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Photosynthesis and assimilate partitioning characteristics of the coconut palm as observed by ^{14}C labelling

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ABSTRACT

A technique was developed on the use of $^{14}CO_2$ rapid labelling of foliage and to ascertain photosynthesis and partitioning characteristics of labelled assimilate into other parts of the coconut palm. An eight-year-old Tall x Tall young coconut palm growing under field conditions at Bandirippuwa Estate and with six developing bunches, was selected for this study. The labelling was carried out on a bright sunny day and soil was at field capacity. Seventh leaf from the youngest open leaf was used for labelling with 5 mCi of $NaH^{14}CO_3$. The results revealed that within 24 hours, 60% of the labelled assimilate was partitioned into other parts of the palm and at the end of the seventh day about 18% of the labelled assimilate still remained in the labelled leaf. Among the developing bunches fifth and sixth bunches from the youngest developing bunch received more labelled assimilate than young developing bunches above them. It was revealed that partitioning of assimilate into various "sinks" is determined by the developmental stage or activeness of the "sink". The proportion of ^{14}C labelled assimilate, partitioned into developing bunches was substantially low compared to the total amount of ^{14}C fixed by the labelled leaf. Further, it was observed that partitioning of assimilated ^{14}C into the young leaves above, as well as the mature leaves below the labelled leaf. The complex vascular anatomy of the palms could be attributed to this pattern of partitioning of assimilates into upper and lower leaves from the labelled leaf.

Key words: Assimilate partitioning, ^{14}C labelling, *Cocos nucifera*, sink activity, source strength

INTRODUCTION

Isotopes are used in research on tree physiology, mainly to investigate photosynthesis, assimilate translocation, nutrient uptake, and transport of water and nutrients. Such studies have broaden the knowledge on subtle processes occurring within plant parts and inherent differences in such processes among different plant species. The ^{14}C technique has been recognized as very useful method to ascertain various inherent processes related to assimilation and assimilate partitioning pattern of plants (Vose, 1980). Distribution of assimilated carbon depends on leaf maturity, phyllotaxy, as well as the source-sink relationship of plants (Larson and Dickson, 1973 ; Forde, 1965). Influence of these plant factors and environmental stress conditions on plant physiological processes also could be investigated with the use of the ^{14}C technique.

The coconut palm (*Cocos nucifera L.*) is a monocotyledonous woody tree species with C_3 pathway of photosynthesis. The low rates of photosynthesis and slow growth are characteristic features of palm species (Jayasekara, 1992). The complex vascular anatomy of palms makes it more difficult to study the translocation characteristics of water/ nutrients in the xylem and photoassimilates in the phloem vascular tissues. Plant species differ widely in their rates of translocation of assimilated photosynthate. The rate of assimilate distribution has a positive correlation with the rate of photosynthesis in many species (Hofstra and Nelson 1969). Similarly, partitioning of assimilates depends on environmental factors such as photosynthetically active solar radiation, relative humidity, temperature, nutrient, and soil moisture availability from soil. Studies on photosynthesis and assimilate partitioning characteristics of ornamental palms, Jayasekara (1985) has shown that about 1/5 of the current assimilate is available to the developing bunches.

The $^{14}CO_2$ labelling of a leaf and estimation of ^{14}C in the constituents of various parts of the plants, provide a reliable and efficient method of describing the fate of assimilated carbon in plants over a period of time. Such tracer studies are useful to ascertain the translocation characteristics of assimilates in coconut. There had been no reported work on such studies for coconut. Hence, this study was conducted to investigate the CO_2 assimilation, usage of assimilated C in metabolic processes and partitioning characteristics of bearing coconut palms

under the field conditions.

MATERIALS AND METHODS

The experiment had a single replicate as it was difficult to select more palms with the same age of leaves and developing bunches as well as to avoid usage of large quantities of labelled carbon within a specific period. Hence, a six-year-old bearing young coconut palm growing under field conditions at Bandirippuwa Estate, Lunuwila, Sri Lanka was selected for this study. The selected palm had five developing bunches and one opened inflorescence with female flowers at the initial stages of receptivity. The seventh leaf from the youngest emerged un-opened leaf, which had no developing inflorescence at its axil was selected for ^{14}C labelling. The lower portion of the leaf was cut to reduce its length and then it was enclosed in a 1 m wide and 4 m long polythene bag (800 gauge). Before enclosing the leaf, a flask containing 5 mCi of $NaH^{14}CO_3$, three battery operated small air circulating fans, were hung on to the petiole. The bag was then tied on to the petiole with plastercene to prevent any leaks. Using a hypodermic syringe, fifty milli liters of 2N HCl was injected into the flask containing radioactive $NaH^{14}CO_3$ and the point that bag was pierced was thoroughly sealed. The leaf was allowed to continue photosynthesis for two and half hours in air enclosed in the bag mixed with $^{14}CO_2$ and then the bag was removed carefully.

On completion of labelling, two leaflets from the labelled leaf were removed immediately to determine the amount of ^{14}C fixed. A respiratory chamber was fixed to a leaflet in the labelled leaf and an air pump was connected to pass air through the chamber. Air leaving the chamber was bubbled through 2N NaOH solution to trap $^{14}CO_2$ evolved by dark respiration. Sampling of plant parts (i.e., leaves, inflorescences, developing nuts, petioles, and roots) was carried out at 1, 3, 5, and 7 days after labelling. Fresh weights of the samples were also recorded. Each sampled material was cut into small pieces and a representative sample of known weight was placed in a paper bag and oven dried at 70 °C for three days until reaching a constant weight.

The dried plant samples were weighed and ground in a rotary mill for the analysis of ^{14}C activity. The analysis of total ^{14}C was carried out by oxidizing 20 mg of plant material with the chromic acid digestion mixture in a tightly closed digestion tube as described by Amato (1983). The carbon dioxide evolved during the digestion was trapped in 2N NaOH and the radio-activity of labelled $NaHCO_3$ was determined using a Packard scintillation counter mixed with "Ultima Gold" (Packard Instrument Co. Inc, USA) as the scintillation cocktail. All the counts were quench corrected and converted to disintegrations per minute (dpm). The specific activity of plant samples was calculated on the basis of unit dry mass.

RESULTS

In labelling dynamic systems such as palms, it is important to label for an adequate period for it to utilize the $^{14}CO_2$ diluted with $^{12}CO_2$ in the enclosed system. Further, the palm should be given an adequate length of time to translocate, assimilated carbon throughout the plant before sampling.

Partitioning of assimilate into leaves

The specific activity of the labelled leaf amounted to 36×10^6 dpm g^{-1} dry weight soon after labelling and then gradually decreased linearly with time as given in Figure-1. At the end of the seven day harvesting period, the remaining ^{14}C activity in the labelled leaf was found to be about 18 percent that of the initial level. Partitioning of ^{14}C labelled assimilate was quite rapid within the first 2 to 3 days and then continued very slowly up to seven days or more.

Respiratory losses of ^{14}C from the labelled leaf was studied at 0-1, 2-3, 4-5, and 6-7 day intervals. The specific activity of ^{14}C in the labelled leaf and total ^{14}C lost as $^{14}CO_2$ by dark respiration from 4 pm to 8 am on the following day are given in Table-1. The ^{14}C activity of NaOH obtained on 0 - 1 day interval was not accurate as the air pump was not working properly for few hours in the early morning. Therefore, the measured respiratory loss of $^{14}CO_2$ at 0-1 day interval was an under estimation. As shown

Table 1. Total ^{14}C activity of labelled leaf (single leaflet) and ^{14}C loss by dark respiration for 16- hour period.

Duration (days)	Respiration losses of $^{14}CO_2$ (dpm)	Total activity of labelled leaflets (dpm)	Percentage loss of ^{14}C respiration
0-1	4800×10^2	4800×10^4	1%*
2-3	4100×10^2	1100×10^4	3.7%
4-5	800×10^2	100×10^4	0.8%
6-7	300×10^2	900×10^4	0.3%

* Under estimation due to technical problem

in Table-1, the utilization of ^{14}C labelled assimilate for metabolic processes decreased with increasing the duration from harvest 1 to harvest 4 after labelling. Nevertheless, it is reasonable to assume that respiratory losses of current assimilate in the studied coconut palms within the 0-1 day period was, 5-10 percent of the initial activity of the labelled leaf, whereas during the 2-3 day period it was about 4 percent.

The distribution of assimilate occurred in both upward and downward directions within the canopy. The seventh leaf from the youngest emerged leaf was selected for labelling and the activity of three leaves just above and below the labelled leaf was studied at each harvest. The specific activities of ^{14}C labelled assimilate in these leaves from day 1 to 7 are given in the Table-2. Comparison of assimilate partitioning

Table 2. Specific activity of ^{14}C labelled assimilate in the labelled source leaf, young leaves above and 3 mature leaves below the labelled leaf (dpm g^{-1} dry wt)

Leaf No.	1 - Harvest (day 1)	2 - Harvest (day 3)	3 - Harvest (day 5)	4 - Harvest (day 7)
4	150×10^2	250×10^2	238×10^2	140×10^2
5	3650×10^2	840×10^2	930×10^2	140×10^3
6	1750×10^2	360×10^2	280×10^2	1100×10^2
7*	14600×10^2	5600×10^2	3300×10^2	3000×10^2
8	180×10^2	250×10^2	370×10^2	130×10^2
9	165×10^2	840×10^2	670×10^2	3870×10^2
10	150×10^2	360×10^2	288×10^2	8000×10^2

*Latelied leaf

Table 3. Activity of ^{14}C labelled assimilate in nut water and the kernel of developing nuts in fifth and sixth bunches

Bunch No.		1 - Harvest (day -1)	2 - Harvest (day -3)	3 - Harvest (day -5)	4 - Harvest (day -7)
5	Nut water	560×10^2	446×10^2	1760×10^2	4800×10^2
	Kernel	20×10^2	1500×10^2	6300×10^2	10000×10^2
6	Nut water	-	-	-	12100×10^2
	Kernel	-	-	-	7200×10^2

to be higher than that in the younger bunches above them. As the duration became longer, the amount of labelled assimilate accumulated in nut water steadily increased. The fourth and fifth bunches of the labelled palm were at the active endosperm filling stage. The nuts of the fourth bunch were at an initial stage of kernel formation. The nuts in the fifth bunch were at the kernel development stage. The kernel material and liquid endosperm of these nuts in fifth and sixth bunches recorded the highest ^{14}C activity at the fourth harvest as given in Table- 4. These results suggest that a significant proportion of ^{14}C labelled assimilate had gradually become incorporated into storage food material in the kernel with time.

DISCUSSION

The results show that partitioning of ^{14}C assimilate from the labelled seventh leaf to the other parts of the coconut palm is a slow process. Within the first 24 hours, nearly 60 percent of the labelled assimilate had been partitioned or utilized for metabolic processes. After 7 days, only about 18 percent of the labelled assimilate still remained in the labelled leaf. Arecoidae palm species like *Chrysalidocarpus lutescens* and *Chamaedorea elegans* leaves export 40-50 percent of the current assimilate from the source leaf within 24 hours (Jayasekara, 1985).

Table 4. Total activity of ^{14}C labelled assimilates in nut water at different harvests

Bunch Number	No. of nuts	1 - Harvest Total volume of nut water (ml)	1 - Harvest Total activity of nut water (dpm)	2 - Harvest Total volume of nut water (ml)	2 - Harvest Total activity of nut water (dpm)	3 - Harvest Total volume of nut water (ml)	3 - Harvest Total activity of nut water (dpm)	4 - Harvest Total volume of nut water (ml)	4 - Harvest Total activity of nut water (dpm)
B1	-	-	-	-	-	-	-	-	-
B2	9	10.5	30×10^2	20	112.7×10^2	20.5	300×10^2	24	3.5×10^4
B3	10	95	50×10^2	100	4.50×10^4	100	400×10^2	148	13.8×10^4
B4	8	280	83.20×10^4	330	15.40×10^4	358	25.3×10^4	325	39.0×10^4
B5	2	450	5.60×10^4	440	4.46×10^4	390	17.6×10^4	390	48.0×10^4
B6	1	-	-	-	-	-	-	300	12.1×10^4

among other a significant amoleaves showed that unt of labelled assimilate in source leaf was transferred to the sixth and ninth leaf, despite the fourth and fifth leaves being the youngest open leaves still growing.

Partitioning of assimilate into nuts

The main emphasis of this study, was to determine the partitioning of current assimilate into developing nuts and to evaluate their sink demand. Hence, nut components were analyzed at each harvest. The activity of ^{14}C assimilate in the mesocarp of the developing nuts is given in Table-3. The ^{14}C activity of the mesocarp increased gradually from day 1 to 7. The activity of ^{14}C assimilates in other nut components *viz.* Liquid endosperm or nut water and developing kernel were significantly higher than that of the mesocarp (Table-4). Within 24 hours, ^{14}C labelled assimilates that have translocated into the nut water in the fourth and fifth bunches were found

These observations suggest that palms in general have similar assimilate partitioning characteristics. A certain proportion of ^{14}C assimilate was transferred into the mature source leaves (Leaf Nos. 8, 9, and 10) below the labelled leaf as well as the immature upper leaves (Leaf Nos. 4, 5, and 6) above the labelled leaf (Fig. 2 and 3). Anderson and Dale (1983) reported that translocation of ^{14}C- labelled assimilate to all parts of barley plants when $^{14}CO_2$ was supplied to the last open mature leaf. Extensive vascular connections between leaves and the main stem have been suggested as the main factor facilitating bi-directional movement of assimilate in *Lolium* (Forde, 1965). The coconut palm also has an extensive vascular system as reported by Parthasarathy and Tomlinson, (1967) and Zimmerman (1973). This explains the movement of assimilate into mature source leaves of coconut, even though they were actively photosynthesising leaves.

Partitioning of assimilate into fruits depends on

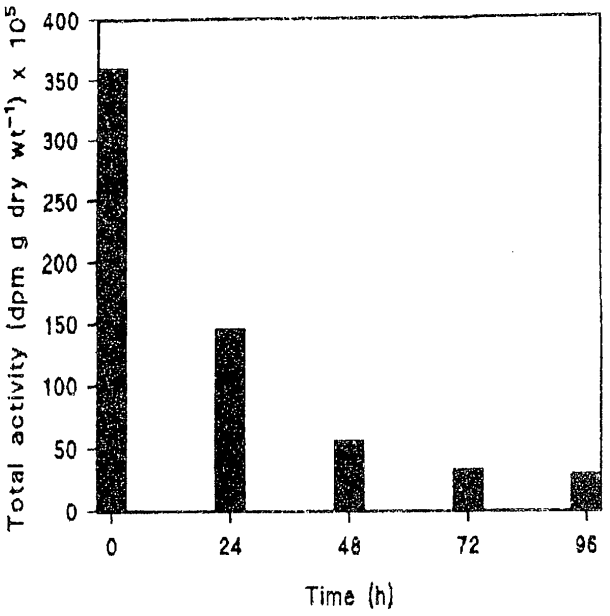

Fig. 1. Depletion of ^{14}C activity in the labelled coconut frond.

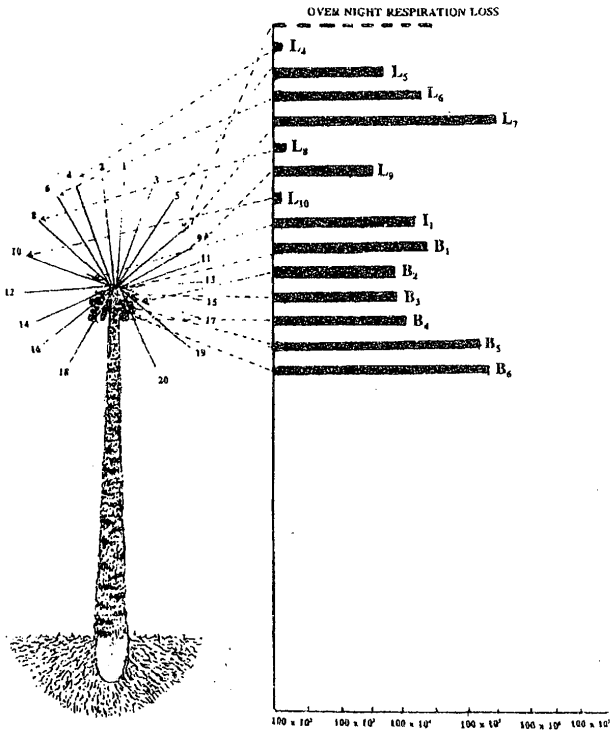

Fig. 2. Distribution of total ^{14}C activity (DPM) 24 hours after labelling

the developmental stage of the sink or fruit size. Fondy and Geiger (1981) demonstrated that partitioning of assimilate into sink tissues depends principally on the demand for assimilate and the distance from source to sink. In this study, it was evident that demand for assimilate by active sinks in palms is governed by the active developmental stage of the sink tissues, rather than the distance from the

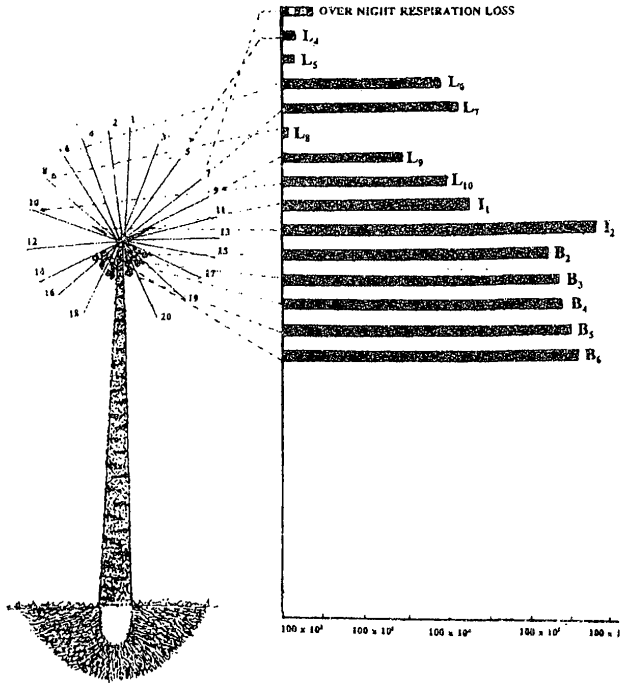

Fig. 3. Distribution of total ^{14}C activity (DPM) 7 days after labelling.

source. Developing fruits in the fourth bunch were five months old and they were at the active endosperm cavity enlargement stage and the cavity was completely filled with water. Where as fifth bunch was six months old and the fruits were at the kernel deposition stage. The nuts in the sixth bunch had about 1 - 2 mm thick kernel. The second and third bunches had very small developing nuts with a cavity of negligible size. These observations revealed that developing fruits in the bunch number 4 onwards have reached the active growth, expansion and kernel formation stage. The activity in ^{14}C labelled assimilate translocated into fourth, fifth and sixth bunches proved actively developing nuts in these bunches have received more labelled assimilate than the developing nuts in the closely located immature bunches (ie. Bunch Nos. 1,2 and 3).

Within first six months of development, generally 2/3 of the developing nuts are shed off and about 1/3 of the button nuts develop into mature nuts (Abeywardena and Mathes, 1971). This initial shedding of developing nuts could be partially or completely attribute to the lesser availability of assimilate for immature bunches due to high "sink" demand exerted by the bunches above fourth bunch. The total quantity of ^{14}C assimilate partitioned into first three developing bunches was low compared to the ^{14}C labelled assimilate partitioned into leaves above and below the labelled leaf. Limitation of assimilates may be a reason for heavy losses of immature nuts during early stages of nut development. Palms such as coconut, which have

simultaneous vegetative and reproductive growth phases depend almost entirely on the current assimilate for their growth and other metabolic processes (Van Die 1974). Hence, any measures to increase partitioning of current assimilate into developing immature bunches would help to increase setting of nuts.

At the fourth harvest, female flowers in the opened inflorescence was at the receptive stage. For the production of nectar, this inflorescence may have received assimilate from other leaves, which caused a sudden increase in ^{14}C activity in female flowers. Within 7 days, all the developing bunches and the opened inflorescence were found to have received a substantial proportion of assimilate from the labelled leaf. During the growth of fruits, sucrose transported into the fruit is converted to complex compounds such as polysaccharides, proteins, and fats. Respiratory losses of carbon during these metabolic processes generally account for about 0.3 g for each gram of carbon and the maintenance respiration in growing fruits has been estimated to be about 15.40 mg of carbon per gram of carbon per day in the fruits (McCree 1976). Net loss of carbon by fruit respiration was not determined in this study. Because of the daily accumulation of current photosynthate, labelled assimilate would be gradually diluted and the quantity of ^{14}C withdrawn from the labelled leaf progressively smaller than immediately after labelling. The dilution process in palm leaves could be responsible for the slow release of labelled assimilate.

In general, the study described here provided considerable amount of information on the assimilate partitioning characteristics of the coconut palm, which were not well known in the past. However the results have to be carefully interpreted as only a single replicate was used.

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