THE DISTRIBUTION OF PHOTOSYNTHATE WITHIN LUCERNE AS INFLUENCED BY ILLUMINATION

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Summary

The influence of exposure of lucerne plants to light and to darkness, after administration of $^{14}CO_2$ to the whole plant, on the distribution of ^{14}C-labelled photosynthate within the leaves, stems, and roots has been studied.

Plants placed in darkness after exposure to $^{14}CO_2$ had a greater quantity in the root system of ^{14}C-labelled material which was soluble in 80% (v/v) ethanol than those left in the light.

The amount of ethanol-insoluble ^{14}C-labelled photosynthate in the leaves increased while the plants were in the light (6 hr) and decreased during their dark phase (18 hr), but it did not vary significantly over the exposure period of 24 hr for plants kept continually in the dark. The decline in the amount of ethanol-soluble ^{14}C-labelled photosynthate in the leaves was more rapid in the light than in the dark.

The loss of ^{14}C from the whole plant was less in the light than in darkness.

I. INTRODUCTION

The effect of different light intensities on the translocation of labelled photosynthate has been studied by Nelson and Gorham (1957) with soybean plants and by Sekioka (1962) with sweet potatoes. They found that a greater proportion of labelled photosynthate entered the root system under high light intensities, than under low light intensities. Thrower (1962) found with soybean that under low light intensities a greater proportion of the assimilate exported from the leaf moved down the stem than under high light intensities. However, the absolute amount of photosynthate moving down was reduced in low light intensities due to a greater retention by the leaf of assimilates. These observations are unlikely to be explained by changes in translocation rates due to differing light intensity since Vernon and Aronoff (1952) found no evidence with soybean to suggest that light intensity influences translocation rates. However, the work of Hartt, Kortschak, and Burr (1964) with sugar-cane plants suggests that continuing photosynthesis causes the movement of newly formed sucrose from the leaves. This would indicate that another light-stimulated mechanism influences the amount of labelled translocate entering the root system despite the apparent greater movement of photosynthate from leaves actively photosynthesizing.

This paper presents the result of an experiment designed to investigate the distribution of ^{14}C-labelled photosynthate as influenced by light and dark treatments.

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II. Materials and Methods

(a) *Treatment of Plants*

Stem tips of clonal lucerne plants (*Medicago sativa* L. cv. Hunter River) were rooted in sand, innoculated with a commercial strain of *Rhizobium*, and grown in a semi-controlled environment glasshouse. The plants were grown in 9-in. plastic pots containing river sand which had been sieved to remove particles greater than 2 mm and washed to remove clay. They were irrigated daily with a nutrient solution described by Arnon and Hoagland (1940) except that Fe-EDTA was used instead of $FeSO_4$.

In all, 24 pots, each containing one lucerne plant, were used in the experiment which was commenced 6 weeks after the cuttings were transplanted. At this stage the plants had unopened flower buds. Two treatments were imposed following administration of $^{14}CO_2$ to the plants, namely a light treatment in which the plants were left in the glasshouse for 6 hr and then transferred to the darkroom, and a dark treatment where the plants were immediately transferred to the darkroom. At 0·5, 1·5, 3·0, 6·0, 10·0, and 24·0 hr after exposure two plants from each treatment were harvested. The experiment was laid out in two blocks with the treatments and harvest times randomly allocated before commencement. For statistical analysis the experiment was treated as a replicated factorial layout.

The administration of $^{14}CO_2$ to the plants involved covering the plants with chambers made from clear Acrylite, 15 in. high and 9 in. in diameter. These chambers were sealed to the rims of the pots with adhesive tape. At 10.30 a.m., 110 μc of $^{14}CO_2$ was released into each chamber by dropping in HCl on $Ba^{14}CO_3$. The exposure period was 30 min. During this time the light intensity outside the chambers was 1600 f.c. and the chamber temperature varied between 23 and 25°C. After exposure the plants were transferred to a temperature-controlled darkroom, or left in the glasshouse under natural illumination for 6 hr, and then placed in the darkroom. During this phase, the mean darkroom temperature was 20·3°C (S.E.$\pm$0·07) and that of the glasshouse was 19·5°C (S.E.$\pm$0·11).

At the predetermined harvest times the plants were dissected into roots, stems, and leaves. Each sample was divided into two approximately equal portions: one-half was used for dry weight estimations by weighing, drying the sample at 90°C in a forced-draught oven, and re-weighing; the other half was weighed (to obtain fresh weight), ground under liquid air, placed in Soxhlet thimbles, and stored at -15°C in a deep-freeze unit.

(b) *Chemical Extraction and Determination of ^{14}C Content of Plant Material*

The material was extracted with 80% (v/v) ethanol under reflux for 6 hr and the residue in the thimble dried in an oven at 80°C. A 1-ml subsample from the ethanol fraction was transferred to small bags made from cellulose dialysis tubing, and then dried under infrared lamps. Also, 0·10 ($\pm$0·05) g of the ethanol-insoluble material was carefully weighed into cellulose bags.

A rapid and accurate measurement of ^{14}C in the samples was obtained by combustion of the samples to CO_2, absorption of the CO_2 into ethanolamine, and

liquid-scintillation counting of the absorbant. The oxygen-flask technique described by Kalkerer and Rutschmann (1961), and modified by Kelly *et al.* (1961) was used. The flasks were mechanically rolled for 30 min after combustion. By adding a constant volume (0·005 ml) and radioactivity of hexadecane-1-^{14}C to different weights of finely ground non-labelled lucerne leaves it was found that absorption of ^{14}C was proportional to sample weight. By calculation of the percentage absorption for various sample weights, calculation of the actual $^{14}CO_2$ produced by each combustion was possible. The absorbing solution was based on that of Jeffary and Alverez (1961), and was a mixture of ethanolamine–methylcellosolve (1 : 9 v/v).

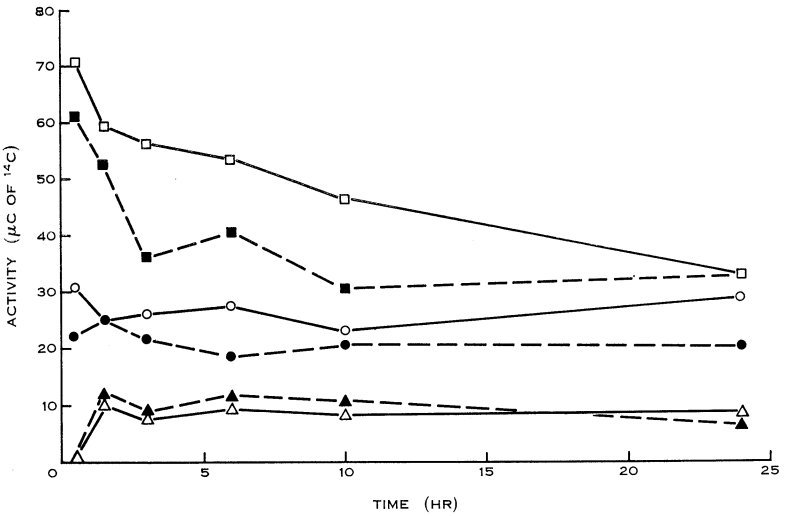

Fig. 1.—Comparison of the change in total ^{14}C, with time, in the leaves (□, ■) stems (○, ●), and roots (△, ▲) for the light (————) and dark (– – – – –) treatments.

(*c*) *Counting of* ^{14}C

A 5-ml aliquot of the absorbing solution was added to 5 ml of scintillator solution [i.e. toluene containing 0·4% 2,5-diphenyloxazole and 0·01% 1,4-bis-2-(5-phenyloxazolyl) benzene] in a glass counting vial. A Nuclear-Chicago liquid scintillation system (model 725) was used for counting. The observed counts were corrected for background radiation, quenching, and counting efficiency. Quenching was corrected for by the channels ratio method outlined in the Nuclear-Chicago scintillation manual.

Quenching curves were prepared with hexadecane-1-^{14}C. The maximum counting efficiency obtained was 59·9%.

After obtaining the total activity of each sample oxidized, the radioactivity contained within each plant portion was calculated.

III. Results

Figure 1 shows the changes with time in the total radioactivities of the leaves, stems, and roots. There was no significant difference between the total activities in

the roots of plants given light and dark treatments but there was a significant difference ($P<0\cdot01$) in the ethanol-soluble ^{14}C content of the roots (see Fig. 5). The total ^{14}C content was significantly different between treatments in the leaves ($P<0\cdot001$) and the stems ($P<0\cdot01$).

The total ^{14}C in the plants was significantly lower at all times ($P<0\cdot05$) in the dark than in the light series. The converse holds for the ^{14}C exported from the leaves, since the combined root and stem activities were significantly higher ($P<0\cdot05$) in the light than in the dark series (Fig. 2).

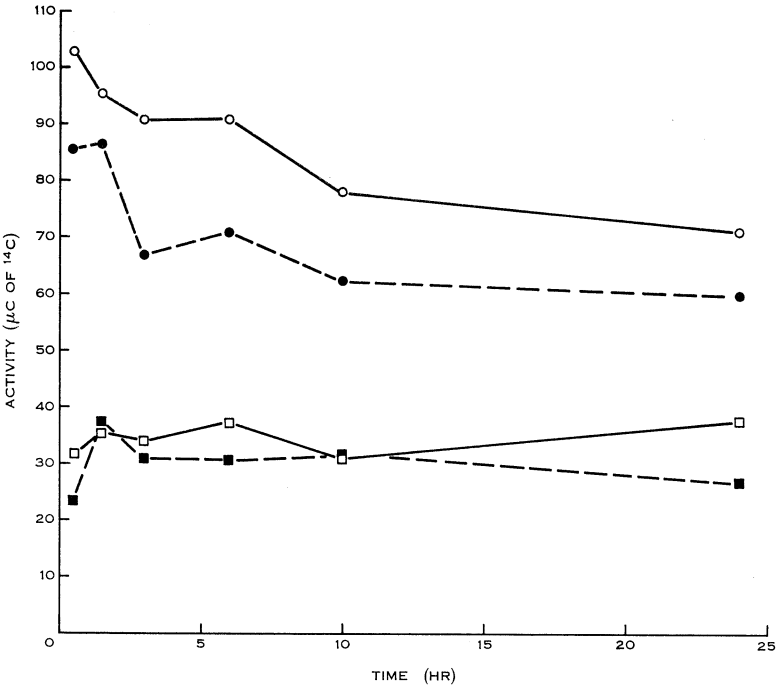

Fig. 2.—Comparison of the change in total ^{14}C, with time, in the whole plant, for the light (○——○) and dark (●- - -●) treatments, and in the combined roots and stems for light (□——□) and dark (■- - -■) treatments.

In the leaves there were significant changes with time in the ^{14}C content of the ethanol-soluble and ethanol-insoluble fractions (Fig. 3). For the soluble fraction there was a more rapid decline during the first 3 hr in the light than in the dark, but by the 24th hr the differences were small. This interaction was significant at $P<0\cdot001$. A significant interaction between illumination treatments and time ($P<0\cdot05$) also existed for the insoluble fraction, the differences being greatest during the first 10 hr. Leaves of plants kept in the light showed a steady increase in the ^{14}C content of the ethanol-insoluble portion up to 3 hr and thereafter declined steadily. For plants kept in total darkness, there was a steady decline for the first 3 hr but thereafter little change resulted.

The ethanol-soluble fraction in the stems did not differ significantly between illumination treatments but showed a significant decline with time as shown in

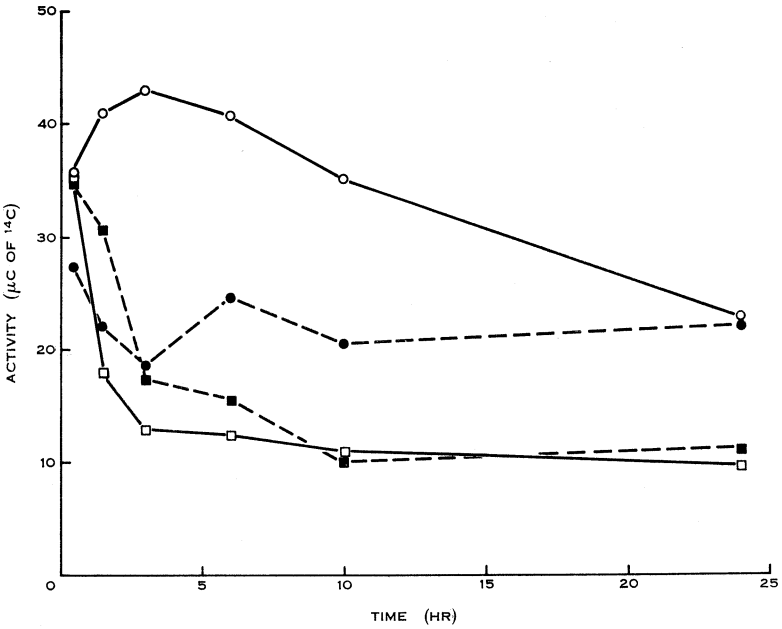

Fig. 3.—Changes in the distribution of ^{14}C, with time, in the leaves for the light (———) and dark (– – – –) treatments. □, ■ ^{14}C content of the ethanol-soluble fraction. ○, ● ^{14}C content of the ethanol-insoluble fraction.

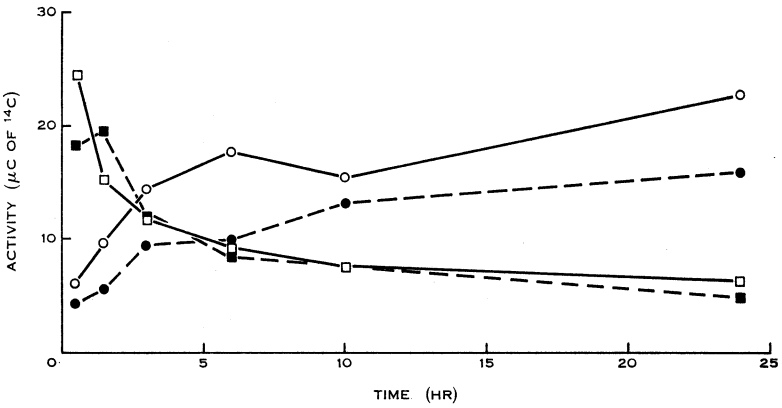

Fig. 4.—Changes in the distribution of ^{14}C, with time, in the stems for the light (———) and dark (– – – –) treatments. □, ■ ^{14}C in the ethanol-soluble fraction. ○, ● ^{14}C content in the ethanol-insoluble fraction.

Figure 4. Analysis of variance showed a significantly higher ($P < 0{\cdot}001$) ^{14}C content of the ethanol-insoluble fraction at all harvest times for plants kept in the light.

Figure 5 shows that the maximum amount of activity in the roots occurred 1–2 hr after exposure to $^{14}CO_2$ and that the majority of the labelled carbon was in the ethanol-soluble portion. Analysis of variance showed that the roots of plants in the light treatment had a lower amount ($P < 0{\cdot}01$) of ethanol-soluble fraction than those of the dark-treated plants. No significant differences were found with time in the ^{14}C content of the ethanol-insoluble fraction between illumination treatments. There was, however, a steady increase in this fraction's radioactivity with time in both treatments.

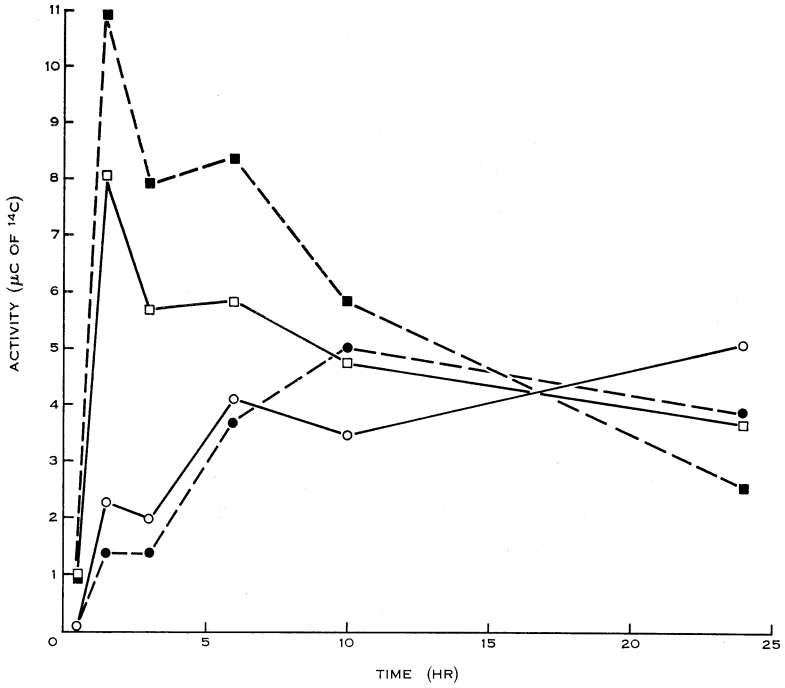

Fig. 5.—Changes in the distribution of ^{14}C, with time, in the roots for the light (———) and dark (– – – –) treatments. □, ■ ^{14}C content in the ethanol-soluble fraction. ○, ● ^{14}C content in the ethanol-insoluble fraction.

IV. Discussion

The total radioactivity of the combined fractions in the roots (Fig. 1) masked substantial differences in the distribution of ethanol-soluble and ethanol-insoluble compounds over a period of 24 hr. Figure 5 shows a steady increase in the ^{14}C incorporated into root structure and storage compounds. It can be seen that the period of maximum accumulation of total ^{14}C was during the first 2 hr. The accumulation in the roots of labelled ethanol-soluble compounds was greater in the plants kept in the dark than the light.

The exact mechanisms by which less ethanol-soluble photosynthate entered the roots of plants placed in the light than the dark-treated plants is not clear. It appeared that the direct or indirect stimulus of light must influence the functioning

of the leaves or stems or both and hence control the rate of movement of photosynthate into the root system. The stimulus of light appeared to have resulted in the formation of a greater quantity of ethanol-insoluble compounds in the leaves, as shown in Figure 3. The radioactivity in this leaf fraction increased during the first 4 or 5 hr in the light and then declined steadily after the plants were placed in the dark. This suggests that ^{14}C-labelled starch was built up in the light and subsequently broken down into maltose and glucose units in the dark. For the plants kept in the dark there was no build-up of ^{14}C content of this leaf fraction over a period of 24 hr. That is, a greater amount of the recent products of photosynthesis were changed into insoluble compounds within the leaves of plants grown in the light as compared with the dark, and these were not immediately available for translocation to the root system.

The experiment showed that the total plant radioactivity at all harvest times was less in plants placed in the dark than those which received the light treatment. Such a loss in the dark-treated plants can only be explained by respiration of a greater proportion of the recently formed labelled photosynthate. This supports the conclusions of Weigl, Warrington, and Calvin (1951), who experimented with detached barley leaves in a closed system. They suggested that "in strong light, respiratory carbon dioxide originates primarily from endogenous sources and only to a very small extent from recently assimilated carbon. In the dark recently photosynthesized compounds are actively oxidized in a fairly constant ratio to endogenous respiration".

It should be noted that the stems and roots of plants left in the light received a greater amount of labelled compounds than plants placed in the dark. That is, the export of labelled photosynthate by the leaves was greatest in the light. However, a larger proportion of these translocated compounds remained in the stems under the light treatment. This agrees with the findings of Hartt, Kortschak, and Burr (1964) and Thrower (1962).

The elucidation of light-stimulated mechanisms which influence the movement of photosynthate within the plant must await further work on translocation and cellular metabolism.

V. Acknowledgments

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VI. References

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