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TRANSLOCATION OF NOCTURNALLY FIXED ^{14}C IN THE CRASSULACEAN ACID METABOLISM EPIPHYTE TILLANDSIA USNEOIDES L.[1]

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Strands of the Crassulacean acid metabolism (CAM) epiphyte *Tillandsia usneoides* L. (Spanish moss) were exposed to $^{14}CO_2$ in situ at night and were collected immediately and at intervals of up to 40 days. Autoradiographs of the exposed strands were made. The tissue was separated into parts, oxidized, and counted in a liquid scintillation counter. The entire surface of labeled portions of the plant took up $^{14}CO_2$. There was very little translocation of ^{14}C out of the labeled portion of the strand at 40 days. Translocation within the labeled portion occurred from leaves and stems to nodal (meristematic) regions. The results are similar to those found with several other CAM plants.

Introduction

Although investigations of the early products of $^{14}CO_2$ fixation in Crassulacean acid metabolism (CAM) plants are numerous (SALTMAN et al. 1956; KUNITAKE, SALTMAN, and LANG 1957; TING and DUGGER 1968; KNAUFT and ARDITTI 1969; KLUGE et al. 1979), there are few studies of translocation of photosynthetically fixed ^{14}C in whole CAM plants. Six hours following exposure of *Opuntia polyacantha* pad sections to $^{14}CO_2$, 77% of the total amount of ^{14}C initially fixed had been lost or was not recovered, and only 4% of the total activity remaining in the tissue had been translocated out of the exposed sections (CHOW, BURNSIDE, and LAVY 1966). Similar results were found with the CAM plant *Opuntia bigelovii* (KLUGE and TING 1978). Even after 6 wk, little translocation of label out of the exposed pad had occurred.

In contrast, rapid and extensive translocation of photosynthate has often been reported in C_3 and C_4 plants (KURSANOV 1963; CRAFTS and CRISP 1971). For example, 60% of the initial amount of $^{14}CO_2$ taken up by a *Beta vulgaris* leaf had been translocated out of the leaf 3 h following exposure (MORTIMER 1965). In non-CAM plants, translocation of photosynthate can occur to many parts of a plant, often depending upon the particular part labeled (BIDDULPH and CORY 1956).

Spanish moss (*Tillandsia usneoides* L.) is a CAM epiphyte in the Bromeliaceae, occurring in warm, humid areas from Virginia south to Argentina and Chile. As in most other CAM plants, Spanish moss has relatively slow CO_2 uptake and growth rates (MARTIN, CHRISTENSEN, and STRAIN 1981). Strands of Spanish moss grow in clumps and may be up to 0.5 m long, yet only the distal 10–30 cm are usually alive. The vasculature of these strands is poorly differentiated; thus, it has been assumed that translocation within a strand is unlikely (BILLINGS 1904; TOMLINSON 1969), though this has never been determined. The purpose of this study was to determine the pattern of translocation of photosynthetically fixed ^{14}C in Spanish moss strands over a period of 40 days.

Material and methods

Spanish moss strands selected for this study were hanging from a small oak tree (*Quercus laevis* Walter) on a sand ridge adjacent to Jones Lake in the coastal plain of North Carolina. Thirty attached strands were exposed to $^{14}CO_2$ in air (315 $\mu l \cdot liter^{-1}$; 22 $\mu Ci \cdot liter^{-1}$) for 1 min in a Plexiglas chamber. Previous studies with Spanish moss (MARTIN et al. 1981) eliminated the possibility of adsorption of $^{14}CO_2$ to the surface of the tissue. The $^{14}CO_2$ air mixture flowed through the chamber at 300 $ml \cdot min^{-1}$. All labeling was conducted from 0100 to 0230 on August 15, 1979. The preceding day was cloudy; temperatures were ca. 27 C during the day and 20 C at night. Each strand was marked with black ink immediately above the labeled portion. The first five strands were labeled and quickly packed in dry ice. The remaining labeled strands were left hanging; then five were harvested after 14 h ("1 day"), 3 days, 7 days, 23 days, and 40 days.

Autoradiographs of the labeled strands were obtained by placing the strands on Kodak No-Screen Tinted Estar Safety Base medical X-ray film which was then stored at 4 C for 48 days. The film was developed using standard techniques. Two unlabeled strands served as controls. There were no contact artifacts.

To quantify the amount of radioactivity in

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different parts of the Spanish moss strands, the strands were separated into labeled and unlabeled segments. Both segments were further separated into stems and leaves (leaf sheaths were left attached to the stem). Each plant part was dried at 60 C for 3 days, cooled in a desiccator, weighed, oxidized, and counted in a Beckman LS250 liquid scintillation counter. The wet oxidation technique and scintillation cocktail were modified from MAHIN and LOFBERG (1970) so as to minimize $^{14}CO_2$ volatilization losses during oxidation (for modifications, see MARTIN et al. 1981). Resultant counts per minute (cpm) per gram dry weight, minus background, were pooled for each tissue part (stems and leaves) for each segment and for each set of five strands harvested at one particular time.

Results and discussion

After a 1-min exposure to $^{14}CO_2$, radioactive CO_2 was taken up by all living portions of a strand of Spanish moss (fig. 1). Approximately half the ^{14}C was found in the leaves and half in the stem (table 1). Irrespective of the time of collection, the amount of label in the leaves was one-third to one-half that in the stems in the labeled portions of the strands. Very little radioactivity moved from the labeled portion to the unlabeled portion. These results are similar to those found with other CAM species (CHOW et al. 1966; KLUGE and TING 1978).

Although translocation of ^{14}C out of the labeled portion of the strands did not occur in 40 days, the distribution of radioactivity in the labeled portion did change with time (fig. 1). No substantial differences in distribution of label were noted 1 day following exposure to $^{14}CO_2$ compared with strands collected immediately after labeling. However, after 3 days, label began to accumulate at the base of the leaves. Meristematic tissue is present in this area (inside the leaf sheath) and most probably accumulated the label. After 7 days, this accumulation had become more pronounced. Twenty-three days following exposure to $^{14}CO_2$, label had accumulated in the meristematic regions and the lower parts of the leaves. Finally, after 40 days, large portions of the basal leaf segments were heavily labeled. Therefore, translocation did occur within the labeled portions of the strands.

TABLE 1

RADIOACTIVITY DUE TO ^{14}C IN LEAVES AND STEMS OF SPANISH MOSS STRANDS HARVESTED AT DIFFERENT INTERVALS FOLLOWING IN SITU LABELING

TIME AFTER LABELING (days)	% RADIOACTIVITY IN				TOTAL COUNTS* $MIN^{-1} \cdot g\ DW^{-1}$
	Labeled leaves	Labeled stems	Unlabeled leaves	Unlabeled stems	
0.....	55.3	44.7	0	0	850,640
1.....	61.5	38.3	0.2	0	1,798,030
3.....	54.6	44.3	0.8	0.3	1,947,970
7.....	32.4	66.2	0.8	0.6	1,404,860
23.....	34.3	64.7	0.9	0.1	1,510,410
40.....	44.8	54.5	0.6	0.1	1,807,360

NOTE.—Values represent pooled results for five strands.

The results of this study indicate that very little ^{14}C was translocated up the strand (basipetally) but do not preclude the possibility that long-distance (over 10 cm) translocation of carbon can occur toward the tip of the strand (acropetally). Short-distance (1–2 cm) translocation from internodes and leaves to nodes occurred in all strands. Such translocation occurred despite the poorly differentiated vasculature (BILLINGS 1904; TOMLINSON 1969).

Although it is not known how much $^{14}CO_2$ was taken up by each group of strands during initial labeling, the total amount of radioactivity found in each group of strands was nearly as high as or higher than the amount found in those strands which were immediately harvested (table 1). Since these strands were labeled 1.5 h prior to the labeling of the strands collected 40 days later, it is difficult to assign meaning to comparisons of these figures because of the probability of changing CO_2 uptake rates during the labeling period.

In summary, 40 days after labeling, translocation of ^{14}C did not occur out of the labeled portion of Spanish moss strands, yet did occur within the labeled portion of strands from leaves and stems to meristematic regions. The results of this study provide a better understanding of several phenomena observed in ecological studies of Spanish moss. The accumulation of radioactivity at the meristematic regions suggests that growth or storage is occurring at each node. Indeed, MARTIN et al. (1981) found that elongation occurred along the entire length of Spanish moss strands. Furthermore, GARTH (1964) found that horizontally oriented strands will produce new shoots from the nodes. Reproduction of Spanish moss by fragmentation is probably a common event. Since little or no long-distance transport of photosynthate was observed in strands, a detached fragment may be relatively autonomous with regard to photosynthetic CO_2 fixation and subsequent redistribution of photosynthate.

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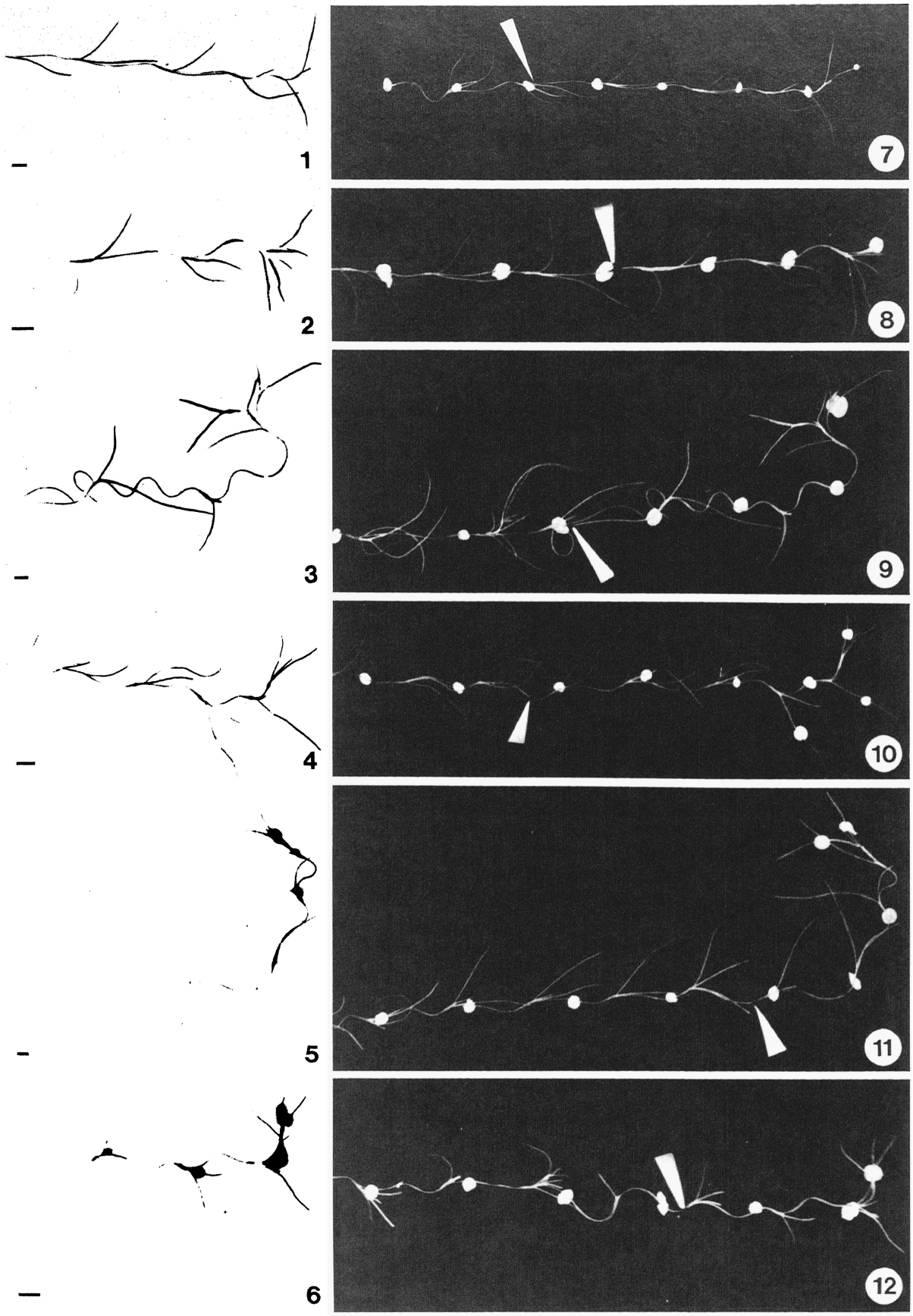

FIG. 1.—Autoradiographs (nos. *1–6*) and respective photographs (nos. *7–12*) of representative Spanish moss strands labeled with $^{14}CO_2$ during the night and collected immediately (*1*, *7*) and at 1 day (*2*, *8*), 3 days (*3*, *9*), 7 days (*4*, *10*), 23 days (*5*, *11*), and 40 days (*6*, *12*) thereafter. The white arrows in the photographs indicate the portion of strand labeled (to the right), and the dark line in each autoradiograph represents 1 cm. The white disks in the photographs were used to hold the strands in place.

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