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*PHOTOSYNTHETIC ASSIMILATION OF $^{14}CO_2$ BY MATURE BROWN STEMS OF THE TEA PLANT (*CAMELLIA SINENSIS* L.)

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The fixation of $^{14}CO_2$ by mature brown stems of the tea plant was studied by supplying $^{14}CO_2$ to selected stems of pruned and intact plants for 24 h under field conditions. Utilization of ^{14}C assimilates for the production of new shoots was also examined. The photosynthetic nature of fixation of $^{14}CO_2$ is demonstrated. The efficiency of this fixation was very low compared with that taking place in leaves. The movement of labelled assimilates from stem bark to the roots was inappreciable, whereas newly emerged shoots on the pruned frame drew labelled assimilates from stem bark.

INTRODUCTION

Tea is a perennial crop which is subjected to a regular cycle of pruning (2-6 years). Some aspects of post-prune growth, particularly the factors contributing to recovery from pruning have received some attention (Tubbs, 1937; Nagarajah and Pethiyagoda, 1965; Selvendran, 1970). Some work has also been done to identify and locate the reserves in the plant which are utilized for early post-prune bud growth. Kandiah (1971) stated that reserves in the stem bark are appreciable and serve to support bud growth on the frame. Selvendran and Selvendran (1972) postulated that during recovery from pruning starch reserves in roots were mobilized and translocated to support growth of new shoots. They also suggested that starch present in the stems may be utilized for new growth. The importance of stem bark as a source of reserves at the beginning of leaf expansion in spring in temperate trees has been reported (Mason and Whitfield, 1960; Tromp, 1970).

Recent work in this laboratory showed that even mature brown stems in a pruned tea plant were capable of fixing $^{14}CO_2$ into soluble and insoluble constituents in light (Selvendran and Selvendran, 1972). This paper presents the results of studies on the nature of the fixation of $^{14}CO_2$ by tea stems and examines the fate and movement of the labelled assimilates in relation to the recovery of the plant from pruning.

MATERIALS AND METHODS

Eight-year-old field plants of clone TRI 2022 carrying a single main stem were used. All branches and foliage were removed so that the height of the pruned plant was 10 cm above the ground except for the single main stem which was pruned at a height of 40 cm. The main stem was about 2 cm in diameter.

The centre position (20 cm) of the stem was enclosed in a polyethylene sleeve the ends of whih were sealed and attached to the stem with adhesive cellophane. $^{14}CO_2$ (20 μCi) was released inside this assimilation chamber by the addition of lactic acid to $Na_2\ ^{14}CO_3$ contained in a small vial inside the chamber. The stem was exposed to $^{14}CO_2$ for 24 h under field conditions (direct sunlight during the day), and at the end of this period the polyethylene sleeve was removed.

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Experiment A

The fixation of $^{14}CO_2$ by the pruned stem in the light was compared with (a) the pruned stem exposed to $^{14}CO_2$ in the dark, and (b) the stem of an intact plant exposed to $^{14}CO_2$ under the same light conditions. In each case the $^{14}CO_2$ treated stem was harvested the following day for further investigation.

Experiment B

The pruned stem was exposed to $^{14}CO_2$ in the light for 24 h. The plant was allowed to recover from pruning and the stem was removed for assay after the appearance of new shoots (60 days). The stem bark and the young shoots on it were removed and prepared for autoradiography as described below.

Experiment C

The photosynthetic efficiency of a dismembered stem portion was compared with that of an excised leaf. Using the treatment chamber described elsewhere (Sanderson and Sivapalan, 1966) both samples of approximately equal surface area were exposed simultaneously to $^{14}CO_2$ (20 μCi) for 30 min in full sunlight. During this period the assimilation chamber was frequently reoriented with respect to the incident light. After the termination of $^{14}CO_2$ feeding, the stem bark was stripped off from the woody portion of the stem. The stem bark and the leaf were extracted separately in boiling 80 per cent ethanol and aliquots used for the estimation of radioactivity in the alcohol soluble fractions.

Extraction of samples (Experiment A)

The stem bark and the stem wood of the $^{14}CO_2$ fed portion were separated, cut into small pieces, dried in an oven for 18 h at 90 °C and ground in a cutter mill to pass a 40-mesh sieve, Sub-samples (500 mg) were extracted repeatedly in hot 80 per cent ethanol until the filtrate gave a negative test for sugars. The alcohol extract was concentrated under reduced pressure at 40 °C and the concentrate fractionated into amino acids, organic acids and sugars as described by Sanderson and Sivapalan (1966). The sugar-free residue was extracted with 52 per cent perchloric acid from which starch was precipitated according to the method of Hassid and Neufeld (1964). The precipitated starch was hydrolysed with 1 N HCl, the hydrolysate neutralized with 1 N NaOH, the mixture passed through a mixed ion-exchange resin column, and the effluent collected. Aliquots of the amino acids, organic acids and sugars from the alcohol-soluble fraction, as well as the hydrolysed starch from the sugar-free residue were planchetted and counted. The sugar fraction and the hydrolysed starch fraction were analysed by one-dimensional paper chromatography using ethyl acetate: pyridine: water (6:2:1), and the radioactive spots located by autoradiography.

Assay of radioactivity

Estimation of radioactivity was by both autoradiographic and counting techniques. Radioactive extracts were counted on an Al planchette as infinitely thin layers using a thin-end window G-M tube. Stem bark specimens were dried between filter papers in the oven at 90°C for 18 h and the mounted specimens masked with cellophane exposed to X-ray film (Kodak medical X-ray film, royal blue) for 30 days.

Experiment A—photosynthetic assimilation of $^{14}CO_2$

The distribution of radioactivity in the stem fractions of the pruned plant fed $^{14}CO_2$ in the light and in the dark is shown in Table 1. Nearly 75 per cent of the soluble radioactivity in the stem bark exposed to $^{14}CO_2$ in the light was present in the sugar fraction. Chromatographic separation of this fraction followed by autoradiography revealed that about two-thirds of the radioactivity of this fraction was in sucrose. It is seen from Table 1 that although there was some fixation of $^{14}CO_2$ in the dark, this is small in comparison to the quantity of $^{14}CO_2$ incorporated in the presence of light. The incorporation of radioactivity in starch was significant. Chromatographic separation of the starch hydrolysate followed by autoradiography revealed only one spot which had an R_F identical to a glucose marker.

TABLE 1 — *Distribution of radioactivity is some products of $^{14}CO_2$ assimilation by mature tea stems*

Radioactivity of plant part expressed as counts^{-1} g dry weight^{-1}

Fraction analysed	$^{14}CO_2$ fed to pruned stem in darkness		$^{14}CO_2$ fed to pruned stem in light		$^{14}CO_2$ fed to intact stem in light	
	Stem bark	Stem wood	Stem bark	Stem wood	Stem bark	Stem wood
Ethanol soluble	19	10	902	126	906	242
Sugars	6	1	666	69	633	152
Amino acid	2	1	60	25	42	24
Organic acid	2	1	94	24	59	20
Starch	1	—	190	6	240	22

It is also seen from Table 1 that pruned and intact stems have a similar pattern of $^{14}CO_2$ assimilation under conditions of equal illumination.

Experiment B—utilization of labelled assimilates for new growth

Plate 1 shows the autoradiogram of the stem bark and the shoots that had developed on it. The new shoots appearing on the $^{14}CO_2$ fed-region are seen to contain the ^{14}C-label. It is also evident that there has been movement of the ^{14}C-label into region A, i.e. above the fed-region. In contrast there has been very little movement of ^{14}C-label into the lower portion C. This would mean that the movement of labelled substrates from the stem bark to the root region was insignificant.

Experiment C—comparison of photosynthetic efficiency

Preliminary observations indicated that the uptake of $^{14}CO_2$ by stems was relatively sluggish, and therefore in the present study only large differences were sought between the photosynthetic abilities of leaf and stem. Sanderson and Sivapalan (1966) found that when a mature leaf was fed $^{14}CO_2$ for 1 h in the light, the total radioactivity incorporated was present in the soluble and insoluble fractions in almost equal amounts. In this study, therefore, the soluble counts alone were taken as an index of ^{14}C incorporation. The results of this experiment showed that the photosynthetic efficiency per unit area of leaf was about ten times that of stem bark.

DISCUSSION

These results show that photosynthetic fixation of $^{14}CO_2$ can occur in mature brown tea stems exposed to light. The presence of labelled starch and also chlorophyll containing tissues just under the bark further support these findings. The photosynthetic efficiency of the stem bark however is small in comparison to that of leaf.

Although pruned and intact stems show similar patterns of assimilation under conditions of equal illumination, it could be argued that most of the stem portion will be shaded in an intact plant under field conditions and photosynthesis by the stem could assume importance only after the foliage is removed. The assimilates thus formed have been shown to be retained in the bark and not transported to the roots. These reserves are available as a ready source of carbon for the newly developing shoots.

During recovery from pruning there is no supply of carbohydrate from the aerial portion of the plant to the roots, because all foliage has been removed. Kandiah (1971) has observed that during post-prune growth, reserves present in roots are largely consumed by the root system itself. This would mean that root reserves would not contribute significantly to shoot development. Selvendran and Selvendran (1972) suggested however, that some of the reserves in the roots are mobilized and translocated to support new growth. Further to these views the finding that stem assimilates are utilized for shoot growth provides new information on the process of recovery from pruning.

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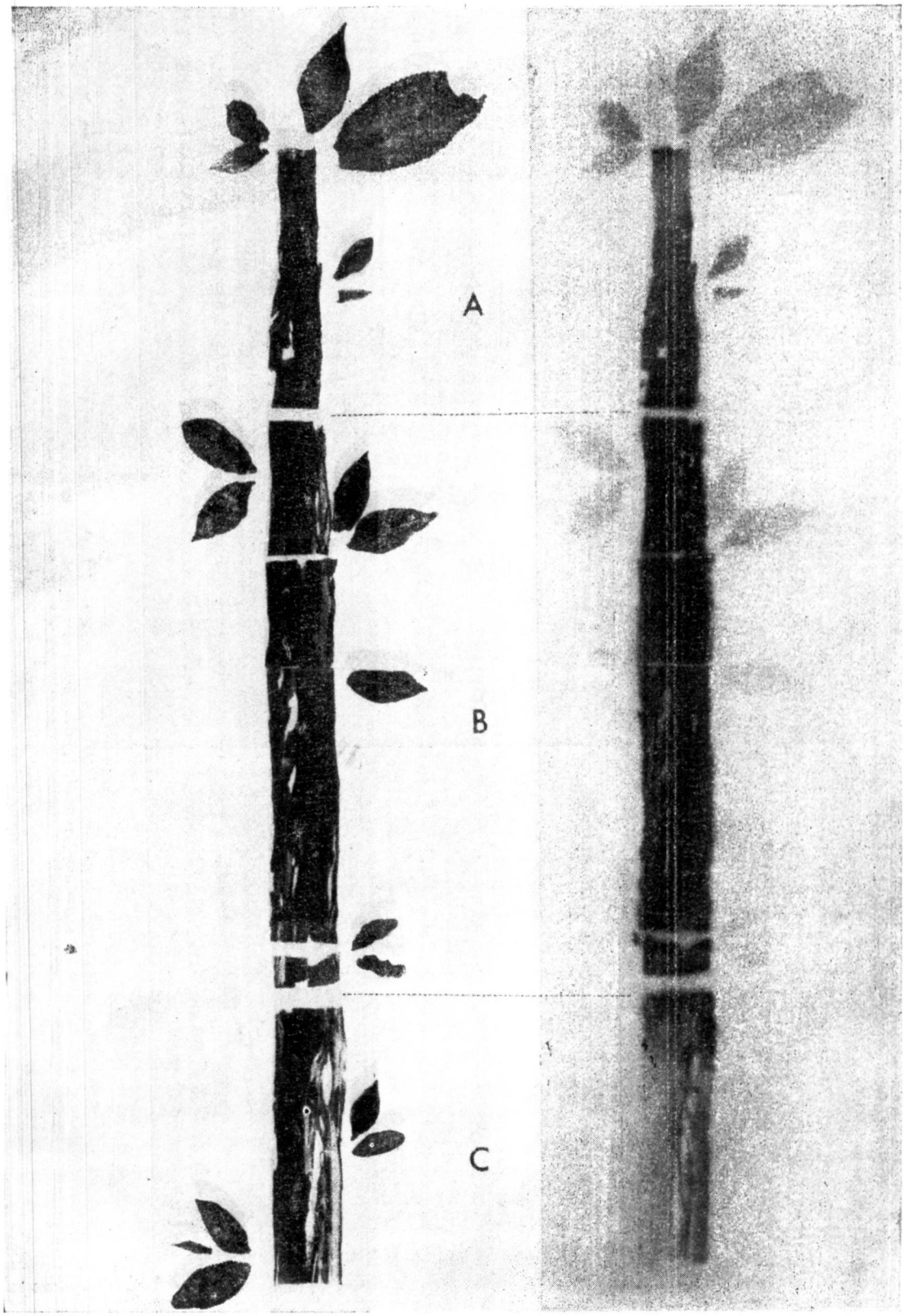

FIG. 1 *^{14}C-assimilate export by mature stem of pruned tea plant. Region B was fed $^{14}CO_2$ for 24 h, the stem was harvested after appearance of new shoots (60 days) and prepared for autoradiography. The plant specimen (stem bark and new shoots) and autoradiograph (right) are shown side by side. Note that while shoots in region A and B show ^{14}C-import, this is not evident in the shoots in region C.*

FIG. 1—*H.E. The President of Sri Lanka at the TRI*

FIG. 2—*The Hon. The Prime Minister of Sri Lanka at the TRI*