Use of Tritiated Water (^3H_2O) as a Tracer to Study the Water Movement in Leaf Scorch Decline (LSD) Affected Coconut Palms

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ABSTRACT

Leaf scorch decline (LSD) is a disorder of coconut *(Cocos nusifera* L) palms in Sri Lanka. The etiology of the disorder is not yet known. The study was aimed to determine the pattern of flow of water from the base of the trunk to canopy and to identify particular places with blockages in LSD affected palms. 15-20 year old, apparently healthy and LSD affected palms at the Bandirippuwa Estate, Coconut Research Institute, Sri Lanka were used for the study. Tritiated water was injected in to the base of coconut palms and transpired water was detected for ^3H for 10 days. LSD affected palms showed a delay in water movement than healthy palms. The highest activity was shown on day 5 and 8, in healthy and LSD-affected palms, respectively.

KEY WORDS: Coconut, Leaf Scorch Decline, Water Movement, Tritiated Water, ^3H_2O

INTRODUCTION

The coconut palm (*Cocos nucifera* L) is one of the most important crops cultivated in Sri Lanka. The coconut industry has a greater impact for the economy in Sri Lanka. It is among main sources of food and foreign exchange earning in national economy. And it also provides employment opportunities. Coconut is used as a raw material for many industries. For instance the value added products from the coconut kernel, husk, shells, leaves, and stems.

Coconut palms are prone to several diseases and disorders that cause reduction of growth and yield and even death of palms in some cases. Leaf Scorch Decline (LSD) is a common disorder found in many coconut plantations. Leaf Scorch Decline of coconut is a disorder first recorded in Sri Lanka in 1955 (Gunasekara, 1973) to be prevalent in the southern province, especially in Gonapinuwala, Baddegama, and Elpitiya areas. The exact cause of the disorder is not yet identified (Ranasinghe, 2005).

LSD reduces the yield within 2-3 years and kills the palm in 5-10 years. The consequent loss to the coconut industry is considerable. The most striking visible symptom of LSD-affected palms is the scorching of leaflets. It starts from the tip of the leaflets and spreads towards the midrib of the frond. Scorched leaflets of lower to middle whorls curl slightly downward. Upper whorls do not show any scorching symptoms. Tapering of the stem just below the crown, reduction of the size of the crown, shortening of fronds, reduction in the size of the inflorescence, number of female flowers and yield, elongated nuts and a decaying root system are the other symptoms in severely effected palms compared to healthy coconut plants (Ekanayaka, 1963).

Water uptake of affected palms compared to healthy ones indicate that affected palms have a lower number of active roots (Jayasekara, *et al.*, 1990). There is also considerable root decay. Secondary roots in affected palms appear to be less than in unaffected palms. Furthermore, root regeneration becomes severely affected with increasing severity of symptoms (Nainanayaka, *et al.*, 1992). This stress condition may be a result of a blockage in the vascular tissues. The functional leaf area of the canopy, rate of photosynthesis, rate of transpiration and leaf water potential is reduced with the development of the disorder (Ranasinghe, 2005).

The xylem water transport system is important for growth of plants. Water uptake and transport in the palms can be studied using radio isotopes as traces (Plamboeck, *et al.*, 1999). In the present study, tritiated water was used as a tracer to study the pattern of water movement from trunk to leaf canopy of coconut palms. The aims of this study are (1) to determine whether there are any obstructions for the transport of water from trunk (stem) to canopy and (2) to identify particular places with blockage in the Leaf Scorch Decline affected palms.

MATERIALS AND METHODS

1. Materials

The experiment was conducted at the Bundirippuwa Estate, Coconut Research Institute, Lunuwila (lat 7° 20′ N, long 79° 53′ E). LSD affected and healthy coconut palms at the age of 15-20 year old were selected for the study. The palms were subjected to uniform fertilizer treatment and cultural practices as recommended by the Coconut Research Institute. Ten palms of moderate LSD affected palms were selected for the experiment. Ten apparently healthy palms were used as the control. The severity of the LSD was determined according to the development of visual symptoms (Ranasinghe, 2005).

Mild stage - only the lower whorls of fronds and 1/3 of leaflets are scorched. Trunk is not tapered.

Moderate stage - Lower and middle whorls of fronds are scorched, 2/3 of leaflets are affected. Tapering of the trunk has started.

Severe stage - Trunk is tapered, Number of inflorescences are very low or not produced. Nut setting is reduced and results a low yield smaller crown and shortened fronds are visible.

2. Treatments

Tritiated water (^3H_2O) was selected as the radioactive trace. Active ^3H_2O solution 185 GB/ml was taken to prepare the stock solution. Stock (1) solution was prepared by using 200 µl of ^3H2O solution and 800 µl of distilled water. 10 µl of Stock (1) solution was diluted up to 5ml to prepare stock (2) solution. Treated Solution was made by diluting 100 µl of stock (2) to 10 ml. Then 5ml of the solution was injected to each of the two drilling wholes made in opposite sides of the trunk of each palms. The activity of injected solution was 42000 cpm (count per minute). Each solution was prepared under room temperature (25^{0}C) and stored in the refrigerator (0^{0}C-15^{0}C). Treated solution was transported in low temperature (in ice) to the field.

3. Methods

3.1. Injection

Coconut palms were drilled (5mm driller) in opposite sides of the trunk in an angle of 45°. Drilling was done at 45-60 cm height from ground level to a depth of 10 cm. Injection was initiated and completed between at 9.00-11.00 am. Injection was done immediately after drilling, by using a micro pipette (1000 µl) and the holes were sealed by using woody pieces to prevent evaporation fraction. Treating solution 5ml was injected in to the two drilling holes of one palm. After the injection of tritium, transpired water was collected for 10 days.

3.2. Sampling

After the application of tritium, polyethylene bags (90-120cm x 7cm and gauge 3) were tied in to the leaflet of the fronds. Two leaflets were selected from opposite side of the frond and leaflets were inserted in to the poly bags and tied by using twine rib. The bags were inserted from 9.00 am to 10.30 am. Bags were collected at 1.30 pm. When transpired water was not enough for analysis, leaflets were kept in sunlight for 1-2 hours for evaporation. Transpired water was collected for 10 days.

3.3. Preparation and analysis

Transpired water was collected from poly bags by using a micro pipette (1000 µl). All transpired water collected from bags was stored in the refrigerator at 0^{0}c until analysis is completed. All the activities were done under room temperature (25^{0}c). 400 µl of transpired water was taken to an eppendorff tube (1.5ml). One ml of Scintillation cocktail (Ultima Gold) was added to the eppendorff and mixed with transpired water and then it was transferred to the scintillation vials for analysis. Tritium was detected for 3 minutes in the Liquid Scintillation Analyzer (Tri.CARB.1000 TR) and cpm value was recorded for analysis.

3.4 *Experimental Design*

Ten palms each of LSD-affected and healthy were used in a Randomize Complete Block Design (RCBD). SAS statistical package was used for data analysis.

EXPERIMENT 1

Determination of the best fronds for sampling

Two fronds from upper whore (3 and 9) and one frond from lower (scorching) whorl were tested. The best fronds for sampling were determined using the methods described in 3.1, 3.2 and 3.3.

Results and Discussion

A higher ^3H_2O activity (cpm) was shown in the 3rd and 9th fronds of crown than the 18th frond. Scorching was found in the fronds of lower whorl where the transpiration rate was very low. Therefore, collection of transpired water was also difficult in the 18th frond (Figure 1). Therefore, 3rd and 9th fronds were selected for sampling.

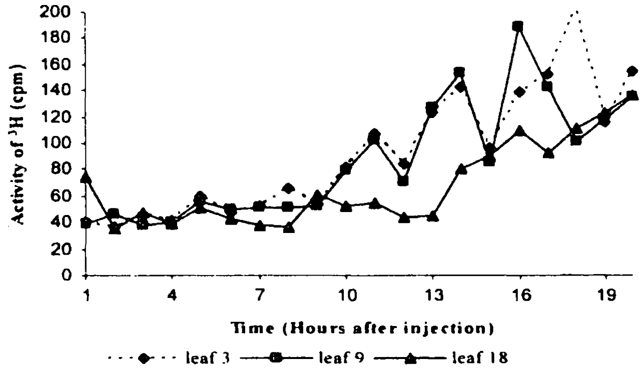

Figure 1. The Patten of changing the activity of ^3H2O in leaf 3, 9 and 18 with time

EXPERIMENT 2

Determination of sampling duration within a day

The same procedure was used to detect the best time duration for sampling. But here three fronds (3rd, 6th and 9th) were considered instead. Samples were collected at 1hr time intervals.

Results and Discussion

Remarkable peaks were identified in 3 fronds regarding the activity of ^3H_2O (cpm) in the second hour after injection. After that activity of ^3H_2O was reduced and a significant difference was not identified. It was decided to take samples in 30 minute interval to identify which 30 minute had a significant participation in the activity of ^3H_2O.

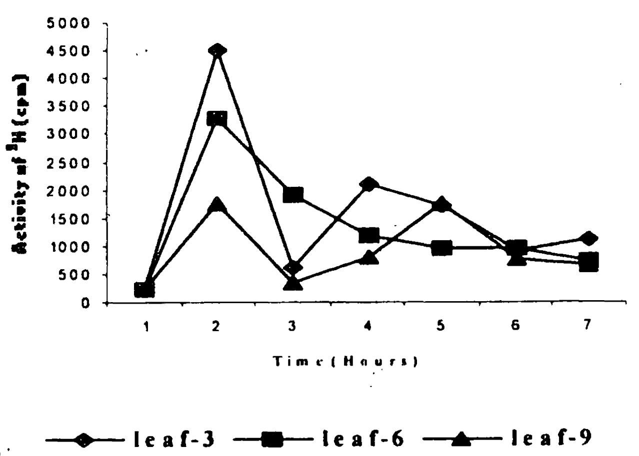

Figure 2. The activity of ^3H_2O (cpm) in transpired water Vs time

According to Figure 3, there was no any significant different in activity of ^3H_2O (cpm) among ½ hours duration. Taking observation once a day was identified as most appropriable for the determination of sampling time. Because obtaining the some in ½ hour or one hour interval tend to create problem.

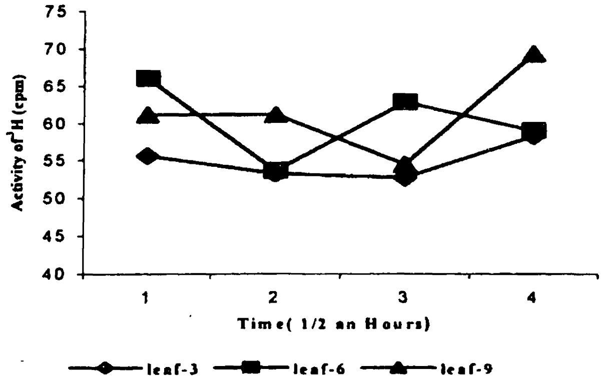

Figure 3. Activity of ^3H_2O (cpm) transpired water vs time

EXPERIMENT 3

Determination the Patten of water movement of LSD affected palms and healthy palms

The above mention methods were used for applying ^3H_2O water (3.1.). The sampling procedure and the analysis of ^3H_2O activity were measured by using above methods (3.2, 3.3 and experiment 1 and 2). Sampling was done for 10 days at 1 day intervals as decided in experiment 2.

Results and Discussion

The Pattern of water transportation

There was a significant difference in water transportation in LSD affected palms compared to healthy palms. According to tritium transportation in healthy palms, the maximum activity was shown 4-5 days after the injection (Figure 4). Then the activity gradually decreased up to the 10^{th} day.

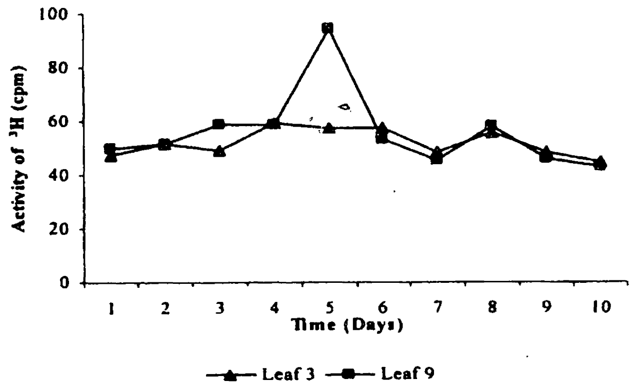

Figure 4. The activity of ^3H in the transpired water of healthy palms with time

In LSD affected palms the maximum tritium concentration was observed on the 8th day after injection. Then it was decreased up to the 10^{th} day after application. The data reveals that there is a delay the transportation of water in the LSD affected palms compared to healthy palms (Figure 5).

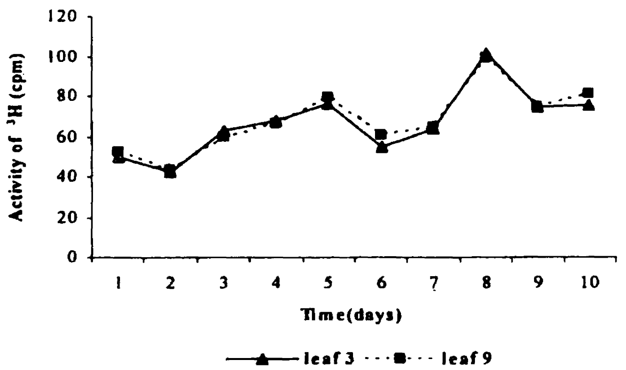

Figure 5. The activity of tritium (^3H_2O) in the transpired water of LSD -affected palms with time

LSD –affected palms showed a slower water movement to canopy compared to healthy palms. On the day 6 and 7, the tritium concentration of both healthy and LSD affected palms was reduced in transpired water. Day 6 was a rainy day and it seems that the xylem sap was diluted during day 6 and 7, and hence the tritium concentration in transpired water has decreased.

Dilution effect of ^3H_2O in healthy and LSD affected palms

The ^3H activity in LSD-affected palms was generally higher than that of healthy palms. Nevertheless, tritium was diluted in healthy palms than in LSD affected palms. On the day of highest activity (day 5) low tritium content (80-90 cpm) was detected in healthy palms and higher activity (90-120 cpm) was detected in healthy palms (day 8) (Figure 4 and 5). Healthy plants have a higher transpiration rate and water absorption compared to LSD-affected palms. Therefore, water content is high in healthy palms. This may cause dilution of xylem sap, which took place inside the trunk of healthy plants. Water absorption of the LSD affected palms is low, therefore the water content is low in the trunk and the crown. The transpired water was collected easily in the healthy palms than the LSD affected palms. The detached leaves of LSD affected palms had to be placed in low sunshine to collect the condensed water. It also explains the water content of the palm and dilution of the tritium water is high in healthy palms.

The total activity of canopy

Table 1. Total mean leaf area and fronds of canopy in healthy and LSD affected palms

Status of palms	No of total fronds	No of affected fronds	Total leaf area (m^2)	Height (feet)
Healthy palms	32.0	-	164.8	16.5
LSD affected palms	25.5	10.3	97.7	14.5

Healthy palms have significantly higher number of fronds, higher leaf area and larger trunk volume than LSD palms (Table 1). This also may explain the lower activity of ^3H in a frond of healthy compared to LSD palms. However, the total activity of the palm should be higher in healthy compared to LSD.

CONCLUSIONS

The study clearly indicated that ^3H_2O can be used as a tracer to study water movement in LSD-affected coconut palms. The upper whorls of the canopy are the most suitable fronds for sampling for ^3H than the lower whorls, as they are the most active fronds. A slower water movement from trunk to canopy was observed in LSD affected palms than the healthy palms. LSD affected plants has shown a delay in the water transportation. Future experiments are needed to be designed to study the same under water-stressed (drought) conditions. The study could be extended using the sap of roots, stem parts, midrib of the leaflet, and nut water from several bunches to determine the pattern of water transport to different organs of LSD-affected palms. The results can also be complimented with the sap flow data of LSD-affected palms.

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