THE VELOCITY AND PATTERN OF ASSIMILATE TRANSLOCATION IN WHEAT PLANTS DURING GRAIN DEVELOPMENT

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Summary

The translocation of labelled assimilates from the flag leaf blade to the ear and other parts of the plant was followed in wheat, 15–20 days after anthesis. The velocity of assimilates moving through the top internode varied from 80 to 100 cm/hr and the velocity of movement down the leaf sheath was about half this value. Removing grains from the head reduced the velocity of the assimilates moving up the stem from the node and increased their downward velocity in the stem. However, the rate of movement out of the leaf was unaffected by the removal of grains. With full grain development assimilates from the flag leaf moved directly from the node of insertion of the leaf up the stem to the ear. The results are discussed in relation to the vascular anatomy of wheat and the mechanism and factors controlling translocation.

I. Introduction

Changes in temperature, light, mineral nutrients, and other features of the environment may effect the utilization of carbon by plants, either through direct effects on growth, or indirectly through the functioning of the photosynthetic tissue. However, it is still not clear whether growth is limited at any time by the capacity of the conducting tissue to transport the necessary volume of assimilates.

The wheat plant during heading provides a useful system for examining growth and assimilation in relation to translocation. Assimilates from the flag leaf move almost exclusively to the ear, through a considerable length of stem, and each part of this system can readily be isolated (Hsia, Wan, and Wang 1963). Also growth may be controlled by the removal of individual grains from the ear with a minimum of disturbance to the remaining grains and the rest of the plant.

In the present experiments a detailed examination has been made of the movement of ^{14}C-labelled photosynthetic assimilates from the flag leaf blade to the ear in wheat, both with maximum grain development and where growth has been reduced by removing two-thirds of the grain from the ear.

II. Methods and Materials

Wheat plants (*Triticum aestivum* cv. Gabo) were grown in Perlite under natural daylight, extended with incandescent lamps to give a 16-hr photoperiod. Temperatures were maintained at 21°C for 8 hr of the light period and at 16°C for the remaining 16 hr (21°C/16°C) of each 24-hr cycle. Plants were selected for uniformity at anthesis and the tillers were removed to eliminate possible water stress. Plants were used in the translocation studies at least 15 days and not more than 20 days after anthesis, when the endosperm was actively accumulating starch.

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There were three labelling experiments which differed in the intervals at which the plants were harvested following the uptake of ^{14}C and also in the sectioning of the plants for analysis. In experiments II and III, grains were removed from two out of every three consecutive spikelets, in the required number of heads, 24 hr prior to the commencement of the $^{14}CO_2$ application.

The method of application of labelled CO_2 was similar in all cases. At 12.00 noon the terminal 19 cm length of the flag leaf blades were enclosed in darkened boxes. Temperatures were held at 21°C. Labelled CO_2 was generated by lactic acid from 100 mg of $Ba^{14}CO_3$ containing 100 μc of ^{14}C, to give an initial concentration of 0·2% by volume. After 5 min, to allow for uniform diffusion of $^{14}CO_2$ throughout the box, covers were removed to expose the enclosed leaves to light of intensity 3500 f.c. The start of the light period is taken as the time of commencement of the $^{14}CO_2$ application in the work that follows. After 10 min in the light, the boxes were flushed with air to remove excess $^{14}CO_2$. In experiment I, the plants were then placed in a 21°C/16°C glasshouse, under natural illumination, while in experiments II and III the plants were retained in an artificially lit cabinet at 21°C and a light intensity of 3500 f.c.

Plants were harvested at varying times from the commencement of the $^{14}CO_2$ application and immediately cut into parts as shown in Figure 1, dried at 70°C, weighed, and ground in a Wiley mill to pass a 40-mesh sieve. Radioactivity measurements were made on 30-mg aliquots of powder for determination of the distribution of ^{14}C in the plant (O'Brien and Wardlaw 1961). The ^{14}C level in any one section is expressed either as "relative specific activity", counts/min/30 mg; or as "percentage distribution", relative total activity of part × 100/relative total activity of whole plant. The "relative total activity" is the product of relative specific activity × weight of the part in grams. This terminology is similar to that used by Hartt *et al.* (1963). Harvest intervals in the three experiments were as follows:

Experiment I: The first plant was harvested 10 min after the commencement of the $^{14}CO_2$ application. Subsequent harvests of individual plants were made 15, 20, 30, 40, 50, 60, and 70 min and then every 20 min until 12 hr from the commencement of application. A further three plants were harvested after 24 hr.

Experiment II: For the harvests taken ½, 1, 2, 6, and 24 hr from the commencement of the $^{14}CO_2$ application, three plants with intact heads and three plants with two-thirds of the grain removed from the head were sampled.

Experiment III: Individual plants were harvested at 10 min intervals from the commencement of the $^{14}CO_2$ application, through a period of 240 min for plants with intact heads and 160 min for plants with two-thirds of the grain removed from the head.

III. Results

(a) *Analysis of the Movement of Labelled Carbon*

The velocity of translocation of labelled assimilates has been determined from a comparison of the pattern of accumulation of labelled carbon in progressive sections

of the leaf sheath and stem with time. This method, although time consuming, does meet the criticisms raised by Canny (1960) regarding the measurements of velocity of translocation with radioactive isotopes.

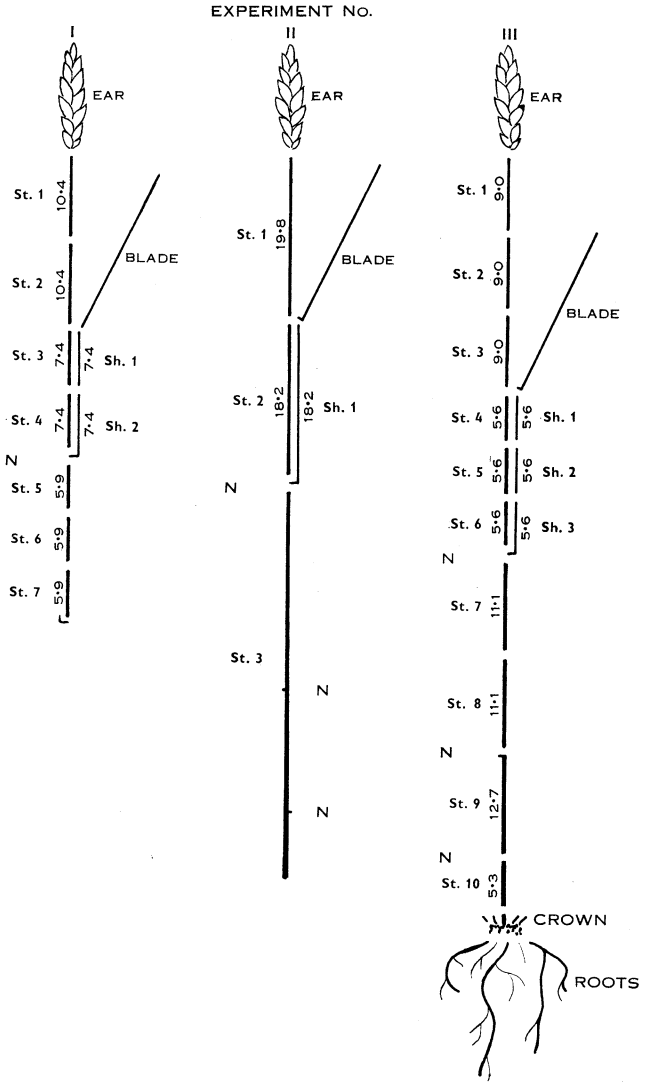

Fig. 1.—Sectioning of plants for ^{14}C analysis. Each section is coded as shown and lengths are indicated in centimetres. St., stem section; Sh., sheath section; N, position of node.

The time relationships of rise in ^{14}C for two sections from experiment I are shown in Figure 2. These profiles compare with the change in time of ^{14}C in aphid exudates or respiration of the willow stem shown by Canny (1961, 1962). The point of half the maximum level of ^{14}C attained by any one section, which is very close to the point of inflexion for each curve, is used as the basis for timing the arrival of the profile in a section.

(*b*) *Pathway of Translocation*

Percival (1921) states that in the wheat plant "the bundles of the leaf-sheath curve inwards into the node and pass through the latter as separate isolated strands without fusing with each other or with the neighbouring bundles which have come

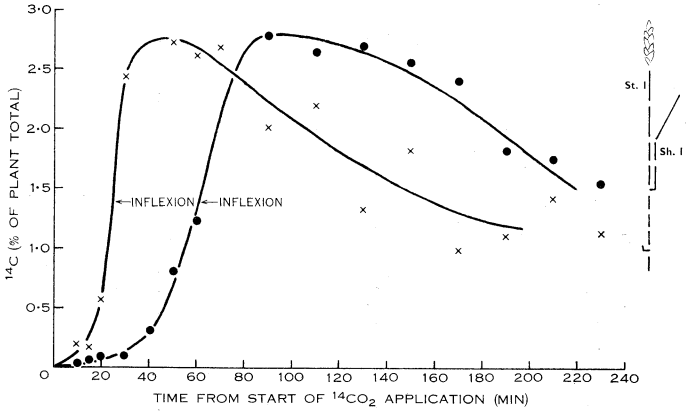

Fig. 2.—Pattern of accumulation of ^{14}C in a leaf sheath and stem section, with time — experiment 1. × Sheath section adjacent to ligule (Sh. 1). ● Stem section adjacent to ear (St. 1). Point of inflexion of profile indicated by arrow.

from the internode above", although the stem traces anastomose with each other at this point. Thus the vascular traces from the leaf do not anastomose with those of the stem until the next node below the point of entry of the leaf. To check the

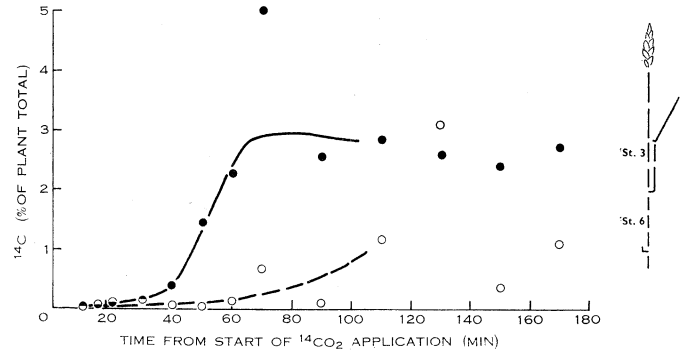

Fig. 3.—Comparison of the change in ^{14}C content, with time, of stem sections above and below the node of insertion of the flag leaf — experiment 1. ● Upper internode section (St. 3). O Lower internode section (St. 6).

validity of the above observations for the material used in the current experiments, blue and red dyes were sucked under vacuum into the xylem of the flag leaf traces and into the traces of the top internode respectively and then down through the

node of insertion of the flag leaf. The distribution of dye within the node confirmed Percival's observations at least for the major bundles of the leaf. Sharman (1942) has described a somewhat similar situation in the maize stem, although here the stem traces anastomose with each other just above the point of insertion of the leaf.

TABLE 1

RESIDUAL ACTIVITY OF SHEATH AND STEM TISSUE 24 HR AFTER $^{14}CO_2$ APPLICATION TO THE FLAG LEAF BLADE: EXPERIMENT I

Replicate No.	Relative Specific Activity (counts/min/30 mg tissue)					
	Sheath 1	Sheath 2	Stem 4	Stem 3	Stem 2	Stem 1
1	126	304	621	498	151	114
2	57	101	677	730	131	72
3	99	171	472	406	75	82
Mean	94	192	590	545	119	89

In Figure 3 (data from experiment I) a comparison is shown of the rise in ^{14}C activity of a stem section above the leaf (St. 3), with a section from the internode immediately below the leaf (St. 6). It appears that the assimilates did not follow

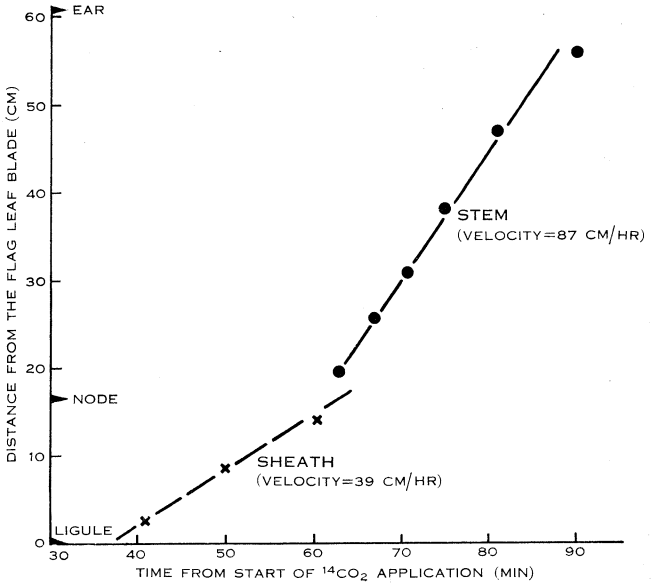

Fig. 4.—Movement of the ^{14}C profile from the flag leaf blade to the ear, with time, in relation to the distance moved — experiment III.

the anatomical pathway, as described by Percival (1921), but moved directly up to the ear from the point of entry of the leaf.

The ^{14}C content of stem and leaf sheath sections rose with time to a peak and then fell back to a steady value. This residual activity is shown, for experiment I,

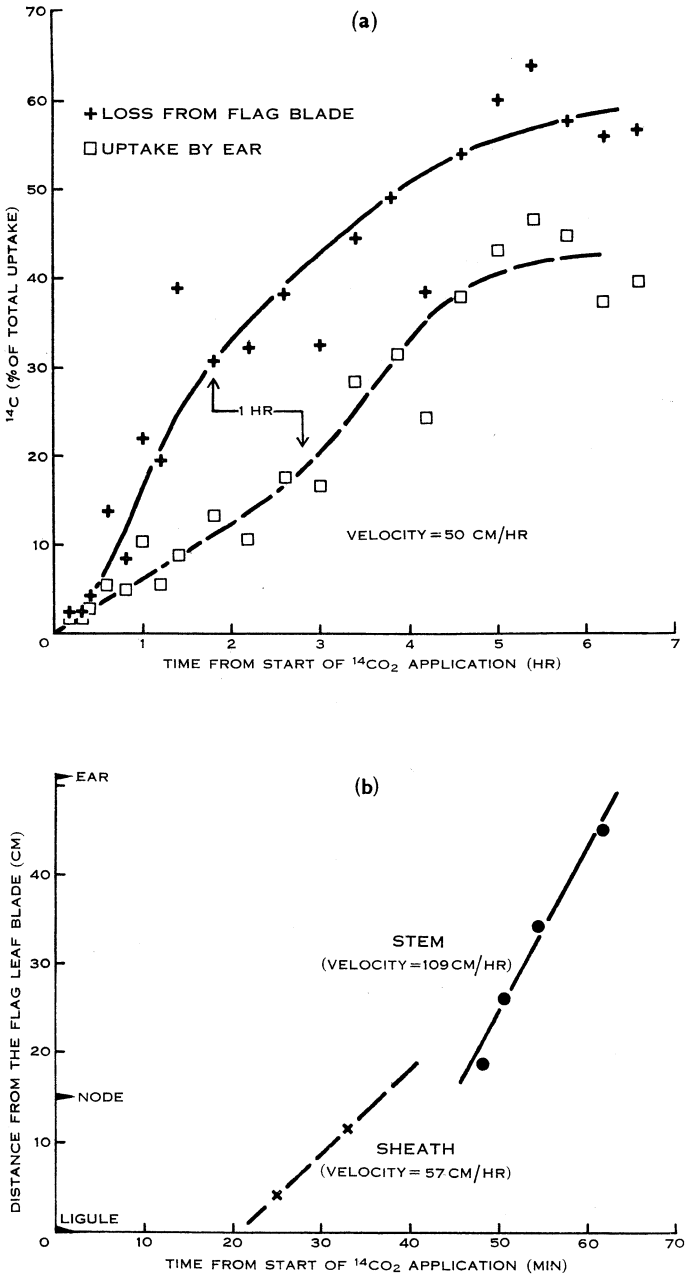

Fig. 5.—(*a*) Relation, in time, of the loss of ^{14}C from the flag leaf blade, to the uptake of ^{14}C by the ear — experiment I. (*b*) Movement of the ^{14}C profile through the sheath and stem with time, in relation to the distance moved — experiment I.

in Table 1, as the relative specific activity of the sections 24 hr after the $^{14}CO_2$ application, and also is shown for experiment II, in Figure 6. The non-green stem tissue enclosed by the leaf sheath (St. 3 and St. 4) had a higher residual activity than

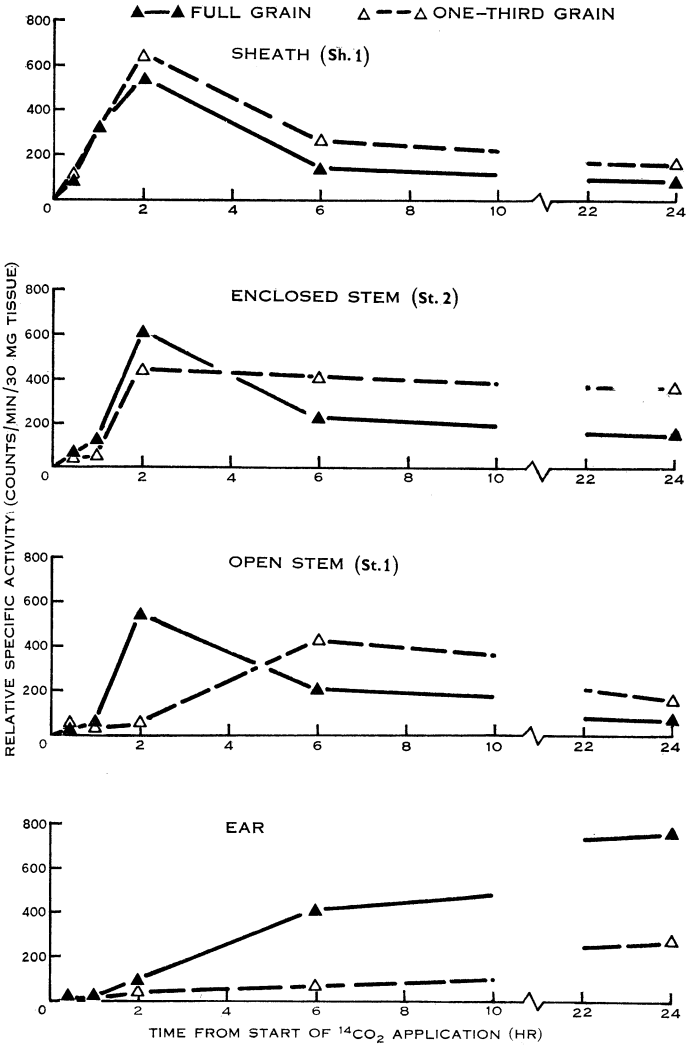

Fig. 6.—Comparison between plants with intact ears and those with two-thirds of the grain removed from the ear of the level of ^{14}C, with time, in the sheath, sections of the upper internode, and in the ear — experiment II.

either the exposed part of the stem or the sheath itself. This difference was true also for ^{14}C activity per unit length of stem. Separate radioautographic studies have indicated that the part of the stem normally enclosed by the sheath assimilates only small amounts of CO_2. Thus the difference in residual activity described could not be due to the reassimilation of ^{14}C accumulating beneath the sheath.

(c) *Velocity of Translocation*

Figure 4 (data for experiment III) shows the time from the commencement of the $^{14}CO_2$ application to the appearance of the ^{14}C profile in each section [see

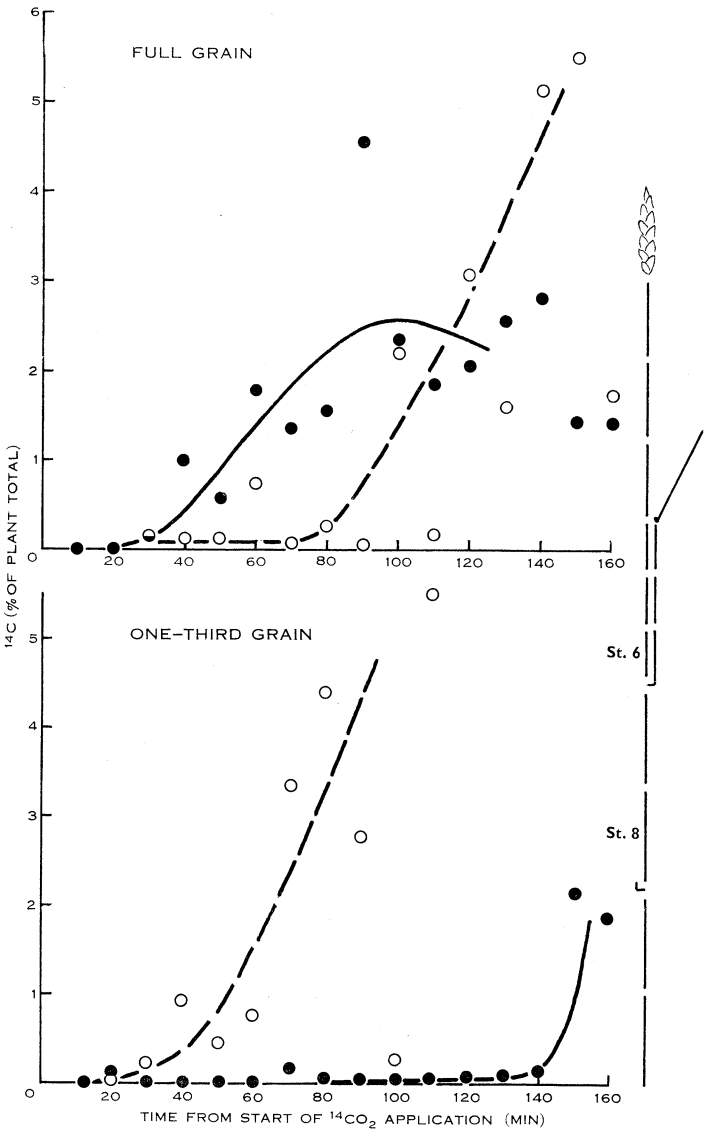

Fig. 7.—Comparison of the change in ^{14}C, with time, in stem sections above and below the node of insertion of the flag leaf, as related to the number of grains in the ear — experiment III. ● Upper stem section (St. 6). ○ Lower stem section (St. 8).

Section III(*a*)], in relation to the distance of the centre of the section from the ligule of the flag leaf. The assimilates moved down the sheath (velocity = 39 cm/hr)

more slowly than up the stem (velocity = 87 cm/hr). A similar difference was observed in experiment I [Fig. 5(*b*)].

The overall velocity of ^{14}C movement between the flag leaf blade and the ear can be determined from a comparison in time of the loss of activity from the blade with the increase in activity of the ear as percentages of total plant activity. This comparison has been made for experiment I in Figure 5(*a*), while the movement of the ^{14}C profile from section to section in the sheath and stem is shown in Figure 5(*b*). The overall velocity of 50 cm/hr is close to that obtained for ^{14}C movement through the sheath.

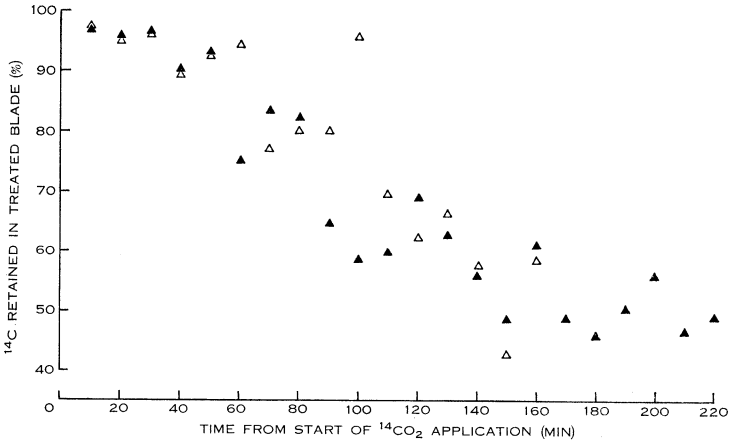

Fig. 8.—Effect of grain removal on the movement of ^{14}C out of the flag leaf blade — experiment III. ▲ Full grain. △ One-third grain.

(*d*) *Translocation in Relation to Grain Number or "Sink Size"*

The effect of removing two-thirds of the grain from the ear on the movement of ^{14}C assimilated by the flag leaf blade is shown for experiment II in Figure 6. There was no obvious difference in movement through the sheath and this was confirmed in experiment III. Movement of ^{14}C up the stem to the ear was slowed to at least one-third of the velocity of the controls with intact heads, although the limited harvest intervals made an exact estimate of velocity impossible. This difference in stem velocity was associated with a smaller total accumulation of labelled carbon by the ear. A comparison of specific activities after 24 hr (Fig. 6) shows that the slower movement was also associated with an increased retention of ^{14}C by the translocating tissue.

In experiment III removal of two-thirds of the grain from the head resulted in the movement of ^{14}C downwards through the internode below the leaf before it entered the stem above the leaf (Fig. 7). The time delay in appearance of ^{14}C in the stem above the leaf was sufficient for the assimilates to have moved to the next lowest node, where the stem and leaf traces anastomose, before ascending the stem again towards the ear.

Although the upward movement of labelled assimilates was reduced by the removal of grains from the head, the movement of ^{14}C out of the leaf blade was not affected (Fig. 8). There was, however, a more rapid movement of ^{14}C assimilates down the stem and a faster accumulation of label in the roots and crown (Fig. 9).

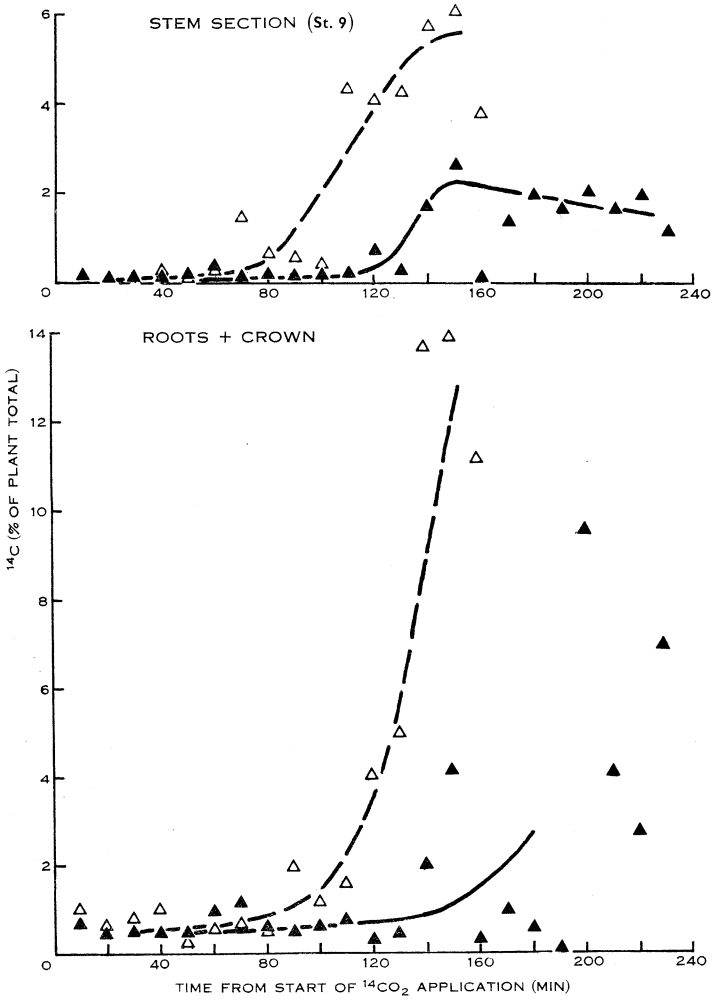

Fig. 9.—Effect of grain removal on the change in ^{14}C, with time, in a stem section below the insertion of the flag leaf and on the accumulation of ^{14}C in the roots and crown — experiment III. ▲ Full grain. △ One-third grain.

IV. Discussion

(*a*) *Path of Translocating Assimilates*

In many cases assimilates have been shown to move from a leaf to the area of their utilization in the plant by a narrowly defined pathway. Thus Zimmerman (1961) claimed that tangential movement in tree trunks was less than 1° and Thrower

(1962) found little lateral displacement of labelled carbon moving down the stem of soybean. Typically, carbohydrate moves from a leaf to its phyllotaxically related part in the top of the shoot (Zhdanova, Lebedeva, and Chvizh 1961; Tsing 1963). However, Hale and Weaver (1962) showed that the fruit spur on a grape shoot initiated the lateral movement of translocated assimilates through non-vascular tissue and Peel (1964) has demonstrated the tangential movement of labelled assimilates through the bark parenchyma of willow stems to an aphid stylet. In the present experiments, a part of the normal movement of assimilates from the flag leaf to the ear appeared to be a lateral transfer between the leaf and stem traces at the node. If, however, the carbohydrate requirement of the ear was reduced by removing grains, the assimilates from the flag leaf could have followed a pathway down to the next lowest node, where the leaf and stem traces are claimed to anastomose (Percival 1921) before ascending to the ear. Since the velocity of assimilate movement through the sheath was about half of that in the top internode, the vascular anatomy of the node may restrict assimilate movement between the leaf and the ear. In rice, where a higher proportion of the grain carbohydrate is derived from the leaves than in wheat (Takeda and Maruta 1955; Lizandr and Brovtsyna 1964), there is a direct link between the leaf and stem traces at the node of entry of the leaf (Inosaka 1958), and the resistance to the movement of carbohydrate from the leaf to the inflorescence may be expected to be less than in wheat.

These results indicate that a closer examination of the vascular anatomy of the cereal stem is required, and particularly of the cells separating the leaf and stem traces at the node.

(*b*) *Movement of* ^{14}C *Profiles and the Retention of Assimilates by the Conducting Tissues*

The maximum rate of movement of ^{14}C out of the flag leaf was reached 20–40 min from the commencement of a 10-min application of $^{14}CO_2$ and considerable movement continued for a period of 3–4 hr. Sheath and stem sections showed a rapid rise in ^{14}C level to a peak, followed by a more gradual decline to a steady minimum or residual activity. The time for the complete wave in ^{14}C activity to pass through any one section and the time for the loss of activity from the leaf were approximately the same. This confirms the important role of the leaf in determining the ^{14}C profile.

Canny (1961) calculated velocities of assimilate translocation in willow cuttings, on the assumption that there was no alteration with distance in the proportionality between translocation, retention by the tissue, and respiration. There is no evidence to suggest that this was not correct in the system he describes. But in the present work, a difference in the retention of translocating assimilates did occur between that part of the top internode enclosed by the flag leaf sheath and the upper exposed green part of the internode. There was a much greater retention of ^{14}C by the enclosed stem where there was no direct supply of photosynthetic assimilates available to the tissue. Canny (1962) noticed an effect of darkened respiratory chambers on the ^{14}C translocation profile in willow stems, which appears to be a similar effect to that of the sheath.

The slower the velocity of movement of assimilates through the tissue the greater was the retention of ^{14}C by the tissue and the less apparent was the peak of activity in any one section. This probably accounts for the blunt ^{14}C profile Canny (1962) has obtained for willow cuttings, where the velocity of movement was much slower than in wheat.

Nelson, Perkins, and Gorham (1959) have reported an extremely rapid translocation of labelled assimilate through the stem of soybean. In experiment I the pattern of accumulation of ^{14}C by the ear did indicate that a small amount of assimilate moved through the stem at velocities far in excess of the main profile. However, there was little evidence for this in either experiments II or III.

(c) *Effect of Growth Requirements on Translocation*

Removing two-thirds of the grain from the ear reduced the velocity of assimilate movement up the stem to the ear from the flag leaf node, and increased the velocity of movement down the stem to the roots. Contrary to results obtained by Shen and Shen (1962), there was no significant effect on the movement of assimilates out of the leaf blade or down through the sheath. Thus the present experiments appear to support the view that translocation is controlled both by the leaf (Whitehead 1962; Hartt *et al.* 1963) and by the demand for assimilates (Aronoff 1955; Hsia, Wan, and Wang 1963). Nelson (1963) has suggested, from a comparison of ^{14}C profiles formed by labelled assimilates, that translocation in the leaf or petiole differs from that in the stem. However, the failure to observe any effect of grain reduction on the movement of assimilates out of the leaf in the present experiments may be related to the restriction in movement between the leaf and stem traces at the node, and the ability of the roots to utilize additional carbohydrate when this becomes available.

The effect of sink size on the velocity of ^{14}C movement in stems is not consistent with the model for translocation put forward by Canny (1962), which was based on the movement of transcellular strands in phloem, observed by Thaine (1961, 1962). In this model any alteration in the utilization of sucrose by the sink might be expected to alter the loading of labelled carbon on the transport system and not the velocity of movement through the system. In addition the known rates of protoplasmic streaming (Swanson 1959), assuming that these could be related to the movement of strands in the phloem, could not account for the velocities of 90 cm/hr reported here. Also since it is unlikely that phloem metabolism is directly controlled by the rate of utilization of assimilates during growth, the above results relating velocity of movement to sink size are also inconsistent with the suggestion made by Kursanov (1961), that translocation is controlled directly by the metabolism of the conducting tissue.

V. Acknowledgments

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