117.46 (10.76)
Partial dry (T_2)	74.16 (8.51)	71.85 (8.50)	73.01 (8.51)
Complete dry (T_3)	0 (0.71)	0 (0.71)	0 (0.71)
Mean	59.77 (6.49)	67.01 (6.82)	63.49 (6.66)
CD(P=0.05)	T= 1.58	M= NS	T × M =NS
	45th Day of application		
Normal (T_1)	62.60 (7.88)	118.51 (10.90)	90.56 (9.39)
Partial dry (T_2)	34.90 (5.94)	107.05 (10.34)	70.98 (8.14)
Complete dry (T_3)	0 (0.71)	0 (0.71)	0 (0.71)
Mean	32.5 (4.84)	75.19(7.31)	53.85(6.07)
CD (P=0.05)	T=0.910	M= 0.743	T × M = 1.28

Figures in parenthesis are the transformed values.

in the latex as in the case of 30th day of observations indicating lack of metabolic utilisation of phosphorus or non functioning of the laticiferous system and that the latex biosynthesis is hindered. The latex harvested from the opposite side of the completely dry trees might be the stored latex.

CONCLUSION

Movement of phosphorus from the applied source was traced through radiotracer studies. Samples of leaves and bark were collected on the 15th, 30th and 45th day and ^{32}P activity was measured. Radioactive phosphorus could be recorded in the leaves, bark and latex. In the latex, ^{32}P activity was recorded on the 20th day of sampling in the normal, partially dry and completely dry trees. On the 30th and 45th day of sampling ^{32}P activity could not be traced in the completely dry trees indicating non incorporation of phosphorus from the applied source for latex biosynthesis and regeneration. The study indicated that in the TPD affected trees, the xylem and phloem transport mechanism is active up to the laticiferous system but the latex biosynthesis and regeneration is affected or impaired.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to Dr. James Jacob, the Director of Research, Rubber Research Institute of India, Kottayam for granting permission to conduct the experiment and for providing encouragement and support. The authors express their sincere gratitude to Dr. A. Alexander, former Director of Research, Kerala Agricultural University for the kind permission and support for conducting this study at the rubber plantation of the Kerala Agricultural University and at the Radiotracer laboratory of the Kerala Agricultural University, Vellanikkara, Thrissur. The cooperation and support provided by the staff and workers of the Kerala Agricultural University farm, Vellanikkara, is gratefully acknowledged. Assistance provided by Smt. Meena S., Scientific Assistant, of the Regional Soil Testing Laboratory, Thrissur in processing the latex sample is acknowledged. The support provided by Sri. Ramesh B. Nair, Joint Director, Statistics and Planning, Rubber Board, in the statistical analysis of the data is acknowledged with thanks.

Date of receipt: February 2012
Date of acceptance: July 2012

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