An Approach to the Study of the Control of Translocation in Higher Plants

by

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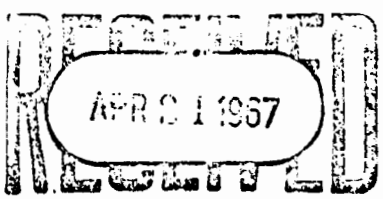

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Abstract

The objective of this investigation was to gain some insight into what limits or controls translocation in higher plants.

Photosynthesis and translocation were studied using $^{14}CO_2$. Over 75% of the assimilated ^{14}C was incorporated into sucrose via aspartic acid and malic acid. About 20% of the ^{14}C went into ethanol-insoluble compounds. Lowering the temperature to 8° delayed the incorporation of the ^{14}C into sucrose, but had little effect on the rate or amount of incorporation.

In young corn plants all the leaves exported ^{14}C, most of which went to the roots and the growing regions of the plants. All the leaves imported some ^{14}C assimilated in other leaves.

The only translocated organic compound in corn was sucrose. It was translocated down the leaf blade at a velocity in excess of 150 cm hour^{-1}. The translocation profile in the leaf blade was logarithmic and was found to be due to a reversible accumulation of the translocate in the vascular tissue.

Corn leaves were used to establish a method for measuring translocation from the leaf. Measuring the amount of ^{14}C remaining in the fed area of leaf at various times after feeding $^{14}CO_2$ provided information on 3 characteristics of translocation; the relative rate of translocation from the leaf, the total percentage of the assimilated ^{14}C that is translocated, and the turnover time of the translocation pool. From these measurements the translocation in corn at 2600 ft-c was calculated to be 327 ugm of sucrose dm^{-2} min^{-1}. The turnover time of the translocation pool in corn was 80 minutes.

This method was used to study the effect of different factors on translocation from the leaf. A decrease in temperature

decreased the rate of translocation more or less linearly from 26 to 7°. The turnover time of the translocation pool and the percentage of the assimilates translocated increased with a decrease in temperature. Light had no significant effect on translocation. The rate of translocation and the percentage of the assimilates translocated changed with the age of the leaf.

The method of measuring the ^{14}C remaining in the fed area of leaves was used in a comparative study of translocation in a number of species. The relative rate of translocation and the percentage of the assimilates translocated varied with the species. Corn, sorghum, millet and sunflower translocated between 70 and 90% of the assimilated ^{14}C in 24 hours, whereas the other species translocated only between 40 and 60%. The compounds in which the ^{14}C was held back in the leaf varied with the species. The turnover time of the translocation pool divided the species into 2 groups; tomato and radish had a turnover time of 40 minutes, all the other species had a turnover time of about 80 minutes.

The result of the investigations suggest that the limiting process in translocation is the transfer of the translocate from the assimilating cells to the phloem. It is speculated that the main part of the control of translocation is at the membranes of the exporting cells.

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I wish to express my sincere appreciation to Dr. C. D. Nelson for suggesting the problem and for his guidance and encouragement during the research and preparation of the thesis.

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Introduction

With the rapidly increasing world population, production of food is becoming more critical. One approach to the problem has been to increase primary productivity through plant breeding. In this way the percentage of the incident light intercepted by the crop and utilized in the fixation of CO_2 has been greatly increased. There are still great differences in the over-all efficiency of photosynthesis among the crop plants. Corn is able to utilize at least twice as much of the incident light for CO_2 fixation as most other crops (Gaastra 1963). Whereas most crops are light saturated at less than 1/3 maximum mid-day light intensities corn is not saturated under maximum mid-day light conditions. Corn is able to utilize all the CO_2 in the air when placed in a closed volume whereas most plants cannot utilize the last 30 to 100 ppm.

For most crops only specific plant parts are harvested for consumption. It is as important to improve the production of storage organs that are harvested as it is to improve the production of total dry matter. This involves increasing the percentage of the assimilates translocated from the leaves, altering the destination of the translocate and perhaps altering the nature of the storage products to increase their caloric value.

Before food production can be greatly increased, the factors that are limiting or controlling CO_2 fixation and translocation of assimilates to the storage organs must be known and understood. The present research problem was set up to gain some

insight into what controls the assimilation and translocation of carbon, first in corn and then in a number of different plant species.

The problem of the control of translocation was approached in the following way. First, the literature was surveyed and a summary presented of the overall process of translocation and its control. Secondly, the techniques of ^{14}C labelling of organic compounds were used to investigate the production of the translocate in the corn leaf, the destination of the translocated ^{14}C in the young corn plant and the translocation of sucrose in corn. Thirdly, a technique was established for measuring the pattern of translocation from the leaf using corn. This technique was used to study the effects of different environmental factors on translocation and to compare translocation from the leaves of different species. Fourthly, the data obtained in the experiments described in this thesis is discussed from the point of view of what controls the several separate processes that make up translocation of sucrose in plants.

Translocation of organic substances in plants has been reviewed by many authors (Swanson 1959, Kursanov 1961, Crafts 1961, Zimmermann 1961, Nelson 1963 and Devlin 1966). However, translocation has not been reviewed from the point of view of the control of translocation. In the following review some of the more pertinent aspects of translocation that need to be known for a consideration of control will be mentioned briefly.

To study control we must know the tissues and the cells involved in translocation. The ringing experiments of Cotta and Knight about the turn of the nineteenth century and the discovery of the sieve tubes by Hartig in 1837 pointed to the phloem as being the conducting tissue for organic compounds (see Swanson 1959). Because of their specialized structure the phloem sieve cells have been considered to be the cells involved in transporting organic substances. More direct evidence for the localization of the translocate in phloem has come from tissue autoradiography using the radioisotopes ^{35}S, ^{32}P, ^{14}C and ^{3}H (Biddulph and Cory 1960, 1964; Gage and Aranoff 1960; Mortimer 1965; Nelson et al. 1959; Webb and Gorham 1964b, 1965). These workers found that near the translocation front labelled translocate was present in the sieve cells, companion cells and the parenchyma cells associated with the phloem. So far the techniques have not been refined enough to be able to pinpoint the cells involved in conduction. Although there is still considerable controversy as to the cells or parts of cells through which translocation occurs there is no doubt that the process is associated with the complex tissue, phloem. Any assessment of what is controlling translocation must take this into account.

The chemical properties of the organic compounds translocated could be very important in the control of their translocation. Identification of the compounds translocated in the phloem is well advanced. Zimmermann (1961) found that the higher plants could be classified into 3 groups on the basis of the sugars that were found in phloem exudates and which were supposedly the

translocation sugars. The first group contained only sucrose. The second group, the majority of plants, contained mainly sucrose with traces of oligosaccharides. In the third group, the oligosaccharides were more abundant than sucrose. The oligosaccharides are raffinose, stachyose and verbascose, all containing sucrose with 1,2 and 3 galactose units respectively. Trip et al (1965) found that non-reducing sugars were the only ones that could be translocated. Also several non-reducing sugar alcohols, such as mannitol and sorbitol, have been found to be translocated by some plants. Plants, therefore, have a mechanism whereby they can select and control the compounds translocated. This mechanism is selective for the non-reducing sugars, mainly sucrose and the sucrose containing oligosaccharides. Whether this selectivity exists at the site of entry into the conducting vessels or in the conducting vessels themselves is not known.

Many attempts have been made to measure the rates of translocation, the sugar concentrations in the sieve tubes and the velocity of translocation in the hope of elucidating something of the mechanism of translocation.[1] Various methods have been used to measure the rate at which assimilates are moved into or out of a plant organ. Canny (1960) has summarized some of these results and expressed them all as grams dry weight cm^{-2} of phloem $hour^{-1}$ (Table I).

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[1]A distinction is made here between rate and velocity of translocation in that the term 'rate' is used to denote weight transfer per unit time and 'velocity' as the distance travelled per unit time.

Table I. Rate of translocation as measured by mass transfer of dry weight (Canny 1960).

Plant system	Specific mass transfer (gm dry wt. cm^{-2} of phloem hr^{-1})	Authors
A. Stems		
Solanum tuber stem	4.5	Dixon & Ball (1922)
Dioscorea tuber stem	4.4	Mason & Lewin (1926)
Solanum tuber stem	2.1	Crafts (1933)
Kigelia fruit peduncle	2.6	Clements (1940)
Cucurbita fruit peduncle	3.3	Crafts & Lorenz (1944)
Cucurbita fruit peduncle	4.8	Colwell
Gossypium bark flaps (probably injured)	0.4 - 0.64	Mason & Maskell (1928)
B. Petioles		
Phaseolus petiole	0.56	Birch-Hirscheld (1920)
Phaseolus petiole	0.7	Crafts (1931)
Tropaeolum petiole	0.7	Crafts (1931)

There is a striking similarity in the values obtained in different plant-stem systems and similar agreement in the petiole systems where the values are lower. A possible explanation for the lower values obtained on Gossypium could be that the cut bark did not give true values.

The concentration of sugar in the sieve tubes has also been measured. The values obtained for different plants are fairly consistent and fall mostly between 10 and 20%. Movement of sugars as a solution could account for the rates of movement noted in Table I.

The velocity of translocation has been measured in many species. Although the measurements obtained are somewhat variable, mainly due to the shortcomings of the techniques used, they are all in the same order of magnitude from 50 to 300 cm $hour^{-1}$. Values as low as 2 cm $hour^{-1}$ have been found by Canny (1961) but the experimental technique of using cut willow twigs is somewhat in question. Devlin (1966) has summarized some of the velocities that have been found by different workers (Table II).

Thus as far as the actual movement in the phloem is concerned there appears to be little difference among the various species that have been examined. Any of the small differences that exist are differences of degree rather than qualitative and may be due to experimental error.

Many people have used the above mentioned results to postulate the mechanism of translocation. Several hypotheses have been proposed over the last 50 years to account for all the results obtained in translocation studies . Most of these hypotheses have

Table II. Translocation velocities in different plant species obtained through the use of radioactive tracers (Devlin 1966).

Plants	Velocity, cm hr^{-1}	Authors
Phaseolus vulgaris	107	Biddulph & Cory (1957)
Beta vulgaris	85 - 100	Kursanov et al. (1953)
Vitis labrusca (Concord)	60	Swanson & El-Shishiny (1958)
Salix sp.	100	Wheatherly et al. (1959)
Saccharum officinarum	270	Hatch & Glaziou (1964)
Saccharum officinarum	84	Hartt et al (1963)
Cucurbita melopepo	290	Webb & Gorham (1964)
Glycine max	86	Vernon & Aranoff (1952)
Cucurbita pepo	40 - 60	Pristupa & Kursanov (1957)

been discarded as inadequate as more information became available. There are 3 main hypotheses held by various workers today.

The mass or pressure flow hypothesis was proposed by Münch (1930). He postulated a unidirectional flow of water and solutes through the sieve elements of the phloem under the driving force of a turgor pressure gradient. This hypothesis rests on the assumptions that the movement of metabolites is passive and along a concentration gradient giving a unidirectional flow.

Van den Honert (1932) in his interfacial-flow hypothesis suggests that the rapid rate of transport reported for phloem translocation might be accounted for in terms of a flow of sucrose along the phase boundary between cytoplasm and vacuole in the sieve tubes.

The activated-diffusion hypothesis of Curtis (1935) has its modern counterpart in the metabolically-activated translocation proposed by Kursanov (1961). These hypotheses are not committed with respect to the mechanism of translocation. Their principal value has been to emphasize the fact that translocation is dependent on metabolism.

None of the above hypotheses at the moment seems to explain all the evidence that has accumulated, yet because of the identical nature of all species as to the aspects of translocation that have been mentioned, probably the mechanism of translocation is the same in all species. In that the mechanism of translocation is still unknown is no major deterrent in the study of the control of translocation. If the mechanism of translocation is the same

in all vascular plants nothing can be done to improve it through breeding and selection. But, since the transport in the phloem is under the control of metabolism in the phloem, learning something of what controls translocation may give a clue as to the actual mechanism of translocation.

Plants evolved many specialized structures as they moved out onto the land. The organs above ground became specialized for CO_2 fixation, whereas the organs below ground became adapted to more effecient uptake of water and minerals. This specialization made the different plant organs dependent on each other and the vascular tissue became the connecting pathway for the movement of material from one plant organ to another. The water and minerals were transported upwards in the xylem and the carbohydrates moved in the phloem from the leaves (the source) to the roots, growing points and storage organs (the sink).

Physiologically, the plant organs form an integrated whole, one part being dependent on and affecting the other parts of the plant. This source-sink relationship is present in all vascular plants and yet this relationship is still poorly understood. The degree or extent to which the source and sink affect each other appears to differ from species to species. This interrelationship also appears to be affected by the physiological condition of either the source or the sink and this condition in turn is affected by the environment. Thus this relationship is very complex. At the same time because it does change and is affected by the environment it should be subject to experimentation and to improvement through either plant breeding or manipulation of the environment. As it appears to involve the major part of the

control of translocation, the source-sink interrelationship could provide one of the keys for the improvement of the production of the economically important organs in the plant.

Over the last decade some knowledge has begun to accumulate on source-sink relationships, but little direct work has been done on the control of translocation although many results imply control. Some workers are beginning to recognize the importance of the source-sink relationship in translocation. Kursanov (1961) stated that the transport of organic materials over long distances is dependent not only on the metabolism of the conducting strands but also on the activities of the organs at either end. He quoted the work by I. F. Belikov with soybean plants. Belikov found that the leaves of mature soybean plants supply specific organs, or zones with their assimilates. The products of low level leaves are translocated mainly to the roots but as the fruit develops an ever increasing number of leaves translocate their assimilates to it, and at length even those at the lower level follow suit and cease almost entirely to feed the roots.

There are many examples of changes in the translocation pattern during the development of an organ or entire plant. Young developing leaves act as sinks until they become self-sufficient which in the case of the soybean plant is at the time the leaves are about 50% expanded (Thrower 1962). In tobacco plants the young leaves import assimilates up to the end of the growing period (Jones et al. 1959). Both the soybean and tobacco

plants pass through a stage where they import and export assimilates at the same time. In both cases it was found that once the leaves were mature they lost their ability to import assimilates from other leaves. There must be a drastic change in whatever is controlling the direction of translocation.

There are several pieces of evidence showing that the sink affects the distribution of assimilate from the leaves. Lupton (1966) studied the pattern of translocation of wheat plants and the changes that occurred in this pattern during the development of three varieties of wheat. Leaves or shoots of wheat plants were supplied with $^{14}CO_2$ at various stages of plant development, from the time of maximum tillering until 5 weeks after anthesis. The distribution of ^{14}C in the plant was determined 2 to 7 days after dosing with $^{14}CO_2$.

Lupton found that before the stems elongated, ^{14}C moved from the treated leaves to all parts of the plant, although the treated shoots contained more ^{14}C than the other shoots. Later, little ^{14}C moved out of the treated shoot, even when this was dying and translocation was mostly upwards. Translocation from the flag leaf was entirely towards the ear, and there was no movement of ^{14}C from the ear. There was little translocation from the leaves below the second leaf into the ear. Translocation from the second leaf was mainly downward in the early stages of ear development. About 29 days after anthesis the pattern of translocation from the second leaf had changed and was entirely directed toward the grain. Not only was the pattern of translocation from the second leaf changed, but there was also a marked increases in the

efficiency of translocation from the flag leaf and from the ear during the first weeks after anthesis, followed by a decline as the ear approached maturity. The percentage of assimilates translocated from the flag leaf increased from 63% at 7 days after anthesis to 81% at 21 days after anthesis.

From the above results it is apparent that the activity of the sink influences the pattern of distribution of assimilates and in the case of the flag leaf and the ear it also increases the efficiency of translocation.

How the sink controls the pattern of distribution of assimilates from the source is still virtually unknown. There are two pieces of recent evidence that give a clue to the specific mechanisms of control. Hew (1965) and de Stigter (1961) suggest that translocation from the source to the sink could at least in part be influenced or controlled by hormones from the sink or a 'factor' produced in the leaves. De Stigter (1961) used various graft combinations of Cucumis melo (muskmelon) on Cucurbita ficifolia root stock as his test system. When a melon scion was grafted on a Cucurbita stock, the plant could not translocate ^{14}C fixed in the melon leaf across the graft union down into the root, even though histological evidence showed that there was a healthy union between the 2 species. However, if the plant was doubly grafted cucurbita/melon/cucurbita the plant was able to translocate into the Cucurbita root from either scion. This worker postulated that some 'translocation factor' was produced in Cucurbita leaves which is necessary for the functioning of the Cucurbita phloem.

Undoubtedly, different species have different auxin concentrations and gradients in their stems. It would appear feasible that a proper hormone gradient must be maintained in the phloem for it to function normally. An abrupt change in concentration, as could occur at a graft union of 2 different species, could disrupt this auxin gradient and thus block the normal functioning of the phloem. The double graft with a small section of melon stem between 2 curcurbita stems could alter the auxin level to somewhere between the 2 species, allowing both to function.

There is no direct evidence from de Stigter's work that a hormone is involved in translocation. However, these experiments do indicate that there is something in the stem or leaves that controls translocation and that this is species specific.

The best evidence to date for a hormonal control over translocation comes from the work of Hew (1965) on young soybean plants. In these experiments the apical meristems were removed and replaced either with water or dilute solutions of GA or IAA, then $^{14}CO_2$ was offered to one of the primary leaves. The effect of applied hormones on translocation was assessed by measuring the total amount of ^{14}C translocated from the leaf, determining the distribution of the translocated ^{14}C in the plant and by calculating the rate of translocation of ^{14}C.

It was found that both IAA and GA affected the 3 aspects of translocation mentioned above. IAA almost doubled the amount translocated from the leaf and GA tripled the amount.

Both IAA and GA increased translocation toward the root. Translocation upward was not affected, probably due to the removal of the sink, although the author does not speculate on this point. Both IAA and GA increased the rate of translocation downward in the stem.

The author postulates that the effects of IAA and GA on translocation may be through the effects of the hormones on the metabolism of the phloem tissue. It has been shown by many workers that translocation is dependent upon metabolic energy in the phloem. Therefore, changes in metabolism should reflect in changes in translocation.

Lupton's work indicated that the activity of the sink can affect both the distribution of translocates and the total amount translocated from the leaf. He obtained no data on the rate of translocation. However, if there is an increase in the amount translocated with no decrease in the rate of photosynthesis then the rate must be increased. Therefore, the effects of the highly active sink on metabolism is the same as the effects on translocation obtained by applying hormones to a cut stem. These results suggest that it is possible that the effect of an active sink on translocation could be through hormones. It has been shown that hormones can have the same effects on translocation as an active sink.

The literature survey indicates that translocation is a complex and poorly understood group of processes. To get any information on how translocation is controlled in higher plants we must take into account the tissue in which the translocation

material is generated, the anatomy of the conducting tissue, the form in which organic material is translocated, the rates and velocities of translocation, the source-sink relationship which determines the pattern of distribution and the possible sites where the control mechanism may be working. These factors have been kept in mind during the experimental work which is reported in this dissertation.

Material and Methods

Only the materials and the general methods used throughout the experiments are described in this section. Specific experimental details are outlined before each experiment.

Materials. Plant material used for the experiments was grown in the greenhouse in pots in a 1:1 mixture of sand and vermiculite which was fertilized with a solution of HiSol 20:20:20 fertilizer. One day prior to the experiments, the plants were transferred to growth chambers. Environmental conditions for most of the experiments were: light intensity, 3000 ft-c; temperature, 26°; relative humidity, 75%. The plants were placed in the light at least 2 hours before feeding of $^{14}CO_2$.

The following plants were used in this study: corn (Zea mays L. var. Pride V; Dent hybrid, W103x44-10-6-3-1; Flint hybrid, Goudster 6-2 x Gelber 2-1), sorghum (Sorghum vulgare Pers. Mor-Su hybrid), millet (Panicum miliacium L. var. Crown Millet), radish (Raphanus sativus L. var. Early Scarlet Globe), soybean (Glycine max L. var. Comet), tomato (Lycopersicon esculentum Mill. var. Ace), sunflower (Helianthus annuus L. var. Russian Giant), castor bean (Ricinus communis L. var. Sanguineus), and nicotiana (Nicotiana affinis Moore var. White Bedder).

Measurement of CO_2 exchange. These measurements were made on 3 corn varieties using a Beckman Infrared Analyzer, Model 215. The CO_2 compensation points were determined using a closed-circuit apparatus as described by Lister et al. (1961). Rates of apparent photosynthesis were measured by passing air sequentially

through the reference cell of the analyzer, over the leaf, through the sample cell of the analyzer and then to the outside of the apparatus.

Feeding apparatus. In the closed circuit apparatus (Fig. 1 A,B,C) air was circulated by means of a variable speed peristaltic pump. All the CO_2 was first removed from the system by passing the air through an Ascarite column. The total CO_2 concentration in the system was brought up to 0.035% by generating CO_2 in a flask (G) by the action of acid on sodium carbonate. The specific activity of the carbon was 51.1%.

The feeding chamber was constructed from plexiglass and designed so that a strip of leaf 3 mm wide was exposed to $^{14}CO_2$. A relatively large air space above and below the leaf allowed for mixing of the air. A 2-liter reservoir allowed about 15 feedings to be carried out before recharging with $^{14}CO_2$.

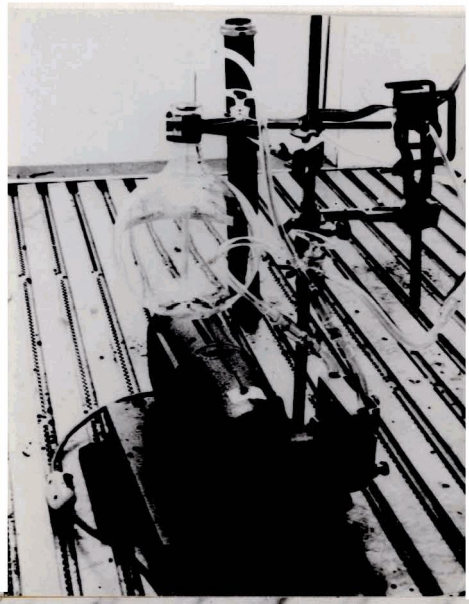
Measurement of translocation. To obtain a measure of the rate of translocation and of the total amount of fixed carbon translocated from the leaf, a narrow section of leaf was exposed to $^{14}CO_2$ for 1 to 4 minutes and the amount of radioactivity remaining in the fed area measured at given time intervals after feeding. Readings were obtained by placing a G-M tube (Nuclear Chicago model D-34), covered with a shield containing a 3 mm slit, directly over the fed area. The G-M tube was placed over the fed area only at the time the readings were taken to allow for near normal conditions throughout the experiment. Readings were expressed as a percentage of the fed activity (i.e. the initial reading) and the values were plotted against time to give a measure of the rate of loss and the

Fig. 1. Apparatus used to expose a restricted area of a leaf to $^{14}CO_2$.

A. Reservoir and circulating pump.

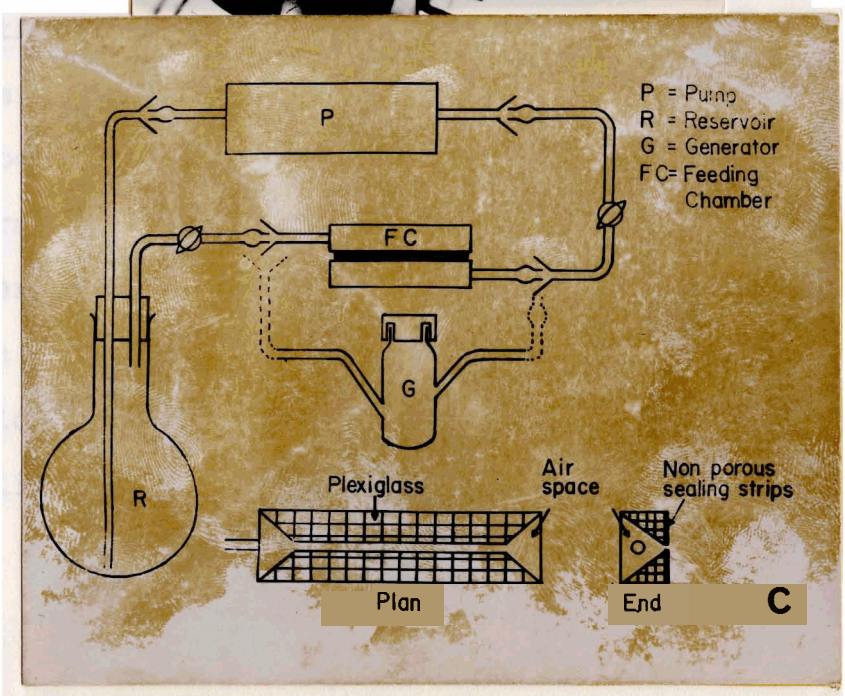
B. Feeding chamber.

C. Diagram of complete apparatus.



A

B


P
FC
G
R
P = Pump
R = Reservoir
G = Generator
FC= Feeding Chamber
Plexiglass
Air space
Non porous sealing strips
Plan
End

C

total amount translocated.

Analysis of the ethanol-soluble and insoluble fractions. The plant material was extracted twice in boiling 80% ethanol. The ethanol-soluble extracts were combined and evaporated to near dryness, and then taken up in a known volume of water. The pigments and lipids were separated out by shaking the extract with chloroform.

The activity in the ethanol-insoluble fraction was determined by digesting the residue to CO_2 according to the wet combustion method of van Slyke (van Slyke et al. 1951). The CO_2 was absorbed in a solution of ethanolamine-ethylene glycol monomethyl ether (Jeffay and Alverez 1961, see Rapkin 1962) and aliquots counted in the liquid-scintillator spectrometer, Packard Tri-Carb Model 3003, using Bray's scintillating mixture (see Rapkin 1963). Values were expressed as a percentage of the total activity in the soluble and insoluble fractions. The activity in the ethanol-soluble fraction was determined by counting an aliquot in Bray's scintillating mixture. The ethanol-soluble fraction was further analyzed by separating the radioactive compounds using 2-dimensional paper chromatography (M. Shiroya et al. 1961). Chromatogram positions of the radioactive compounds were identified by autoradiography. The relative activity in each of the spots was determined by cutting out the radioactive spots, placing them in vials containing 2, 5-diphenyloxazole and 1, 4-bis-2-(4-methyl-5-phenyloxazole)-benzene in toluene, and determining the radioactivity in the liquid scintillation counter.

Photosynthesis in Corn.

Introduction.

There are many aspects of carbon assimilation in corn that have not been investigated. Before studies on translocation could be set up, it was necessary to understand as much as possible about the production and the availability of the translocate in the leaf.

In this part of the research the following aspects were investigated: the CO_2 exchange characteristics of 3 corn varieties; the distribution of fed ^{14}C in different compounds at various times after a short term $^{14}CO_2$ assimilation and the effect of temperature on this distribution.

Experiments and Results.

CO_2 exchange in corn. Some preliminary experiments were conducted with 3 varieties of corn to compare their rates of apparent photosynthesis and CO_2 compensation points with previously reported results. It is known that the rate of apparent photosynthesis doubles as the light intensity doubles over the range 10^3 to 1.4×10^6 ergs cm^{-2} sec^{-1} (Björkman 1967). Also, corn is able to remove all the CO_2 from the air when placed in a closed volume (Tregunna et al. 1964) giving a "compensation point" of zero.

Three varieties of corn were checked. These were Pride V and the Dent and Flint single cross hybrids. Using the infrared analyzer, rates of apparent photosynthesis were measured at 900, 1800, and 2600 ft-c. and at an air temperature of 24°. The CO_2 compensation points were determined at 2600 ft-c and at 10, 15, 20, 25, and 30°.

The results of these experiments for all 3 varieties were similar to those previously reported. Table III shows the rates of apparent photosynthesis for Pride V. The rates doubled with a doubling in light intensity. The compensation points at all the temperatures were below 2 ppm, the lower limit of detection for the apparatus used. It is possible that these varieties were able to reduce the CO_2 content to zero. The 2 single cross hybrids of Flint and Dent had not been previously checked for these CO_2 exchange characteristics. They are similar to other hybrids that have been studied extensively (Forrester et al. 1966).

This preliminary work shows that these varieties can efficiently fix CO_2 in the light and that they do not release any of the fixed CO_2 in the light. This means that any charge of ^{14}C fixed in the light can be completely accounted for after a period of time as long as the plants remain in the light. Therefore, these plants make good material for studying the translocation of assimilated carbon.

The products of photosynthesis in corn. One of the processes that may be affecting or controlling translocation in the plant is the synthesis of new compounds in the leaf. When the leaf is exposed to $^{14}CO_2$, the ^{14}C is incorporated into a number of different compounds. Since sucrose is the only labelled compound translocated in most plants (Zimmermann 1961), the distribution of ^{14}C among the assimilates and the changes in the distribution with time are important factors in determining how much of the assimilated carbon is available for translocation.

Table III. The effect of light intensity on the rate of apparent photosynthesis in corn, variety Pride V.

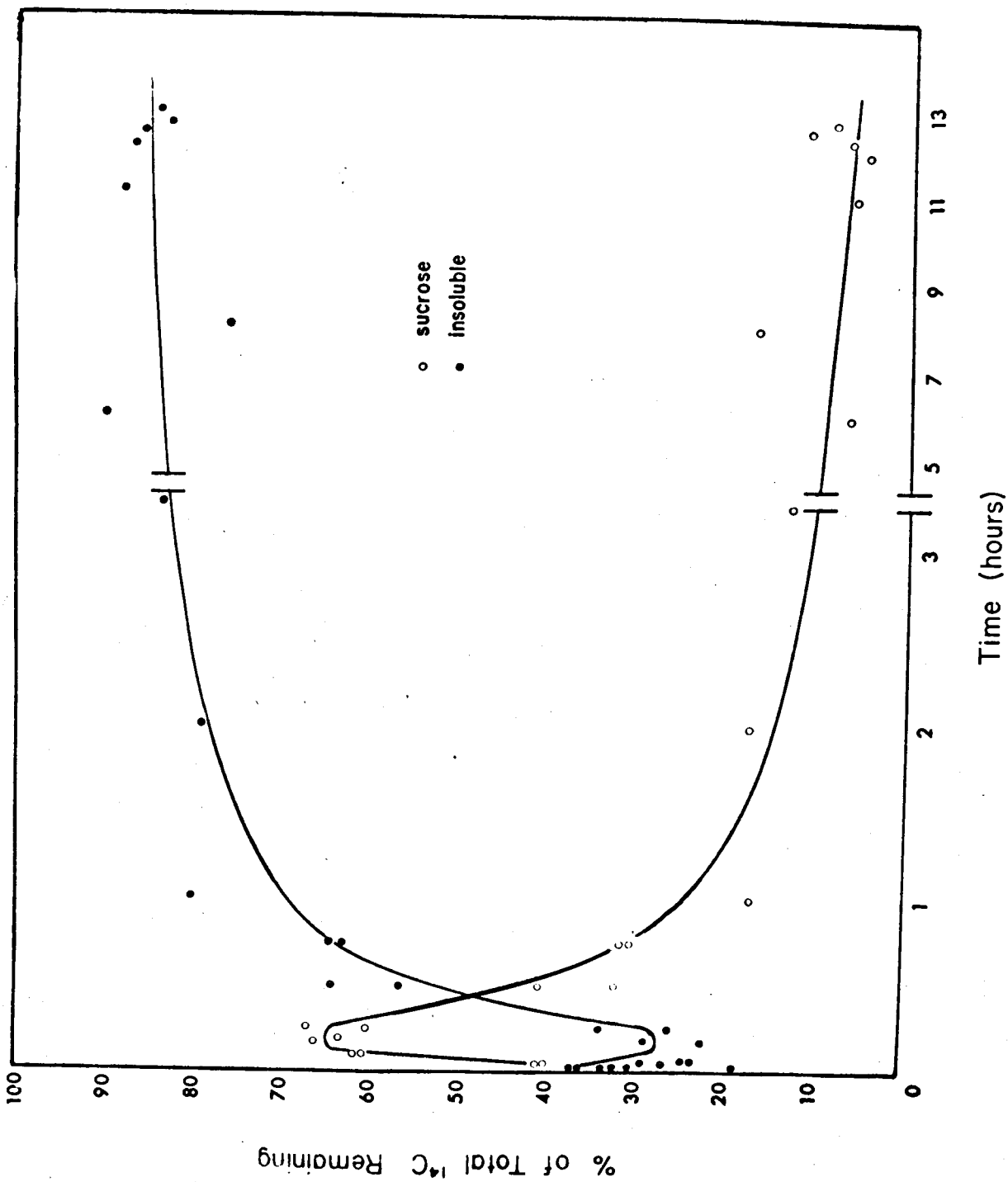
Light Intensity (ft-c).	Rate* (mgm CO_2 dm^{-2} min^{-1})
900	0.19
1800	0.38
2600	0.56

*Mean of 2 experiments

Sections of corn leaf of variety Pride V, 7 cm long, were exposed to light and $^{14}CO_2$ for 15 seconds, 30 seconds or 1 minute followed by light and $^{12}CO_2$ for various time intervals from zero to 4 minutes. The leaves were killed and extracted in boiling ethanol. The distribution of ^{14}C between the ethanol-soluble and insoluble fractions was determined and the radioactive compounds in the ethanol-soluble fraction were identified.

Fig. 2 shows the amount of ^{14}C in the insoluble fraction and in sucrose as a percentage of the ^{14}C recovered from the fed area at various intervals of time after feeding. The amount of ^{14}C in the insoluble fraction was about 40% initially, fell off to about 25% at 10 minutes and then increased rapidly to about 80% in 2 1/2 hours. After 2 1/2 hours there was a gradual increase to about 85% over the next 10 hours. Initially there was about 25% of the ^{14}C in sucrose. The activity in sucrose increased to about 65% at 10 minutes and then fell off to about 10% in 12 hours. The two curves are virtually mirror images of each other. During the first 10 minutes there was a shift of ^{14}C from the insoluble fraction to sucrose. After 10 minutes translocation of sucrose accounts for the increase in the percentage of activity in the insolubles since it is virtually all that is left in the fed area after the sucrose is translocated out. Sucrose and the insoluble fraction account for all but 5% of the total ^{14}C left after 12 hours. The results from paper chromatography show that most of the remaining ^{14}C is in free glucose and fructose and in 1 or 2 organic acids.

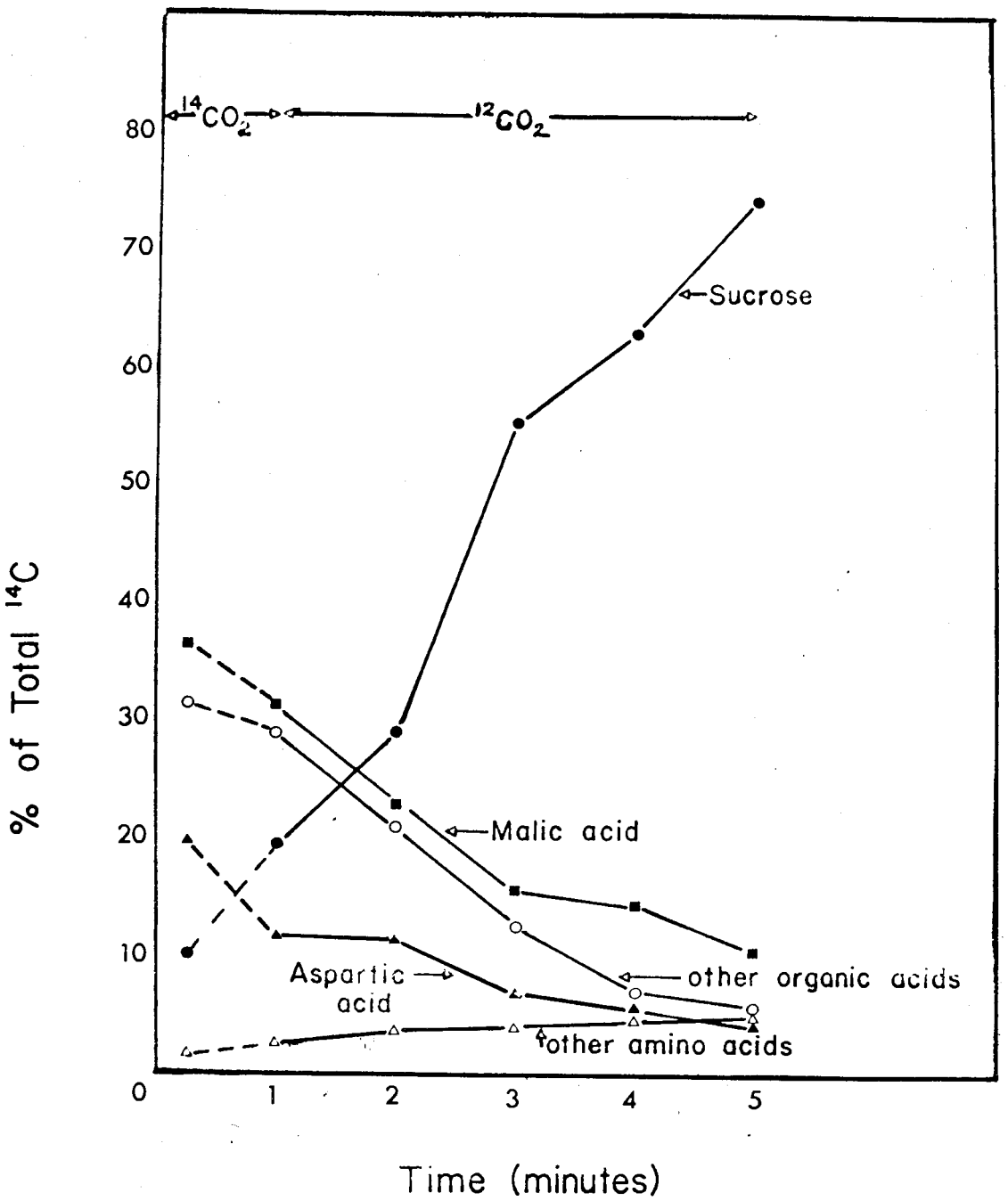
Fig. 2. Distribution of ^{14}C in sucrose and the ethanol-insoluble fraction of the fed area, expressed as a percentage of the ^{14}C left in the fed area at various times after a 1 min. feeding in $^{14}CO_2$ in the light followed by $^{12}CO_2$ in the light.


% of Total ¹⁴C Remaining
100
90
80
70
60
50
40
30
20
10
0
1
2
3
5
7
9
11
13
Time (hours)
sucrose
insoluble

The variability among experiments on different days was about ±10%. The variability in the results appeared to be in the pretreatment of the plants, since the plants that were used on any one day for an experiment behaved almost identically. The effect of pretreatment was not futher investigated.

Analysis of the ethanol-soluble fraction is summarized in Fig. 3. At the end of a 15 second feeding about 65% of the ^{14}C was present in malic acid plus other organic acids, almost 20% in aspartic acid and about 10% in sucrose. About half of the activity in the organic acids was in malic acid. α-keto-glutarate and glycolate were identified and contributed 5 and 2% respectively of the activity in the organic acid fraction. At the end of the 1 minute exposure to $^{14}CO_2$ about 60% of the activity was in the organic acids, about 15% in aspartic acid and about 20% in sucrose. The remaining 5% was mainly in phosphorylated compounds with traces in other amino acids. Analysis at succeeding 1 minute intervals showed that the activity in sucrose increased rapidly with time, reaching 75 to 80% of the total soluble activity in 5 minutes. The activity in the organic acids and aspartic acid decreased correspondingly rapidly. At the end of the 15 second feeding the amount of activity in α-ketoglutarate was about twice the amount present in glycolate. At the end of the 1 minute feeding the relative amount of activity in the 2 compounds was reversed. The percentage activity in glycolate remained high over the following 4 minutes. The 2 amino acids, α-alanine and glycine, contained a small amount of ^{14}C after the 1 minute feeding and the amount slowly increased with time.

Fig. 3. Distribution of ^{14}C among compounds of the ethanol-soluble fraction of a corn leaf fed $^{14}CO_2$ at 24° for 1 min. in the light followed by $^{12}CO_2$ in the light.


$^{14}CO_2$
$^{12}CO_2$
Sucrose
Malic acid
Aspartic acid
other organic acids
other amino acids
% of Total ^{14}C
Time (minutes)
0
10
20
30
40
50
60
70
80
1
2
3
4
5

It is clear that an initial pulse of ^{14}C is incorporated mainly into the organic acids and amino acids. This ^{14}C is rapidly converted to sucrose, the translocation compound. Of the initial ^{14}C fixed, 80% is in the soluble fraction after 5 minutes and of this 80% is in sucrose, thus making 64% of the fixed ^{14}C potentially available for translocation. With further time, additional ^{14}C is mobilized into sucrose. Whether or not all of this sucrose is translocated, this calculation shows that within 5 minutes the sucrose pool in the fed area is extensive.

According to Donovan (1965) the Flint single corn hybrid has a greater degree of tolerance to low temperatures than the Dent hybrid. These hybrids were used in a study in the products of photosynthesis in corn at a lower temperature of 8°.

The 2 hybrids were grown for 3 weeks in a growth chamber under 16 hours light and 8 hours dark, 13° day temperatures and 5° night temperatures, and at 2000 ft-c. and a relative humidity of 75 to 85%. During the first week there was little difference between the growth of the Dent and Flint hybrids. All the seedlings were pale green in color. During the second week the Dent hybrid developed necrotic lesions on the leaf tips and margins, and there was little further growth of these seedlings at this low temperatur The Flint hybrid however, produced no lesions but remained green and continued to grow slowly (Fig. 4). Thus the Flint hybrid exhibited a greater tolerance to low temperatures as Donovan reported.

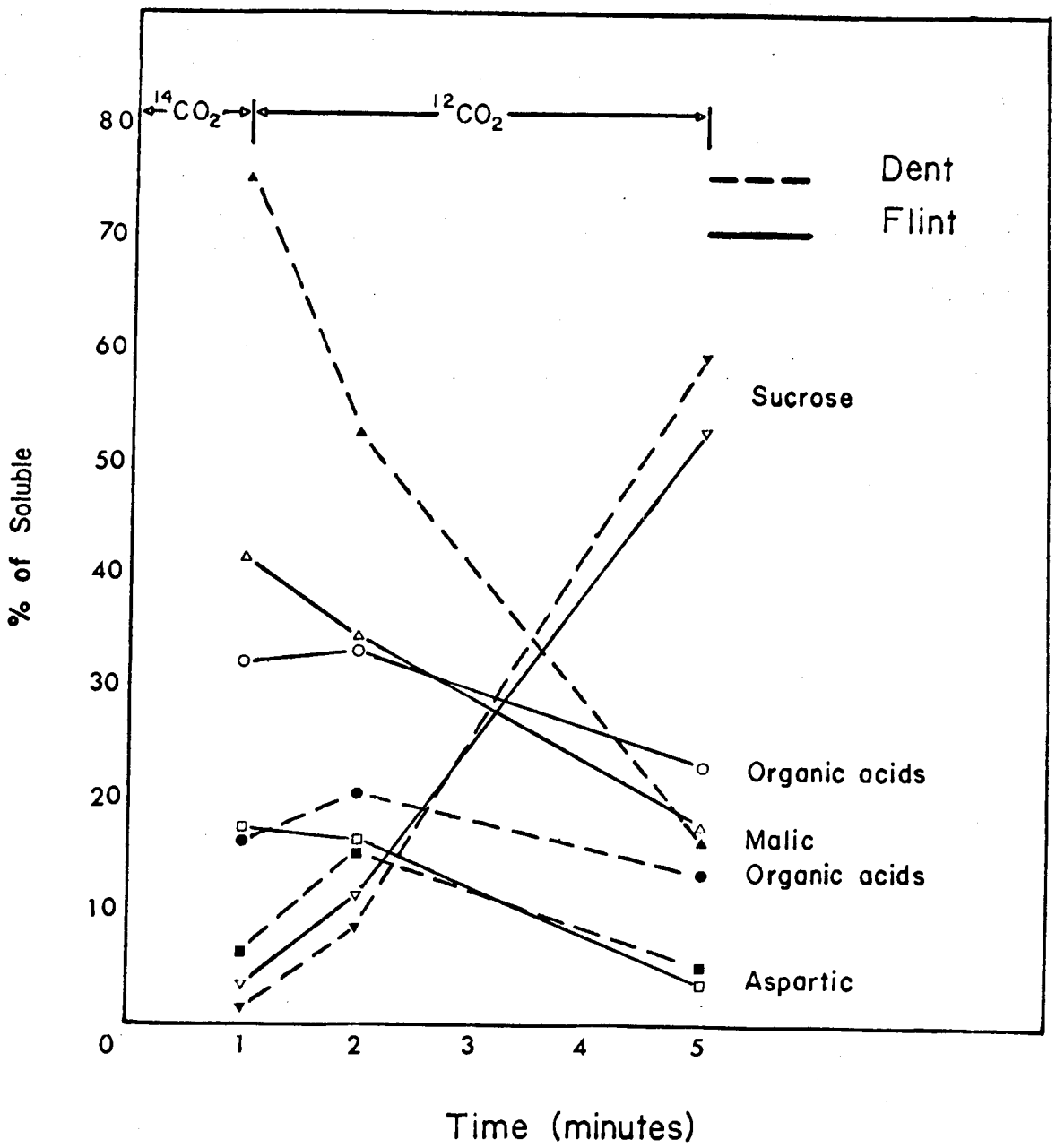
Fig. 4. Corn plants grown at day temperature of 13° and night temperature of 5°C. Left; Flint. Right; Dent.

The plants used for the anlaysis of the products of photosynthesis at 8° were grown in the greenhouse for 6 weeks and the plants were transferred to the growth chamber at 8° 1 hour before feeding. Analysis of the products of photosynthesis at 8° showed that the rate of sucrose production did not differ greatly between the 2 hybrids. The major difference was in the amount of the ^{14}C present in malic acid immediately after the 1 minute feeding (Fig. 5). At the end of the 1 minute feeding the Dent hybrid had almost 2 times more ^{14}C in malic acid than the Flint hybrid. Correspondingly, there was much less radioactivity in the other organic acids in the Dent hybrid. In both hybrids about 15% of the activity went through aspartic acid.

When the changes in the distribution of ^{14}C in the products of photosynthesis at 8° are compared with those at 24° it is noted that the amount of sucrose produced during the 1 minute feeding at 8° was less than at 24°. The rate of sucrose production during the next 4 minutes was the same at the 2 temperatures. In the Dent hybrid the amount of activity in malic acid at 8° was double the amount present at 24°.

Although at the lower temperature the production of sucrose was somewhat delayed, sucrose was still the main product of photosynthesis. The lower temperature had a greater effect on the pattern of carbon fixation in the Dent hybrid in that a much greater proportion of the ^{14}C went through malic acid. There was little difference between the Dent and Flint hybrids in sucrose production and there was little effect of temperature on the amount of sucrose made available for translocation in 5 minutes.

Fig. 5. Distribution of ^{14}C among compounds of the ethanol-soluble fraction of corn leaves (Dent and Flint variety) fed $^{14}CO_2$ at 8°C for 1 min. in the light followed by $^{12}CO_2$ in the light.


$^{14}CO_2$
$^{12}CO_2$
Dent
Flint
Sucrose
Organic acids
Malic
Organic acids
Aspartic
% of Soluble
Time (minutes)
0
10
20
30
40
50
60
70
80
1
2
3
4
5

Discussion

Corn was found to utilize all the CO_2 in a closed volume of air as had also been found by Tregunna et al.(1964), Forrester et al.(1966), and El-Sharkawy et al.(1966). This has been interpreted to mean that corn does not carry on respiration in the light. Although most species do evolve CO_2 in the light (photorespiration), (Tregunna et al. 1964, Forrester et al.1966) corn is not the only plant that has not apparent respiration in the light. Hartt (1965) found no compensation point in sugarcane and El-Sharkawy reports that another monocot, sorghum, and a dicot, Amaranthus edulis, also release no CO_2 to the atmosphere in the light.

The assimilation of CO_2 involves the enzymatic coupling of CO_2 to an organic compound thought to be ribulose diphosphate. This process is immediately followed by the synthesis of new compounds in the leaf. The results of the experiments show that in corn most of the fixed CO_2 is converted to sucrose, the translocation compound.

In the investigations on the availability of the ^{14}C for translocation some interesting information on the path of carbon in corn came to light. According to Calvin and Bassham (1962) CO_2 is coupled to ribulose-1,5-diphosphate by the enzyme ribulose-1,5-diphosphate carboxylase with the subsequent formation of phosphoglyceric acid (PGA) and 3, 4, 5, 6 and 7 carbon sugars. The results from the investigation in corn on the distribution of ^{14}C at various times after a $^{14}CO_2$ feeding do not support the above pathway. Malic and aspartic acid were the main labelled compounds after a brief exposure of the leaf to $^{14}CO_2$. The

formation of these compounds in large quantities without the formation of PGA suggests that CO_2 fixation in corn occurs via a different pathway by a different set of enzymes.

Recent evidence by Björkman (1967) indicates that the rate of photosynthesis in corn does not coincide with the activity of the enzyme ribulose-1,5-diphosphate carboxylase as it does in other species he examined. Other enzymes appear to be involved in CO_2 fixation in corn. Further evidence for the existence of a different enzyme(s) comes from the work of Kortschak et al, (1965) and Hatch and Slack (1966) on sugarcane. The products of photosynthesis in corn and sugarcane are very similar in that after a 15 second exposure to $^{14}CO_2$ of leaves of either species, most of the radioactivity is found in malic and aspartic acid. Hatch and Slack (1966) suggest that the enzyme ribulose-1,5-diphosphate carboxylase is inactive in sugarcane and that the primary carboxylation reaction is via some other enzyme. In the sugar beet Burma and Mortimer (1957) found that the carbon is fixed by the photoreduction cycle proposed by Calvin and Bassham, but that there is also some CO_2 being fixed into malic acid independently of the other photoreduction cycle.

The initial high percentage of the total ^{14}C in the ethanol-insoluble fraction followed by a decline in activity suggests a direct incorporation of $^{14}CO_2$ into the ethanol-insoluble compound(s). The nature of the insoluble compound(s) remains to be investigated. Neither Kortschak et al nor Hatch and Slack found any evidence of a similar incorporation of $^{14}CO_2$ into ethanol-insoluble compounds. In sugarcane there was a gradual

incorporation of the ^{14}C into the ethanol-insoluble fraction suggesting that the insolubles in sugarcane are secondary products of photosynthesis. On the other hand in the sugar beet leaf Burma and Mortimer (1957) found that the ethanol-insoluble residue contained 15 to 20% of the total incorporated ^{14}C. In squash Webb and Gorham (1964) found that after a 15 second feeding over 60% of the ^{14}C was in the ethanol-insoluble fraction, followed by a rapid decline during the first 5 minutes and then remained relatively unchanged for the next 2 to 3 hours. Trip et al. (1964) found that in celery 75% or more of the ^{14}C was in the ethanol-insoluble fraction with feeding times of less than 15 seconds. Similar but smaller amounts were found in maple, corn, lily of the valley, avocado, birch and lilac (Trip et al.1964).

Studies of the distribution of ^{14}C in the various compounds at 8° indicate that low temperatures do not alter the path of carbon qualitatively in corn. The production of sucrose was delayed considerably, however. The labelled compounds at 8° were the same as those at 24° and they also appeared in the same order. It appears that a decrease in temperature slows down the overall reaction of carbon fixation and as such should be a valuable approach in determining the path of photosynthesis in corn.

After the shortest feeding time (15 seconds) at 24°, 3 amino acids were present in the ethanol-soluble fraction. These were aspartic acid, α-alanine and glycine. The amount of activity in aspartic acid decreased rapidly with time, whereas the activity in α-alanine and glycine increased slowly. At 8° at the end of the 1 minute feeding aspartic acid was the main amino acid labelled with only a trace of activity in α-alanine.

The amount of activity in α-alanine increased with time and glycine became radioactive. These results indicate that aspartic acid is a very early intermediate in carbon fixation whereas α-alanine and glycine are secondarily labelled. It is not known whether aspartic acid is produced by a separate carboxylation reaction or whether it is formed by transamination from malic acid. The amount of activity in aspartic acid decreases more or less at the same rate as that in malic acid, indicating that either aspartic acid is not produced from malic acid or the 2 compounds occur in equilibrium with each other. In the sugar beet there did not appear to be any definite association between malic and aspartic acid (Burma and Mortimer 1957).

From these distribution experiments there is no evidence of the path followed by carbon from malic and aspartic acid to sucrose. Sucrose is, however, the main product of photosynthesis that accumulates in large quantities in the leaf. Sucrose makes up upwards to 85% of the ethanol-soluble fraction or about 65% of the total ^{14}C in the fed area after 10 minutes. In sugarcane about 70% of the ethanol-soluble fraction was in sucrose (Kortschak et al.1965), in soybean leaves a maximum of 55% (Nelson et al.1961), in tobacco from 25 to 70% depending on the stage of development of the leaf (M. Shiroya et al.1961) and in pine seedlings about 70% (T. Shiroya et al.1962b). Compared to other species corn has a large pool of sucrose produced in a short time to serve as the translocation substrate. Fig. 2 indicates that most of the sucrose is rapidly lost from the fed area of the leaf but between 5 and 10%

of the remaining ^{14}C is still in sucrose even 12 hours after feeding. A similar compartmentalization or separation into different sucrose pools has been observed by other investigators in other plant species. Older tobacco leaves which were very rich in sucrose translocated only a small percentage of the sucrose, the rest remaining in the leaf not readily available for translocation (M. Shiroya et al 1961). The sugar beet leaf still contained 40 to 50% of the assimilated ^{14}C, mostly in sucrose, after translocation had virtually ceased (Mortimer 1965). In pine seedlings it was found that sucrose occurs in 2 forms in the leaves, either as storage or as transport sucrose (T. Shiroya et al 1962b). Compared to other species corn has a large percentage of its sucrose as translocation sucrose.

Destination of the Translocated ^{14}C in Corn.

Introduction.

Translocation involves a movement of assimilate from the source (the leaf) to the sinks (accumulating areas). Before the actual process of translocation was considered, the distribution of the translocate throughout the plant was investigated.

Evidence indicates that the sinks influence the translocation from the leaves (Lupton 1966, Hew 1965). The destination of the translocate from each of the leaves of corn was investigated to determine the distribution of the sinks throughout the plant, and if possible to determine how the sinks influence translocation from the leaves.

Experiments and Results

The plants that were selected for this study were about 2 months old and had 7 or 8 leaves. At this age the lowest three leaves are fully expanded and another 4 or 5 leaves are present in various stages of development. The youngest leaf displayed a blade about 10 cm long. The plants were allowed to carry on translocation for 24 hours after feeding $^{14}CO_2$ to a 7-cm section of one leaf for 5 minutes. A period of 24 hours (16 hours light, 8 hours dark) was chosen to allow most of the translocated sucrose to move to the active sinks. The pattern of translocation from the various leaves was determined by cutting the plant into pieces and analyzing each piece separately for its ^{14}C content as shown in Fig. 6 A-G. Since the plants were grown in vermiculite it was difficult to obtain all the rootlets for

Fig. 6. Distribution of ^{14}C in the various parts of the corn plant after 24 hours of translocation from different leaves expressed as a percentage of the total ^{14}C in the plant. Leaves were fed $^{14}CO_2$ for 5 min. in the light in the areas shown, followed by 24 hours in $^{12}CO_2$ in the light for 16 hours and in the dark for 8 hours.

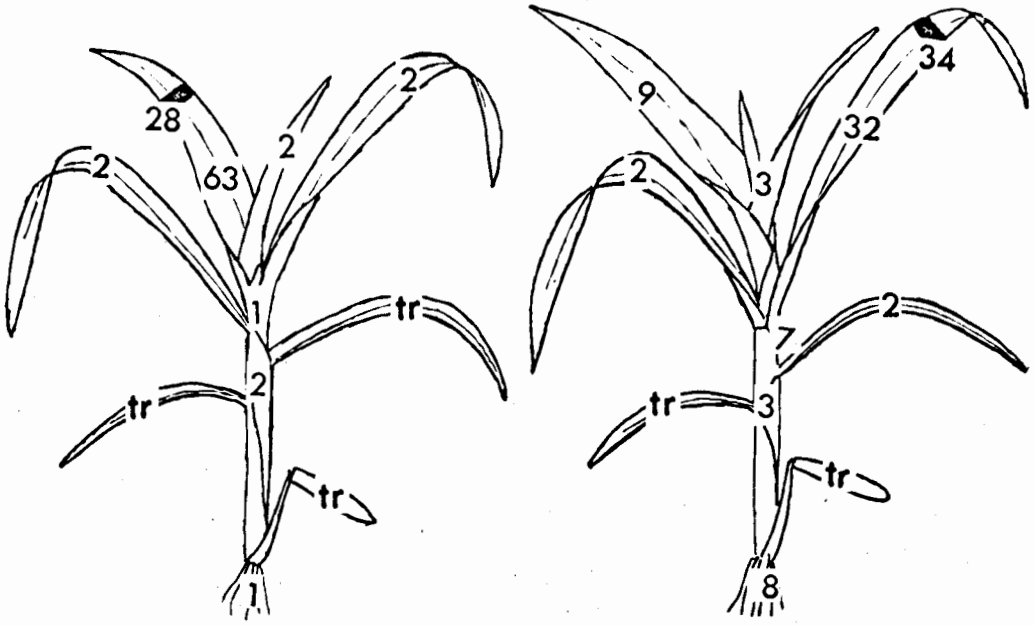
A leaf 1

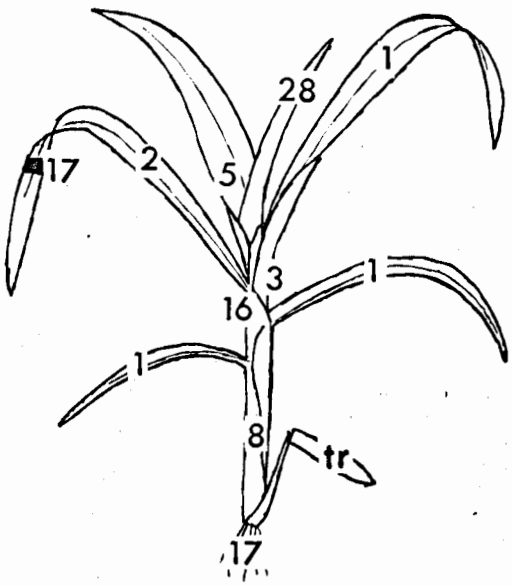
B,C leaf 3

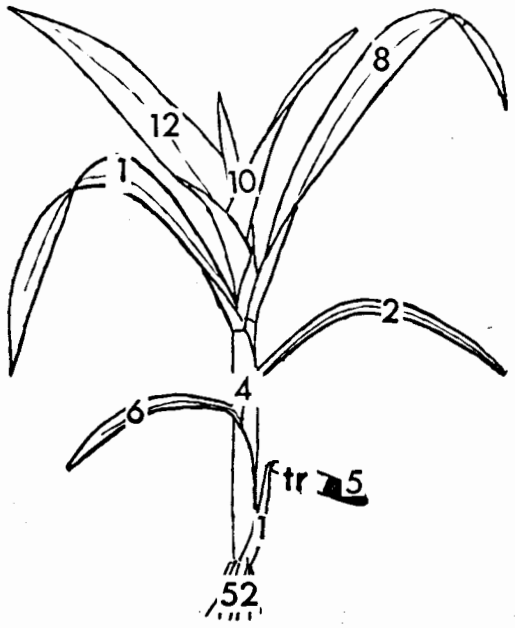
D,E leaf 4

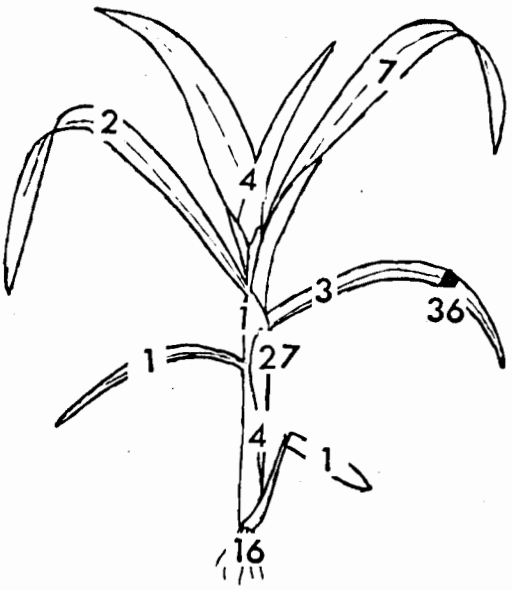
F leaf 5

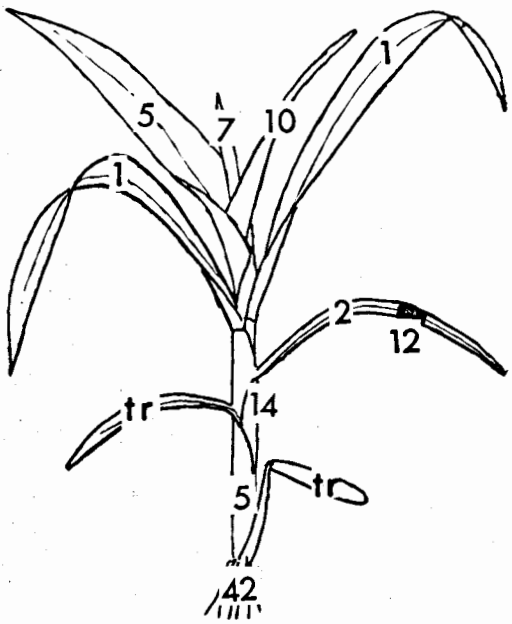
G leaf 6

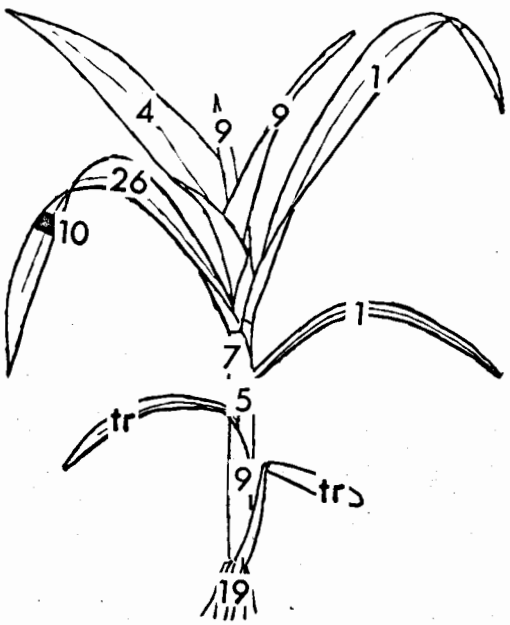

28
2
2
63
2
2
1
tr
2
tr
tr
1
9
34
32
2
3
2
7
tr
3
tr
8


1
28
17
2
5
3
1
16
1
8
tr
17


12
8
1
10
2
4
6
tr
5
1
52


7
2
4
3
36
1
1
27
4
1
16


5
7
10
1
1
2
12
tr
14
5
tr
42


4
9
9
1
26
10
1
7
5
tr
9
tr
19

analysis. The radioactivity found in the roots is thus probably lower than the actual amounts present.

The results are summarized in Fig. 6 A-G. All of the leaves exported ^{14}C even though some of the leaves were only partially expanded and must therefore have been separated from the stem by a meristimatic region. The amount of material leaving an immature leaf however, was small compared to the mature leaves. The more mature the leaf the more photosynthate was translocated from the leaf to other parts of the plant (Table IV). In very immature leaves (less than 1/3 expanded) a large percentage of the radioactivity was retained in the leaf blade outside the fed area. Not until the blades were fully expanded were large amounts retained by the expanding leaf sheaths. Fully mature leaves retained at least part of the radioactivity in the leaf outside the fed area. The amounts retained were generally in the order of about 15%, most of this being in the ethanol-soluble fraction.

All of the mature leaves, whether above or below the fed leaf, imported some of the translocate. However, the further the leaf was from the fed leaf the less it imported from that leaf, regardless of the age of the leaf. Most of the radioactivity imported by mature leaves was recovered in the ethanol-soluble fraction. The average percentage of the radioactivity in the soluble fraction in these mature leaves was 66.4 ± 10.8 (n=16). This is in contrast with the other parts of the plant where the radioactivity was distributed more or less 50:50 between the 2 fractions.

Table IV. Translocation of ^{14}C from corn leaves of various ages in 24 hours.

Leaf No.	Percentage Translocated		
	From the Fed Area	From the leaf	To the roots
7 (youngest)	71.8	8.6	1.0
6	66.1	27.6	8.4
5	64.4	34.4	15.8
4	90.2	56.8	18.6
3	83.5	65.3	16.6
2	87.7	71.7	42.4
1	94.8	93.5	52.2

In all but 2 cases more radioactivity was translocated towards the roots than upwards to the growing point (Table V). The 2 exceptions were leaves on which the sheaths were just about fully expanded. The growing leaves appeared to produce most of their own substrate for growth and little was imported from the leaves below. The lower the leaf was on the plant the more of the exported photosynthate was recovered in the roots.

Discussion.

The study of the destination of the translocated ^{14}C revealed the sinks and the relative importing activity of the sinks in the young corn plants. A young growing corn plant has many meristimatic regions, the roots, stem apex, the growing leaves and the growing stem. It was found that all of these regions acted as active sinks and imported ^{14}C from all of the leaves. Of the sinks, the roots imported the greatest percentage of the translocated ^{14}C. It is interesting to note that even fractionally expanded leaves exported at least some of their assimilates to the roots. As in other species that have been investigated, soybean (Thaine 1959, Thrower 1962) and wheat (Lupton 1966), the corn leaves closest to the roots translocated the highest percentage of their exported assimilates to the roots (Table V).

After the roots, the stem apex imported the next largest percentage of the assimilates. Of the assimilates that were exported from the leaves, from 10% from the oldest to 45% of the youngest leaves was translocated to the apical meristimatic region. This pattern is similar to the one for wheat and soybean.

Table V. Destination of ^{14}C translocated from corn leaves of various ages.

Leaf No.	Percentage Translocated	
	Up	Down
7 (youngest)	2.2	6.4
6	12.0	15.6
5	12.7	21.7
4	28.7	28.1
3	38.8	26.5
2	29.0	42.7
1	41.3	52.2

The growing leaves and leaf sheaths imported some of the assimilates translocated from other leaves, but never more than 10% of the total ^{14}C in the plant was found in any one leaf or sheath, except for the leaf exporting ^{14}C. As the length of the leaf increased, the amount of assimilate imported from other leaves decreased. By the time the leaf was between 1/3 and 1/2 expanded only 1 to 2% of the ^{14}C in the plant was found in that leaf. This amount was only slightly more than that imported by fully expanded leaves. In the partially expanded leaves most of the ^{14}C was found in the growing region of the leaf.

The vascular bundles from all the leaves in corn are interconnected in the stem (Kumazawa 1961). It is therefore not too surprising to find some ^{14}C in all the leaves, but the amount found in mature leaves was relatively small, the total ^{14}C recovered from all the leaves that were 1/2 or more expanded was less than 5% of the total ^{14}C in the plant.

These experiments show that small amounts of the translocate remain in the vascular tissue of the exporting leaves, (being less than 3% of the total ^{14}C in the plant). Over 50% of this activity was recovered from the ethanol-soluble fraction. The mature leaf sheaths on the other hand contained between 1 and 16% of the total activity and again between 50 and 75% of this was in the ethanol-soluble fraction. The amount of ^{14}C found in the stem, not counting the stem apex, was low (being only between 1 and 8.5% of the total ^{14}C), and was divided about 50:50 between the soluble and insoluble fractions. Thus radial translocation

from the transport stream varies with the plant organ, being the lowest in the leaf blade and the highest in the leaf sheath. The localization of this radially translocated and accumulated material was not investigated.

The corn plant has many sinks which import translocate from all the leaves. The activity of any one of those sinks could influence the translocation from the leaves. The roots comprise the largest sink in the young plants and influence translocation from all the leaves. However, it appears that any meristematic region in corn is a major sink for the import of translocate.

Translocation of Assimilated ^{14}C in Corn.

Introduction.

Before a system could be set up that could be used to study the control of translocation, it was necessary to understand as much as possible about translocation in corn.

In this part of the research the following aspects were investigated: the form in which organic compounds are translocated in corn; the velocity of translocation in corn; the sucrose profile in the leaf blade and changes in the profile with manipulation of the source or the leaf blade; and the localization of the translocate in the leaf blade.

Experiments and Results

Identification of the translocate. To determine the form in which carbon is translocated in corn, a leaf segment was exposed to $^{14}CO_2$ for 5 minutes and then placed in air for 5 minutes. At the end of this time the leaf was cut into 2.5-cm sections, killed and extracted in boiling 80% ethanol and the radioactive compounds identified by 2-dimensional chromatography and autoradiography.

The only radioactive compound that was detected in the corn leaf below the fed area was sucrose. During the course of this work, this and similar tests were carried out about 20 times with the same results. It is concluded that sucrose is the form in which organic carbon fixed in photosynthesis is translocated in corn. Loomis (1945) made the same conclusion from studies of the soluble material in corn plants. He found that changes in carbohydrate content of leaves and stems was mainly due to loss

or accumulation of sucrose. From this he concluded that in corn, sucrose is the main translocation sugar.

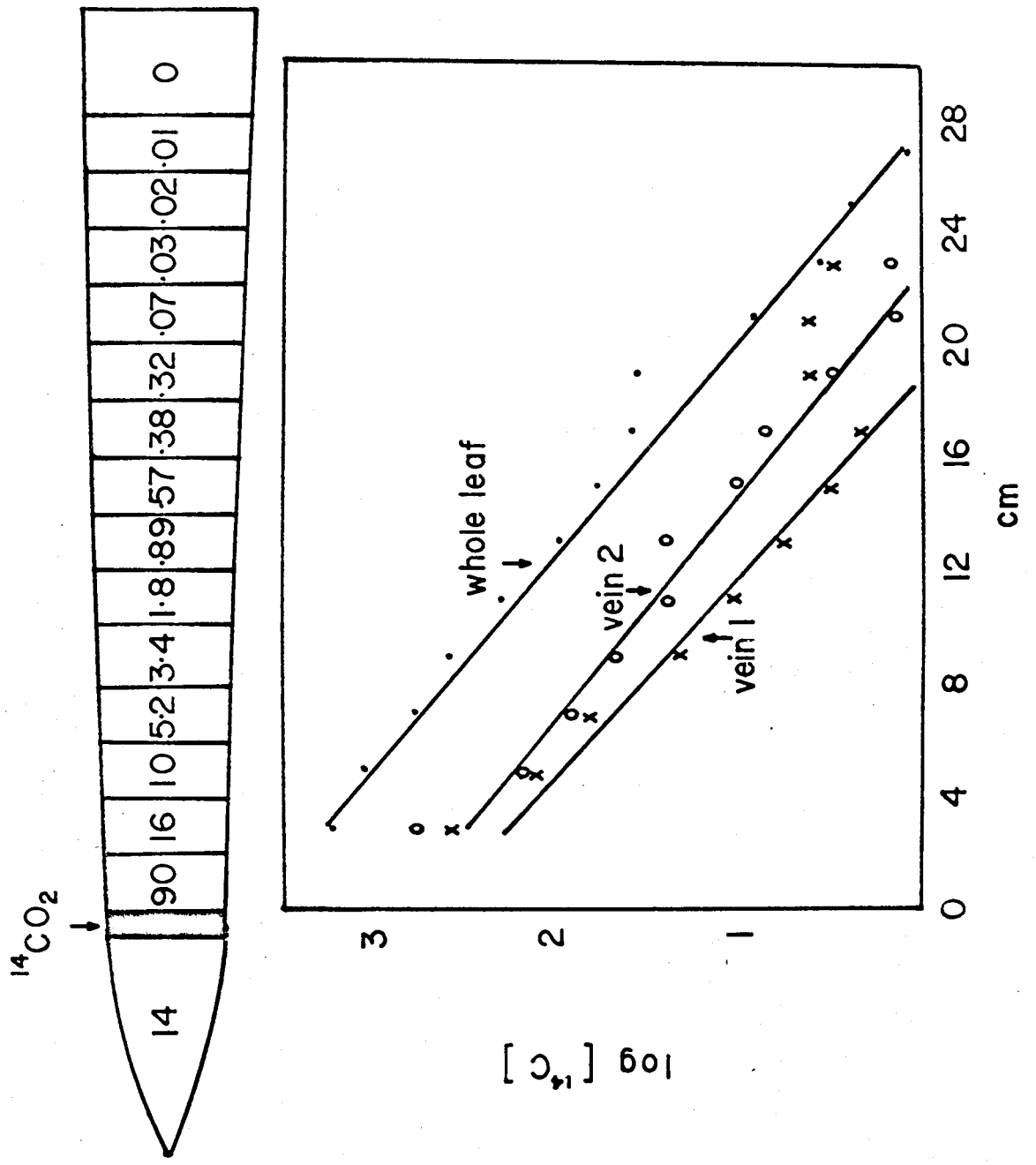
The translocation velocity and profile. Two measurements are useful in describing the characteristics of translocation of organic compounds originating in photosynthesis. These are the velocity of translocation of ^{14}C and the profile of ^{14}C concentration along the leaf.

A narrow segment of corn leaves was fed $^{14}CO_2$ for 1 minute. The leaves were then left to carry on photosynthesis in air for 9 minutes, and then cut into 2-cm sections each of which was killed and extracted in boiling 80% ethanol. The radioactivity in each section was determined by counting aliquots of the extract in the liquid scintillation counter. In all sections of the leaf, other than the fed section, the 80% ethanol-soluble fraction accounted for more than 95% of the total radioactivity. In addition, sucrose was the only ^{14}C labelled compound recovered.

The velocity of translocation was estimated by measuring the distance travelled by the sucrose-^{14}C during the experiment. Sucrose-^{14}C was detected 26 cm down the leaf blade 9 minutes after feeding. This gives a velocity of 150 cm per hour. Such a velocity is in the range noted in Table I.

The profile for the whole leaf is shown in Fig. 7. The sucrose-^{14}C decreased logarithmically from the point of feeding down the leaf blade. Similar profiles have been found in the stems of dicotyledonous plants (Nelson et al. 1959, Canny 1961, and Clauss et al. 1964).

Fig. 7. Distribution of ^{14}C in a corn leaf blade and single veins after feeding $^{14}CO_2$ to the restricted area for 1 min. in the light followed by 9 min. in $^{12}CO_2$ in the light. Values in the diagram of the corn leaf express relative radioactivity in the different parts.


$^{14}CO_2$
14
90
16
10
5·2
3·4
1·8
·89
·57
·38
·32
·07
·03
·02
·01
0
whole leaf
vein 2
vein 1
log [^{14}C]
3
2
1
0
4
8
12
16
20
24
28
cm

It is of interest to examine the profiles in the individual veins that make up the profile for the whole leaf blade to see whether the rates of translocation differ in the different veins. Profiles for 2 separate veins are shown in Fig. 7. The veins were pulled or cut from the leaf blade, cut into 2-cm sections and the sucrose-^{14}C in each section determined. The radioactivity in each vein decreased logarithmically down the leaf from the point of introduction. Logarithmic profiles for single veins were to be expected since only the sum of the logarithmic profiles for single veins will give the logarithmic profile in the whole leaf. However, since the slopes of the profiles are a reflection of the rate of translocation, the rates were different in the 2 veins examined.

The effect of removing or darkening the fed area on the translocation profile. The logarithmic profile down the leaf blade remains for at least 30 to 40 minutes after feeding. To determine how this profile is maintained, the fed areas of corn leaves were either darkened or removed 5 minutes after a 1 minute $^{14}CO_2$ feeding. The fed areas were removed by cutting off the leaf 2 cm below the fed area or the fed area was darkened by covering with aluminum foil. The leaves were then left for various periods of time before they were cut into sections, extracted and the amount of sucrose-^{14}C determined in each section.

Within 5 minutes after feeding the logarithmic profile extended to at least 15 cm below the fed area (Fig. 8A). Within 5 minutes after the fed area had been removed the profile had

Fig. 8. Effect of the removal of the source on the translocation profile. Restricted areas were fed $^{14}CO_2$ in the light for 1 min., left in the air in the light for a further 5 min., the fed areas removed and the profiles determined after various intervals of time.

A 0 min.

B 5 min.

C 10 min.

D 15 min.

E 20 min.

F 30 min.

Fig. 8. Effect of the removal of the source on the translocation profile. Restricted areas were fed $^{14}CO_2$ in the light for 1 min., left in the air in the light for a further 5 min., the fed areas removed and the profiles determined after various intervals of time.

A 0 min.

B 5 min.

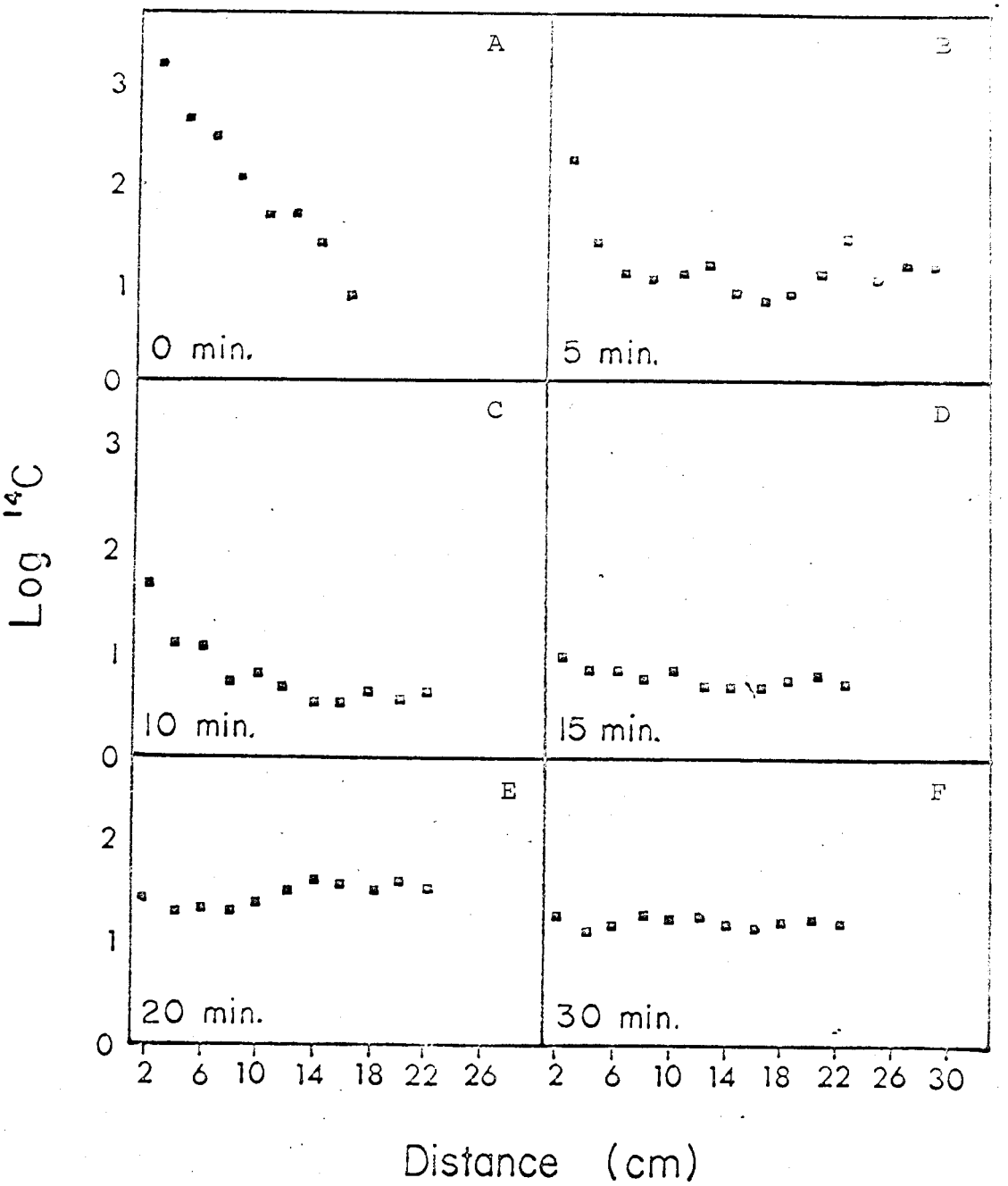
C 10 min.

D 15 min.

E 20 min.

F 30 min.

Fig. 8.


A
B
C
D
E
F
0 min.
5 min.
10 min.
15 min.
20 min.
30 min.
Log ^{14}C
Distance (cm)
0
1
2
3
2 6 10 14 18 22 26
2 6 10 14 18 22 26 30

changed (Fig. 8B). The section next to the fed area still contained the highest amount of sucrose-^{14}C, while the rest of the sections contained more or less the same amounts of sucrose-^{14}C. After 10 minutes there was little change in the shape of the profile (Fig. 8C). After 15 minutes all sections of the leaf contained the same amount of sucrose-^{14}C to give a profile parallel to the x-axis. This profile was maintained for a least 20 and 30 minutes after the removal of the source (Fig. 8D,C,F).

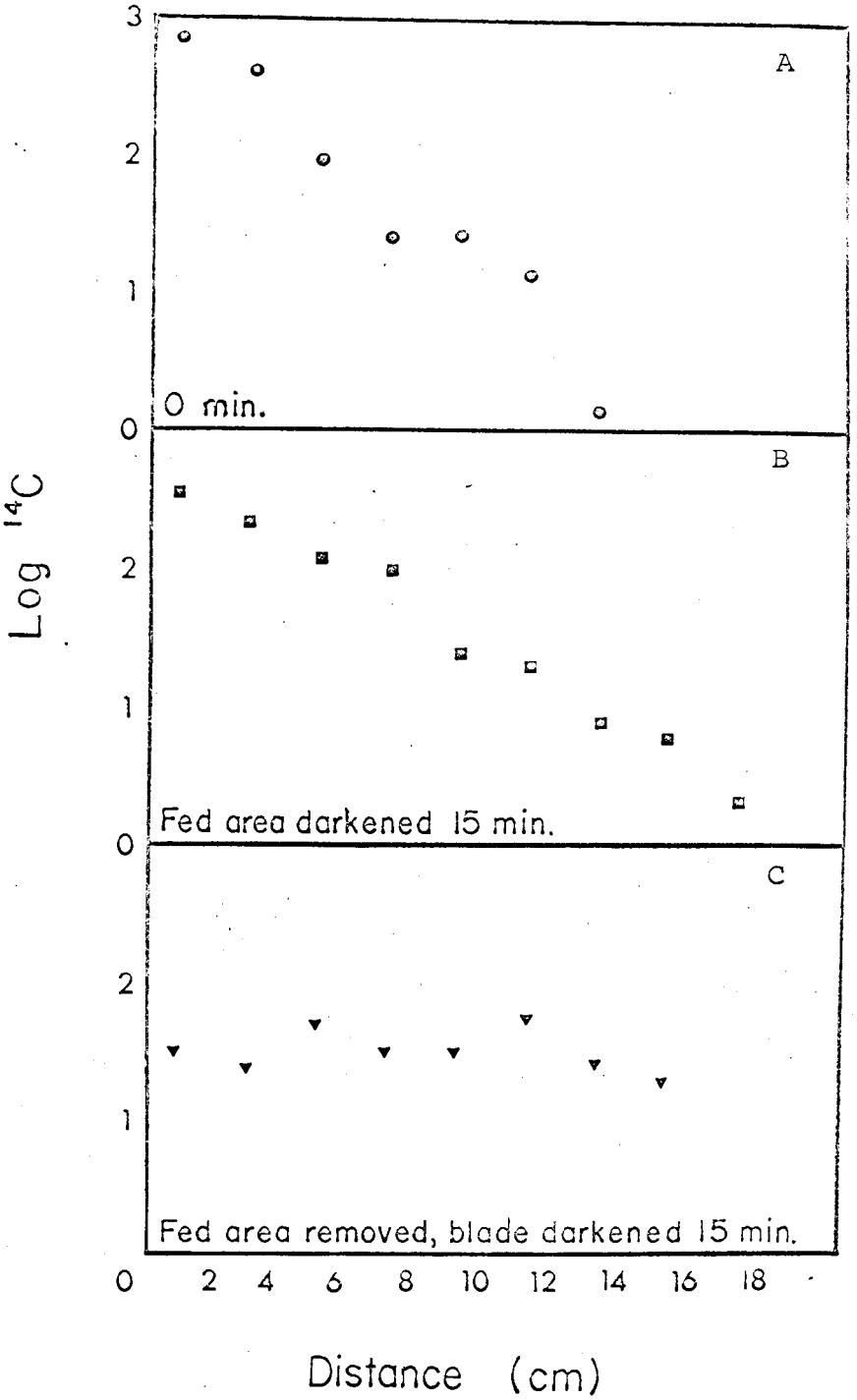
Darkening the fed area for 15 minutes had no noticeable effect on the profile. The profile was still logarithmic over the first 20 cm below the fed area (Fig. 9A,B). Darkening the leaf for 15 minutes after removing the fed area produced a profile (Fig. 9C) similar to that in Fig. 8D where the blade was kept in the light after the fed area had been removed.

From these experiments it appears that the logarithmic profile was maintained by the exporting tissue. As long as the source was translocating, which it did in both light and dark, the logarthmic profile was maintained. As soon as the fed area was removed the logarithmic profile levelled off and remained level. Darkening the leaf blade after the fed area had been removed had no effect.

Localization of the translocated sucrose in the leaf blade. The previous experiment indicates that translocation occurs in the veins. Two approaches were taken to obtain a more precise picture of the localization. In the first approach a leaf was divided into blade and midrib and each analyzed separately

Fig. 9. Effect of darkness on the translocation profiles in corn leaves. A restricted area of the leaf was fed $^{14}CO_2$ in the light for 1 min at 0 distance. A. Leaves were left in the light $^{12}CO_2$ for 5 min. and were analyzed immediately, B. were left for a further 15 min. with the fed area in the dark and the rest of the leaf in the light or, C. the fed area was removed and the rest of the blade was darkened for 15 min.

Fig. 9.


A
0 min.
B
Fed area darkened 15 min.
C
Fed area removed, blade darkened 15 min.
Log ^{14}C
Distance (cm)
0
1
2
3
0
2
4
6
8
10
12
14
16
18

in 10 cm sections down the leaf from the fed area. In the second approach a whole-leaf autoradiogram was made.

Corn leaves were fed $^{14}CO_2$ in a localized area (see Fig. 10) for 2 minutes. After 45 minutes in air the leaf blade was cut into 10 cm sections. The midrib was removed from each leaf section and the blade and midrib extracted separately with 80% ethanol and the radioactivity determined in each (Table VI). In the first section below the fed area more than 90% of the radioactivity was in the blade outside the midrib. Further down the leaf a progressively higher percentage of the radioactivity was present in the midrib (Table VI). There was more ^{14}C in the sheath after 45 minutes than in adjacent sections and this ^{14}C was not concentrated in the midrib.

A study of the venation pattern on the corn leaf reveals that the veins in the leaf converge on the midrib as they go down the leaf blade. When the veins enter the leaf sheath they diverge and the midrib becomes less pronounced. The translocated ^{14}C in the leaf follows this pattern as would be expected if the sucrose remains in the veins that it enters in the photosynthetic area.

For the whole-leaf autoradiogram a narrow section of a corn leaf was exposed to $^{14}CO_2$ for 5 minutes followed by an exposure to air for an additional 25 minutes. The midrib was removed and discarded, the blade was placed between 2 sheets of x-ray film, quick frozen in a mixture of isopentane and dry ice (Nelson & Krotkov 1962) and left to develop at -24° for 1 week. The midrib had to be removed from the leaf to allow close enough contact between the leaf blade and the x-ray film.

Fig. 10. Diagram of a whole leaf autoradiogram showing the localization of sucrose-^{14}C in a corn leaf. $^{14}CO_2$ was fed to the restricted area as shown. Veins in which ^{14}C was located are shown as solid lines, while veins without ^{14}C are shown as dotted lines. The areas diagramed in the 4 fold blow-ups show that considerable ^{14}C is localized in the main veins and a small amount in the smaller veins. Note that the main movement was toward the base of the leaf with a smaller amount localized in the tip.

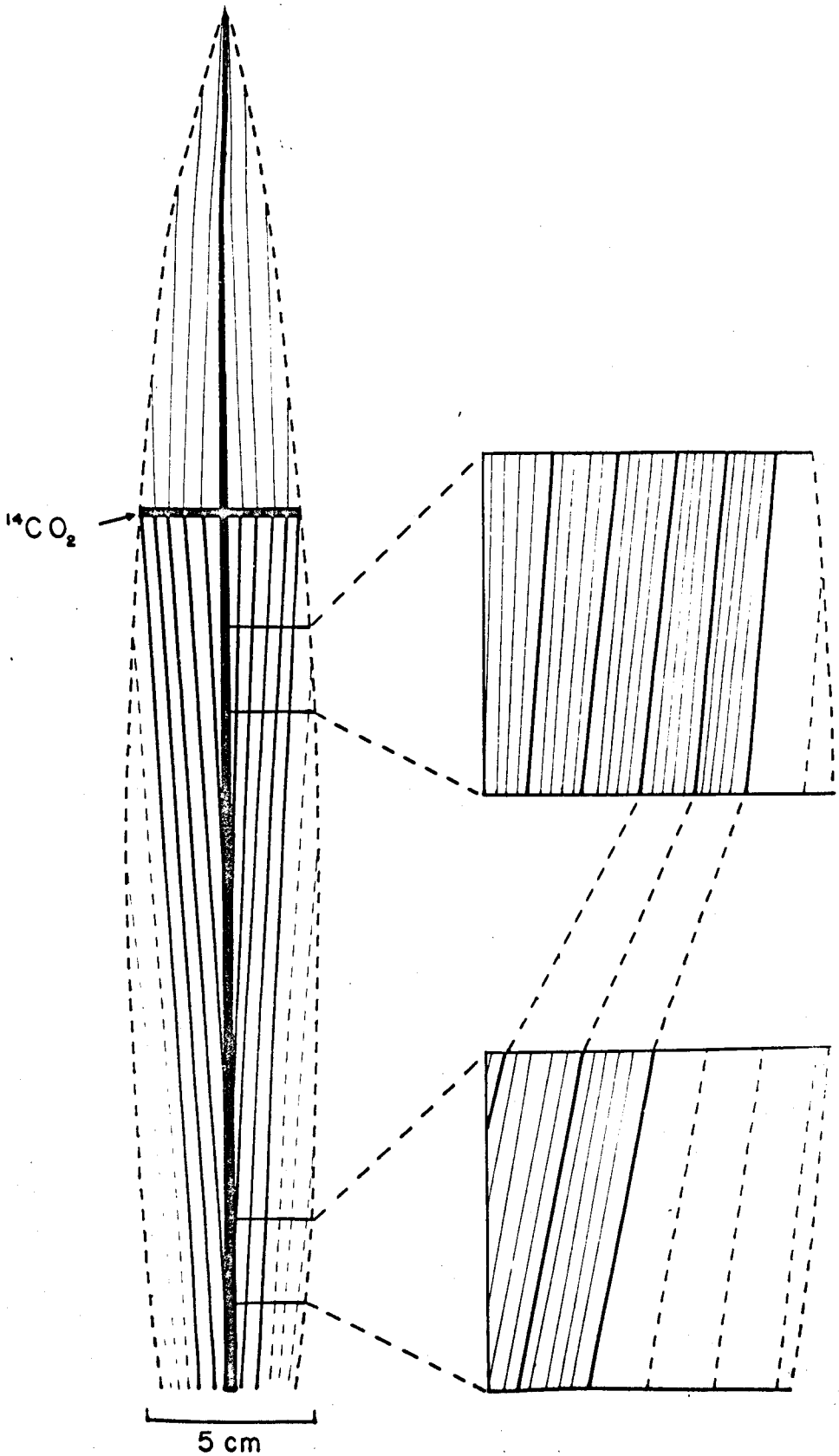

$^{14}CO_2$
5 cm

Table VI. Localization of ^{14}C in the leaf blade 45 minutes after feeding $^{14}CO_2$.

Leaf Section (10 cm)	Leaf 1			Leaf 2		
	Blade a cpm	Midrib b cpm	b/a	Blade a cpm	Midrib b cpm	b/a
1	320	18	0.06	355	26	0.07
2	39	6	0.15	34	13	0.4
3	10	9	0.9	8	12	1.5
4	6	11	1.9	7	11	1.6
5	...	...	...	3	18	6.0
6 (sheath)	59	34	0.6	...	...	...

The whole-leaf autoradiograms are presented diagrammatically in Fig. 10. The radioactivity remained in the veins that it entered in the fed area. Radioactivity was detected in the outermost veins, coming from the fed area, all the way down the leaf blade. Veins originating on the leaf margins below the fed area did not contain enough radioactivity to be detected on the x-ray film. These results are consistent with those obtained by extraction.

Both approaches to the problem of localization of translocated sucrose-^{14}C support the conclusion that sucrose travel straight down the leaf blade in the vascular tissue that passes through the fed area and that there is little or no lateral movement of sucrose from one vascular bundle to another.

Discussion.

In many respects translocation in corn is similar to that in other species. The velocity of translocation was found to be in excess of 150 cm hr^{-1}, a value in the same range of magnitude of velocities found in most species investigated (Canny 1960).

The translocation profile found in the corn leaf was similar to the profiles found in stems by Nelson et al. (1959), Canny (1961) and Clauss et al. (1964). The translocation profile in the squash (Webb & Gorham 1964b) and sugar beet petiole (Mortimer 1965) was found to be linear. Not enough species have been investigated to say whether a linear profile is characteristic of leaf petioles and the logarithmic profiles of stems. Studies of

aphid stylet exudate showed that the amount of ^{14}C in a sieve tube in the stem increases logarithmically when a leaf on the stem is fed $^{14}CO_2$ (Canny 1961). From these results Canny concluded that the logarithmic profile is a characteristic of the translocation process and was not caused by an accumulation of translocate in the conducting tissue.

Webb and Gorham (1964a) found a retention of ^{14}C in the petiole for an appreciable length of time after translocation of ^{14}C from the leaf had ceased, suggesting an accumulation of the translocate in the petiole. Evidence from studies on soybean indicate that the translocation front does not coincide with the distance of travel calculated when the log profile is extrapolated to zero (Nelson et al. 1959). Clauss et al. (1964) found that the only explanation that fitted the logarithmic profile in the stem was an accumulation of the translocate in the stem. Horwitz (1959) in his mathematical treatment of translocation found that he could explain the experimental results on translocation most satisfactorily by an 'en mass' flow of translocate solution through a pipe with irreversible loss of translocate to the surrounding tissue. These authors all suggest that there is an accumulation of material in or near the conducting system but so far no direct evidence for such an accumulation has been obtained.

The results obtained from the studies on translocation in corn indicate that the logarithmic profile is an accumulation profile and is not a characteristic of the translocation process. The studies on the distribution of ^{14}C with time after feeding

(Fig. 2) indicates that the greatest loss of sucrose from the fed area occurred at 15 minutes after feeding. There is an initial rapid rate of loss followed by a decrease in this rate with time. If this exported sucrose travelled 'en mass' down the leaf blade a concentration gradient would be expected with the highest concentration furthest down the leaf blade. The opposite concentration gradient is found in corn leaves. Therefore, the sucrose profile in the leaf does not reflect the pattern of translocation from the fed area, but rather suggests an accumulation of the translocate at a steady rate down the leaf blade giving the highest concentration closest to the source of the radioactive translocate.

Removal of the fed area does not result in a shift of the profile down the leaf blade as would be expected if the profile represented the moving sucrose, but rather it gradually levels off. This change in the profile also indicates that the profile is not caused by an irreversible accumulation as suggested by Horwitz (1958) but rather that at least part of the accumulation is reversible. The results of the studies on the destination of the translocated sucrose-^{14}C showed that some of the activity remained in the leaf blade outside the fed area at least 24 hours indicating that part of the accumulation is more permanent.

The results on the profiles and the loss of sucrose from the fed area can best be explained by an accumulation process in the vascular tissue. The accumulation appears to be a dynamic process. Once the source of the sucrose-^{14}C is removed the

accumulated sucrose is lost from the vascular tissue and is presumably replaced by unlabelled sucrose coming from the photosynthesizing tissue surrounding the vascular bundles. The aphid stylet studies on willow twigs (Canny 1961) which showed that the radioactivity in the exudate increased logarithmically with time suggest that the accumulation of the sucrose is in the sieve tube. If the accumulation of sucrose is in the part of the cell where the aphids feed then the actual transport of the sucrose must be in another part of the cell or in a different cell altogether. This evidence rules out any possibility of a mass flow through the sieve tubes.

As soon as the source of the sucrose-^{14}C is removed the profile levelled off. This means that translocation continues down the leaf veins. This indicates that the vein is able to maintain its translocation without the 'push' of the source. Darkening the leaf blade had no effect on the profile indicating that translocation in the veins is not under light control as suggested by (Hartt et al. 1964, Hartt 1965c, Hartt and Kortschak 1967). Darkening the fed area alone also had no effect on translocation indicating that the transfer of photosynthetically assimilated carbon to the conducting tissue does not require light.

The whole-leaf autoradiograms indicate that the translocated sucrose-^{14}C was localized in the vascular bundles of the leaf. Although the vascular bundles are cross-connected at regular intervals in the leaf (Sharman 1942) both the leaf extraction analysis and the autoradiograms showed that the basipetal translocation was entirely in the veins that pass through the fed area.

Veins originating below the fed area contained no detectable amounts of sucrose-^{14}C. Whether there was any lateral movement toward the midrib could not be determined from these experiments. The techniques used were also not sensitive enough to determine whether there was any radial movement out of the conducting cells to other cells in the vascular bundles.

The studies of translocation in corn indicate that translocation in the vascular tissue involves an exchange of sucrose between the moving sucrose and sucrose stored in the vascular tissue. The transfer of sucrose into the vascular tissue, the movement of the sucrose along the veins and the accumulation of the sucrose in the vascular bundles are not under light control.

Measurement of Translocation by Following the Loss of Photosynthetically Assimilated ^{14}C from a Corn Leaf.

Introduction.

When a small area of a corn leaf is pulse-labelled with ^{14}C the loss of activity from the fed area can be measured with a G-M detector. The amount of radioactivity in the fed portion of the leaf decreases with time. The ^{14}C, fed as $^{14}CO_2$, is incorporated into the photosynthate, which in corn is translocated only in the form of sucrose. Thus the loss of radioactivity from the fed area of the leaf is entirely due to the removal of sucrose from that area. A measurement of the rate of loss of ^{14}C from the leaf therefore gives a measure of the rate of translocation of sucrose, the only translocated organic substance.

There are, however, some inherent difficulties in this method of measuring translocation. Because of the low energy of the β particle emmitted by the ^{14}C, considerable absorption occurs in the leaf especially from the lower layers furthest removed from the G-M detector. Any localized movement of material through the thickenss of the blade would give a change in the reading without actual translocation from the area. However, no such movement is to be expected in the blade except to the vascular bundles, and therefore, presents no large error to the measurement of translocation by this method. The ^{14}C in the insoluble compounds especially in the cell walls gives a higher value than an equivalent amount of ^{14}C in solution (Mortimer 1966). Only if there were a shift of ^{14}C from the soluble to the insoluble fraction or vice versa would the

count be affected, otherwise any loss of radioactivity from the fed area as indicated by the G-M detector would be due to the loss of sucrose from the leaf. As reported under 'products of photosynthesis in corn' the amount of ^{14}C in the insoluble fraction did not change significantly after the first 5 minutes and as such would not be an important factor in this method of measuring the rate of translocation. Assuming that the relation between the leaf and the detector is kept constant this should be a valid method for measuring the rate of translocation and the amounts of assimilates moved from the leaf.

Experiments and Results.

Measurements of the rate of translocation and the amounts translocated in corn. The rate of translocation and the total amount of photosynthate translocated were determined in corn by measuring the amount of ^{14}C left in the fed area of the leaf at various times after feeding. The amounts of radioactivity left at various times after feeding were converted to a percentage of the initial amount, and the percentages were plotted against time. The Dent and Flint single cross hybrids as well as the Dent variety Pride V were used. Typical results are shown in Fig. 11A.

Immediately after $^{14}CO_2$ fixation the loss of radioactivity from the fed area was rapid. Over 50% of the ^{14}C was moved out of the fed area in the first 30 minutes. In 3 to 4 hours a total of 80% of the fixed ^{14}C had been moved out of the fed area. Another 5% of the radioactivity was lost from the fed area during the next 20 hours (Fig.11A). These results agree closely with those obtained by Dudko (1963) on corn.

Fig. 11. A. Amount of ^{14}C left in the fed area of a corn leaf at various times after feeding.

B. Distribution of ^{14}C in fed area 6 hours after feeding $^{14}CO_2$.

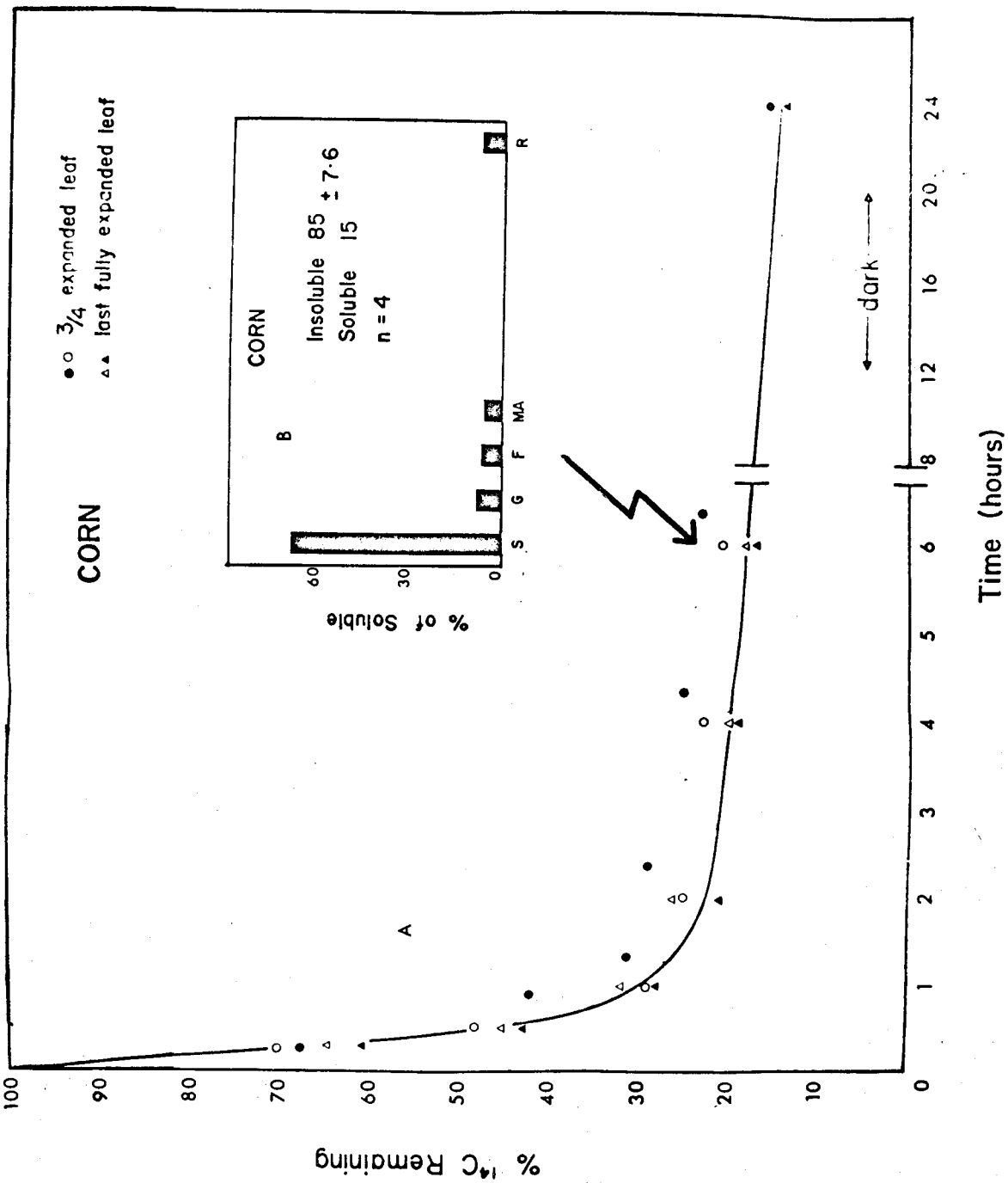
S sucrose

G glucose

F fructose

MA malic acid

R remainder


CORN
● ○ 3/4 expanded leaf
▲ ▲ last fully expanded leaf
A
B
CORN
Insoluble 85 ± 7·6
Soluble 15
n = 4
S G F MA R
% of Soluble
0 30 60
dark
% ¹⁴C Remaining
Time (hours)
0 1 2 3 4 5 6 8 12 16 20 24
10 20 30 40 50 60 70 80 90 100

When the $\log_{10}$ of the amount of ^{14}C remaining in the fed area is plotted against time (Fig. 12) the points fall along 2 intersecting straight lines. The 2 lines intersect at about 80 minutes after feeding. This occurs when the initial high rate of translocation is just dropping off. The analysis of the radioactive compounds show that at this time about 20% of the remaining ^{14}C is still in sucrose and the radioactivity in the insoluble fraction is just beginning to decrease (Fig. 2). When the results plotted in Fig. 2 are recalculated on the basis of the 'loss of ^{14}C from the fed area' curve (Fig. 13) it is noted that the slow rate of translocation after 80 minutes is only slightly faster than the conversion of the insoluble material back to soluble compounds probably mostly sucrose. Thus there is an initial rapid rate of loss which decreases logarithmically, the rate changes to a slower rate after 80 minutes which also decreases logarithmically and which involves the transport of the remaining sucrose in the fed area as well as a conversion and translocation of the ^{14}C stored in the insoluble compounds.

The variability in the rate of loss and the total amount moved, among different corn plants of the same variety and also among the different varieties of corn that were used, was extremely low being in the order of $\pm$ 7%. Thus under optimum conditions the 3 varieties had the same translocation curve. These translocation experiments were performed from 1 1/2 hours after the lights came on in the morning until about 8 to 9 hours from the time of first illumination. Throughout this pretreatment period

Fig. 12. Amount ^{14}C remaining in the fed area of a corn leaf at various times after feeding.

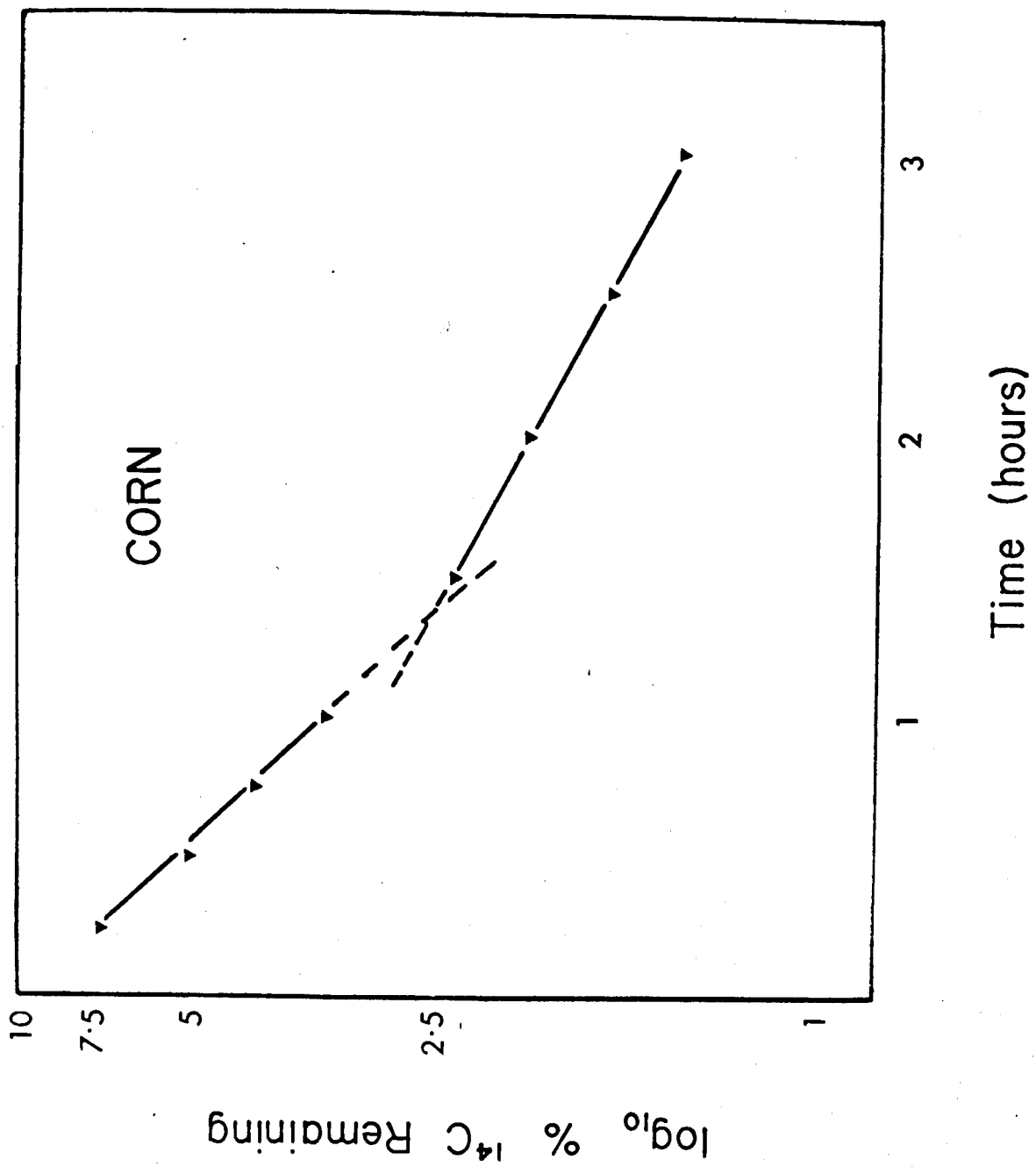
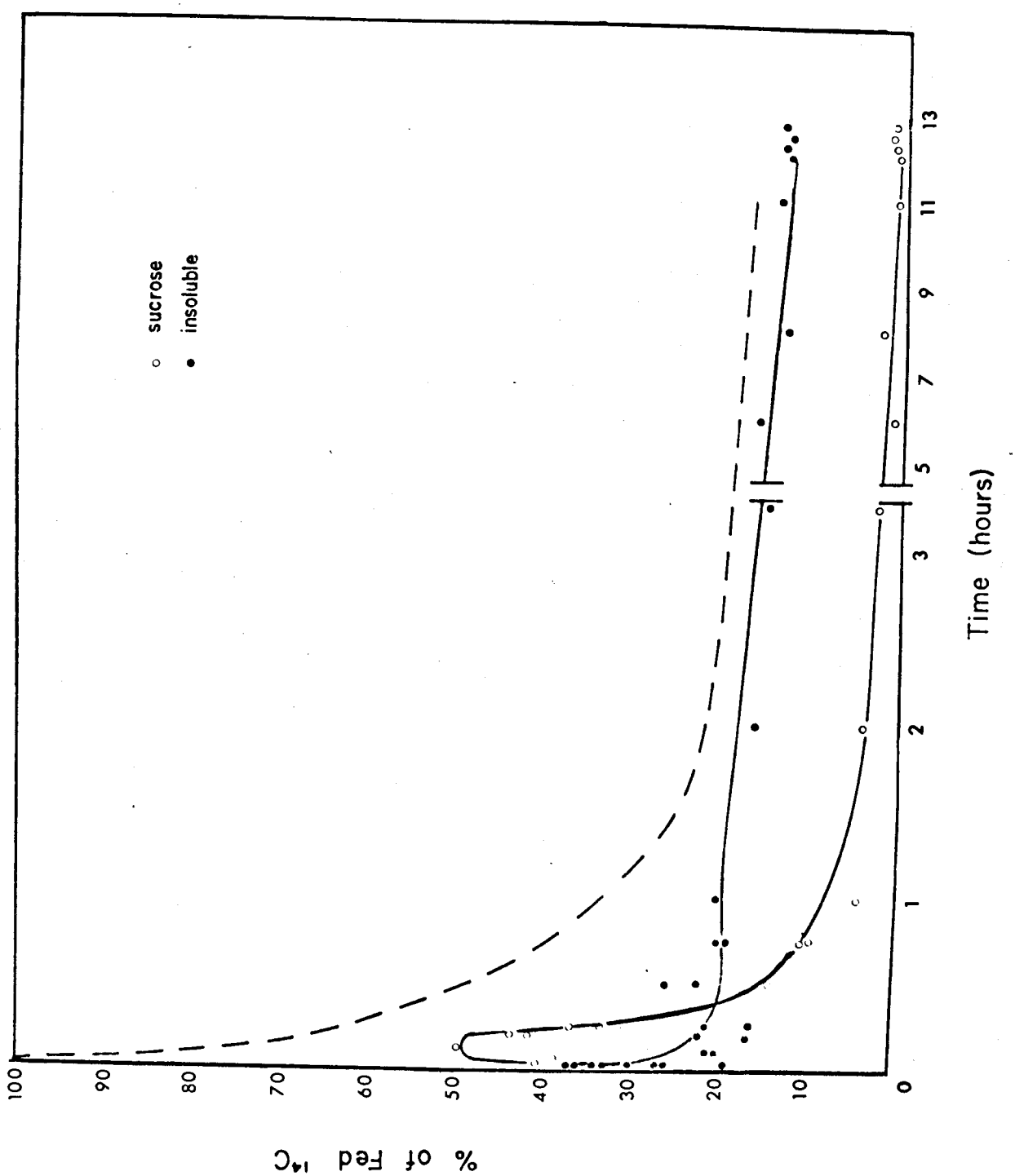

CORN
10
7·5
5
2·5
1
1
2
3
Time (hours)
log10 % 14C Remaining

Fig. 13. Distribution of ^{14}C in sucrose and the ethanol-insoluble fraction of the fed area, expressed as a percentage of the ^{14}C initially present in the fed area, at various times after a 1 min. feeding in $^{14}CO_2$ in the light followed by $^{12}CO_2$ in the light. The dotted line is the amount of ^{14}C left in the fed area superimposed here from Fig. 11 for comparison.


sucrose
insoluble
% of Fed ^{14}C
Time (hours)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
5
7
9
11
13

there were no large differences in the rates of loss i.e. percent translocated in 30 minutes (Table VII). Also the rate of translocation of the ^{14}C was independent of the size of the pulse of ^{14}C fed.

After 6 hours over 80% of the fixed radioactivity had been lost from the fed areas. Of the remaining ^{14}C about 75 to 85% was in the ethanol-insoluble fraction. Of the ethanol-soluble compounds about 70% was still in sucrose, most of the remainder being in glucose and fructose with about 7% in free amino acids (Fig. 11B) Thus the decrease in the rate of translocation corresponds to the disappearance of sucrose from the fed area of the leaf.

From the translocation curve determined by measuring the amount of ^{14}C remaining in the fed area of the leaf 3 characteristics of the translocation process can be assessed. These are the rate at which the ^{14}C is lost from the fed area i.e. the rate of translocation, the total amount of the assimilated ^{14}C moved out, and the length of time required for the bulk of the translocate to be exported.

The validity of the method was checked by comparing the translocation using the rate of loss of ^{14}C from the fed area and direct measurement of the total ^{14}C recovered from the leaf remote from the fed area. In the 2 plants that were checked, 46 and 54% respectively of the total ^{14}C was found outside the fed area. These values are almost identical to the value of $50 \pm 7\%$ obtained by measuring the loss of ^{14}C with the G-M counter.

Table VII. The effect of pulse size and length of pretreatment in the light on the rate of translocation from corn leaves, determined by the percentage of the assimilated ^{14}C left after 30 minutes

Pretreatment in the light (hrs.)	Pulse size (cpm)	% translocated in 30 min.
1 1/2	37,000	50
2	11,000	57
2 1/2	12,500	56
3	45,000	52
3 1/2	5,000	48
4	9,000	53
5	75,000	53
6	25,000	44
7	7,000	47
8	20,000	55
9	30,000	52

The possibility of using the above method to study the effects of different factors on the 3 characteristics of translocation was investigated. For this study the effects of temperature and darkness on translocation were chosen as the environmental factors, since in the literature these factors have been emphasized as being important in translocation. As a third factor the effect of the ontogenetic stage of the leaf on the translocation from that leaf was investigated.

The effect of temperature on translocation. For this study the corn plants were placed in the growth chambers at the desired temperatures 1 hour before feeding $^{14}CO_2$ to a narrow segment of leaf. The amount of ^{14}C remaining in the leaves was measured for a period of 4 hours at temperatures ranging from 7 to 26°. The experiment was repeated at least 20 times at each temperature. The averages of the results are plotted in Fig. 14.

A decrease in temperature below 26° decreased both the rate of translocation as well as the total amount of photosynthate moved out of the fed area of the leaf. The effect of temperature on translocation was much the same in both the Flint and the Dent corn. The differences among the varieties was much less than the variability within any variety. The variability between different feedings increased with a decrease in temperature and was in the order of 15 to 20% at the lowest temperature.

When the $\log_{10}$ of the remaining ^{14}C in the leaf is plotted against time the low temperature curves also become 2 intersecting straight lines (Fig. 15). The time at which the break in the curve

Fig. 14. Effect of temperature on the amount of ^{14}C left in the fed area of leaves of Dent and Flint corn at various times after feeding.

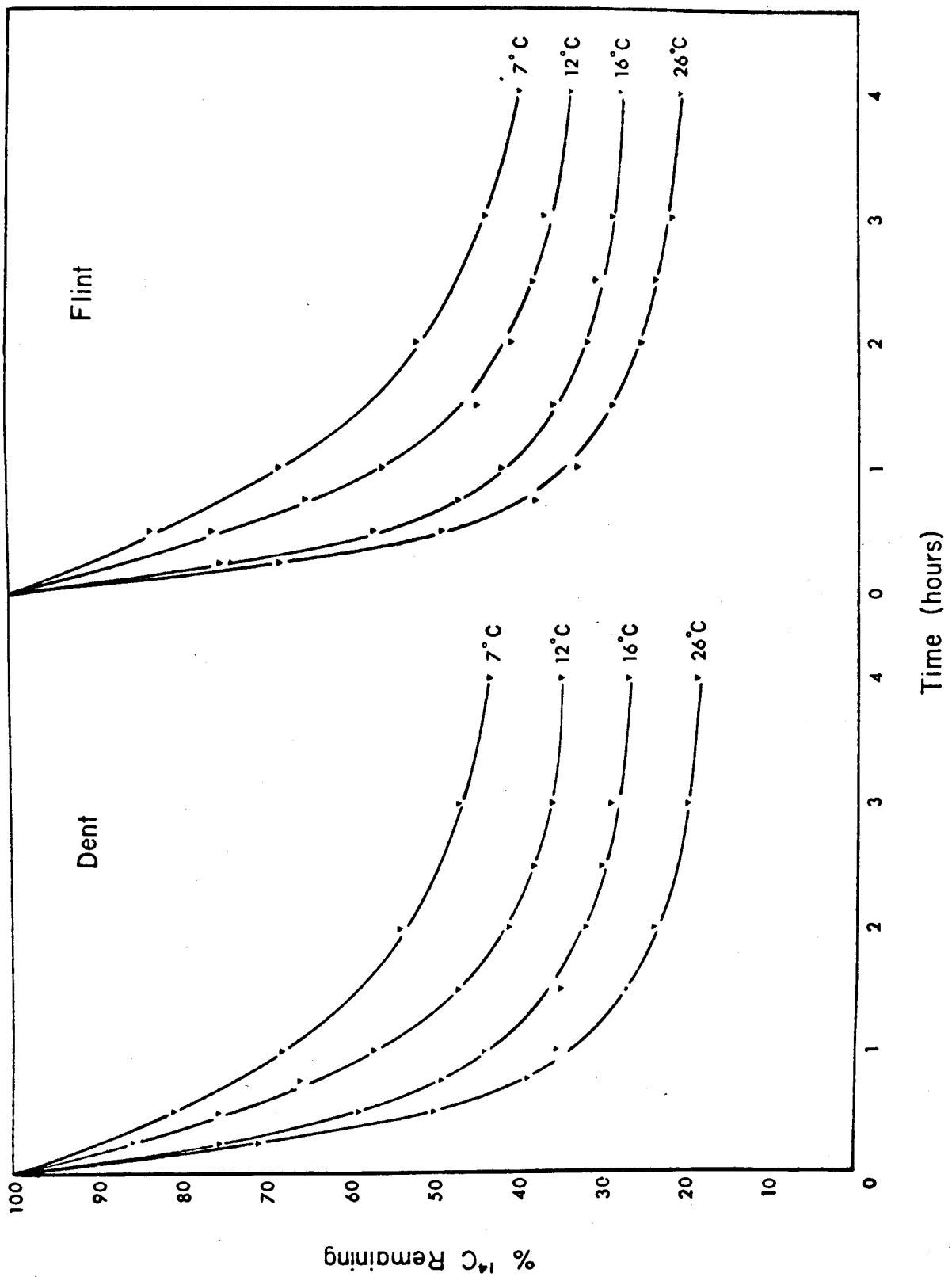
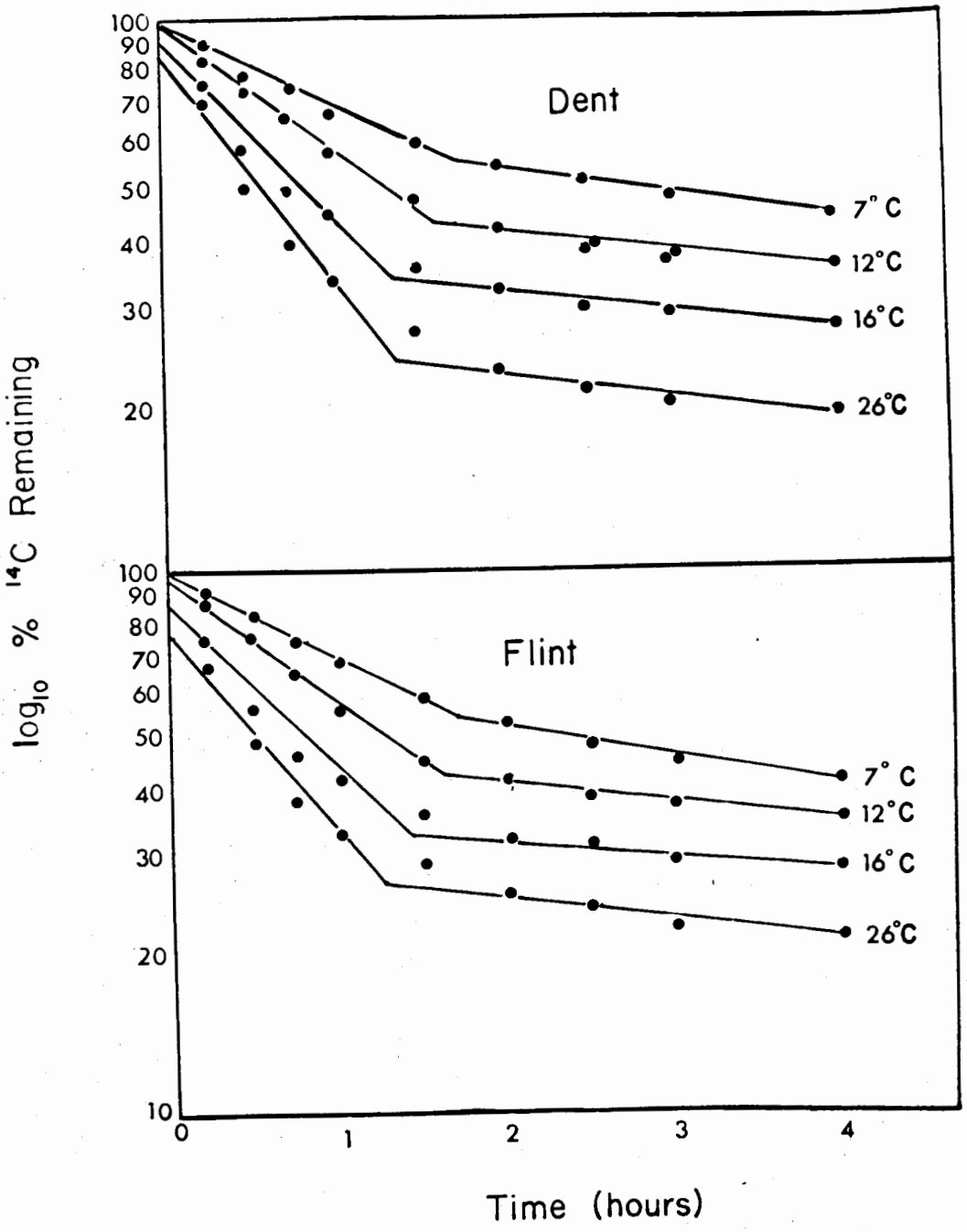

Flint
Dent
7°C
12°C
16°C
26°C
% ^{14}C Remaining
Time (hours)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4

Fig. 15. Effect of temperature on the amount of ^{14}C left in the fed area of leaves of Dent and Flint corn at various times after feeding.


Dent
Flint
7° C
12°C
16°C
26°C
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
log10 % 14C Remaining
Time (hours)

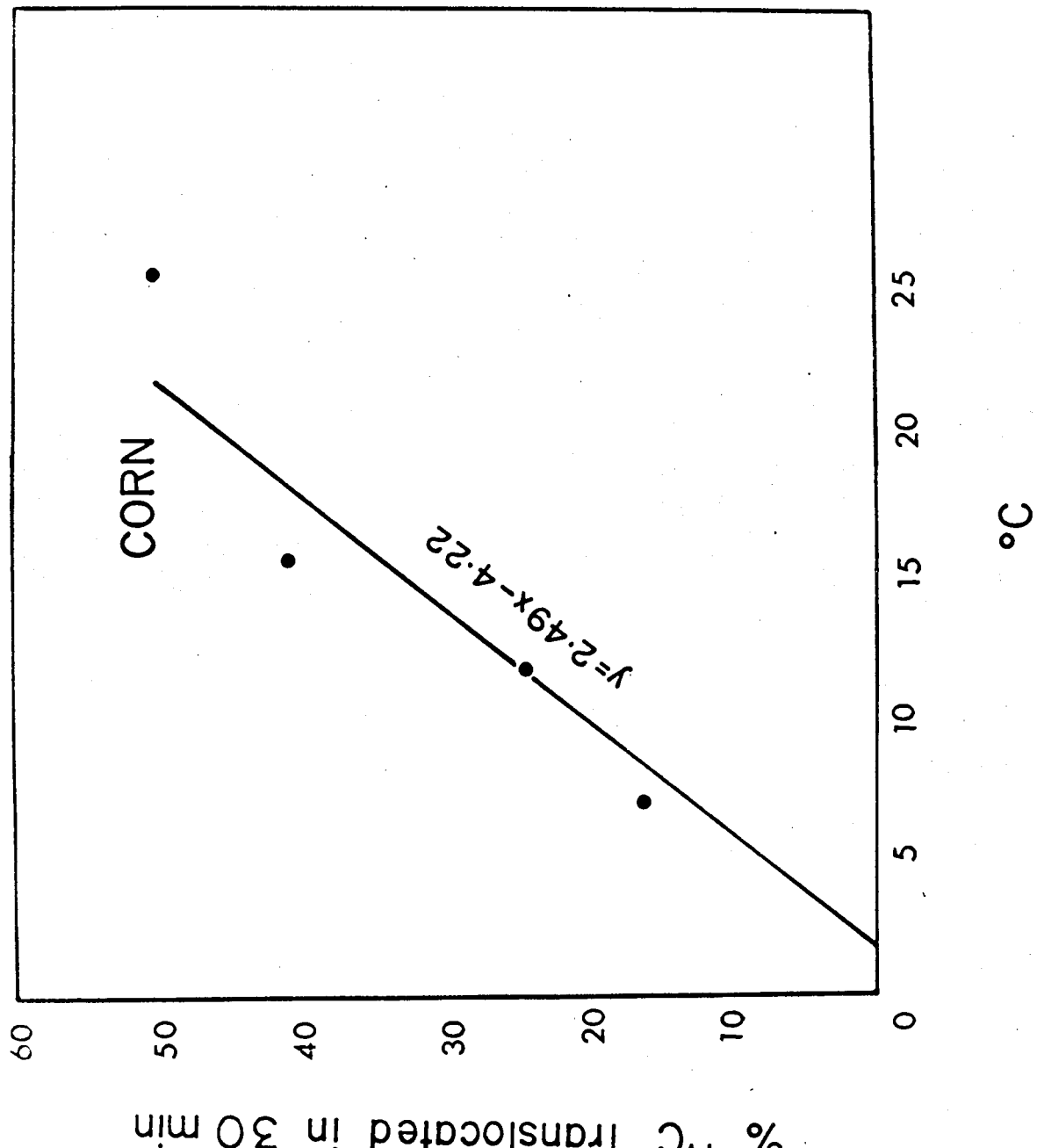
occurs becomes progressively later as the temperature decreases. But again there was little difference between the varieties.

When the percentage of the fixed ^{14}C moved out during the first 30 minutes is taken as the rate of translocation the rate of translocation decreased more or less linearly with a decrease in temperature between 7 and 26° (Fig. 16).

In Fig. 15 all the straight lines drawn through the points do not pass through the 100% point. At the higher temperatures where it is known that there is a substantial shift of ^{14}C from the insoluble fraction to the soluble this discrepancy is more pronounced. At the lower temperatures the lines do pass through the 100% point suggesting that there is no shift from the insoluble to the soluble fraction. These discrepancies have not been further investigated. Another explanation of this discrepancy at higher temperatures is the possibility that a small amount of material is being moved out via the phenomenon of rapid translocation described by Nelson et al. (1959).

The effect of darkening the leaf blade on translocation. It has been reported that translocation from the leaf in sugarcane is under the control of light (Hartt et al. 1964, Hartt 1965, Hartt and Kortschak 1967). A similar suggestion also comes from the work of Bilenko et al. (1964) with corn. An experiment was set up to determine whether measurements of the rate of loss of ^{14}C from the leaf in the dark would give any indication of the control of translocation by light.

Fig. 16. Effect of temperature on the rate of translocation from the fed areas of corn leaves.

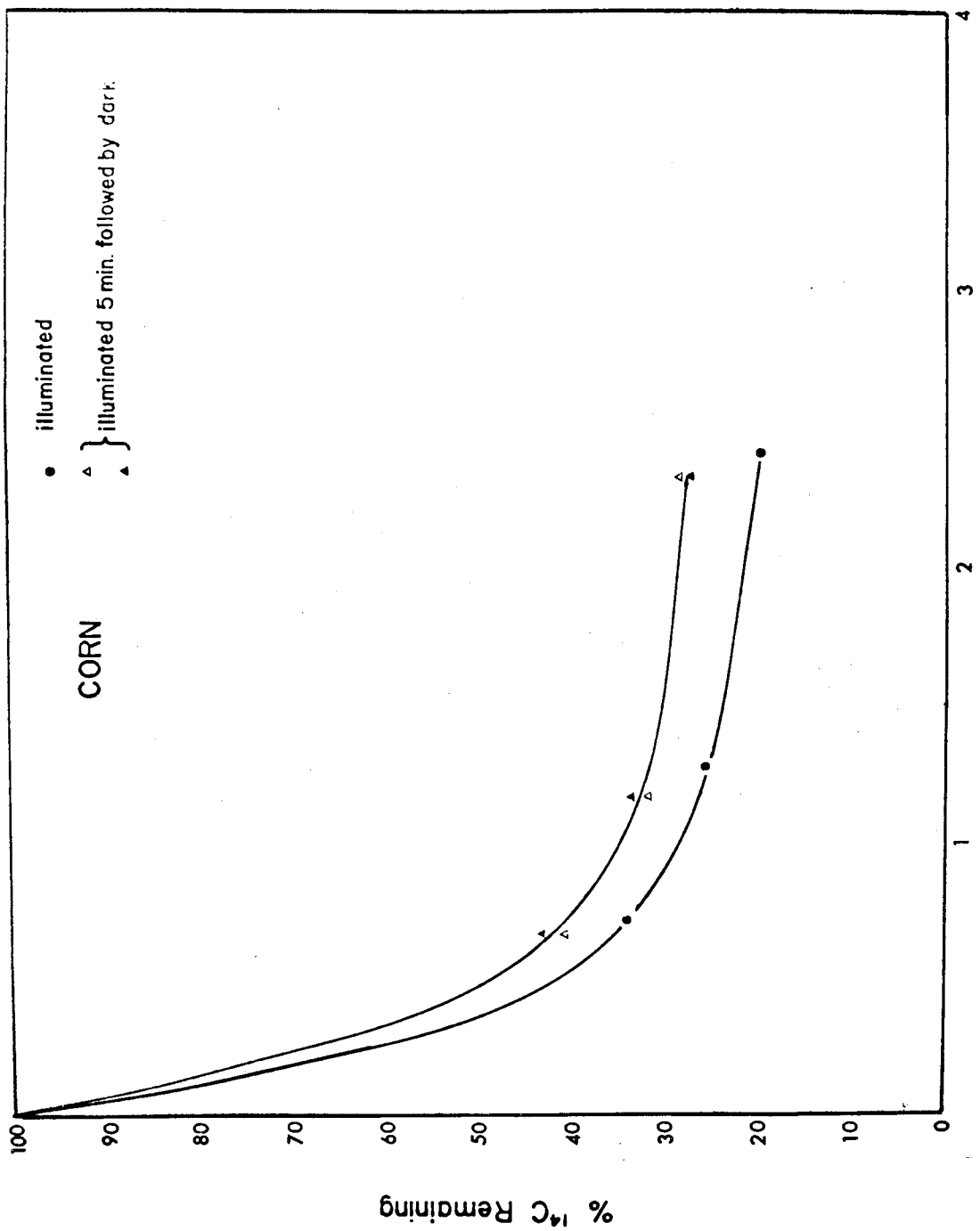

% ^{14}C Translocated in 30 min
60
50
40
30
20
10
0
5
10
15
20
25
°C
CORN
y=2.49x-4.22

Sections of corn leaf were fed $^{14}CO_2$ for 2 minutes, then the leaves were left in the light for an additional 3 minutes to ensure that most of the radioactivity was in sucrose. It has been reported by several workers that the fixed ^{14}C does not go to sucrose if the leaf is placed in the dark immediately after exposure to $^{14}CO_2$ (Vernon & Aranoff 1952, Burma & Mortimer 1957, Hatch & Slack 1966). The leaves were then put in darkness by covering them with sleeves made from aluminum foil. The rest of the plant was left in the light so that any effect of darkness would be on the exporting leaf blade itself.

The rates of translocation were not significantly different in the leaves left in the dark or in the light (Fig. 17). The total amount translocated was about 10% less for the leaves placed in the dark. The smaller percentage moved was probably due to the fact that the transfer of the ^{14}C into sucrose had not been complete by the time the leaf was placed in the dark. This method of measuring the translocation of sucrose indicated that light did not stimulate translocation as has been reported by other workers.

Translocation in various age corn leaves. Translocation studies were carried out on corn leaves of various ages, to determine whether the rate of translocation and the amount translocated changes as the leaf becomes older. For these experiments, plants were selected that had 8 leaves in various stages of development. The oldest leaf was beginning to yellow at the leaf tip and the sheath was split in 2 by the expanding stem. Leaves 6, 7 and 8

Fig. 17. Effect of dark on the amount of ^{14}C left in the fed area of a corn leaf at various times after feeding.


CORN
illuminated
illuminated 5 min. followed by dark.
% ¹⁴C Remaining
Time (hours)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4

were fully expanded and did not show signs of senescence. On the 4th leaf the sheath was about half expanded and on leaf 3 the blade was over half expanded. Leaves 2 and 1 were only partially grown.

Sections near the tips of the leaves were fed $^{14}CO_2$ for 1 minute and the activity remaining in the assimilating areas was measured over a 4 hour period. The results are shown in Fig. 18 and 19.

The developmental stage of the leaf had a very definite effect on the pattern of translocation from the leaf. The tips of the leaves (fed areas) were able to translocate at the fastest rate and were able to translocate the most photosynthate from the time the leaf blade was about 3/4 expanded to the time the leaf sheath on the leaf was fully expanded. Both younger and older leaves translocated more slowly and they also translocated a smaller percentage of the fixed ^{14}C during the 4 hours .

It is clear from these experiments that the stage of development of the corn leaf is an important factor in translocation of sucrose from that leaf.

Discussion

When a section of leaf is exposed to $^{14}CO_2$ the ^{14}C is incorporated into a number of different compounds but most of it goes into sucrose, the only compound translocated from the corn leaf. The ^{14}C in the fed area of the leaf can be measured by means of a G-M detector. The ^{14}C that has been incorporated into sucrose is translocated from the assimilating area, and the rate at which the ^{14}C is lost from this area can be followed by the G-M detector.

Fig. 18. Initial rate of ^{14}C translocation out of fed areas of corn leaves of different ages on 2 month old plants.

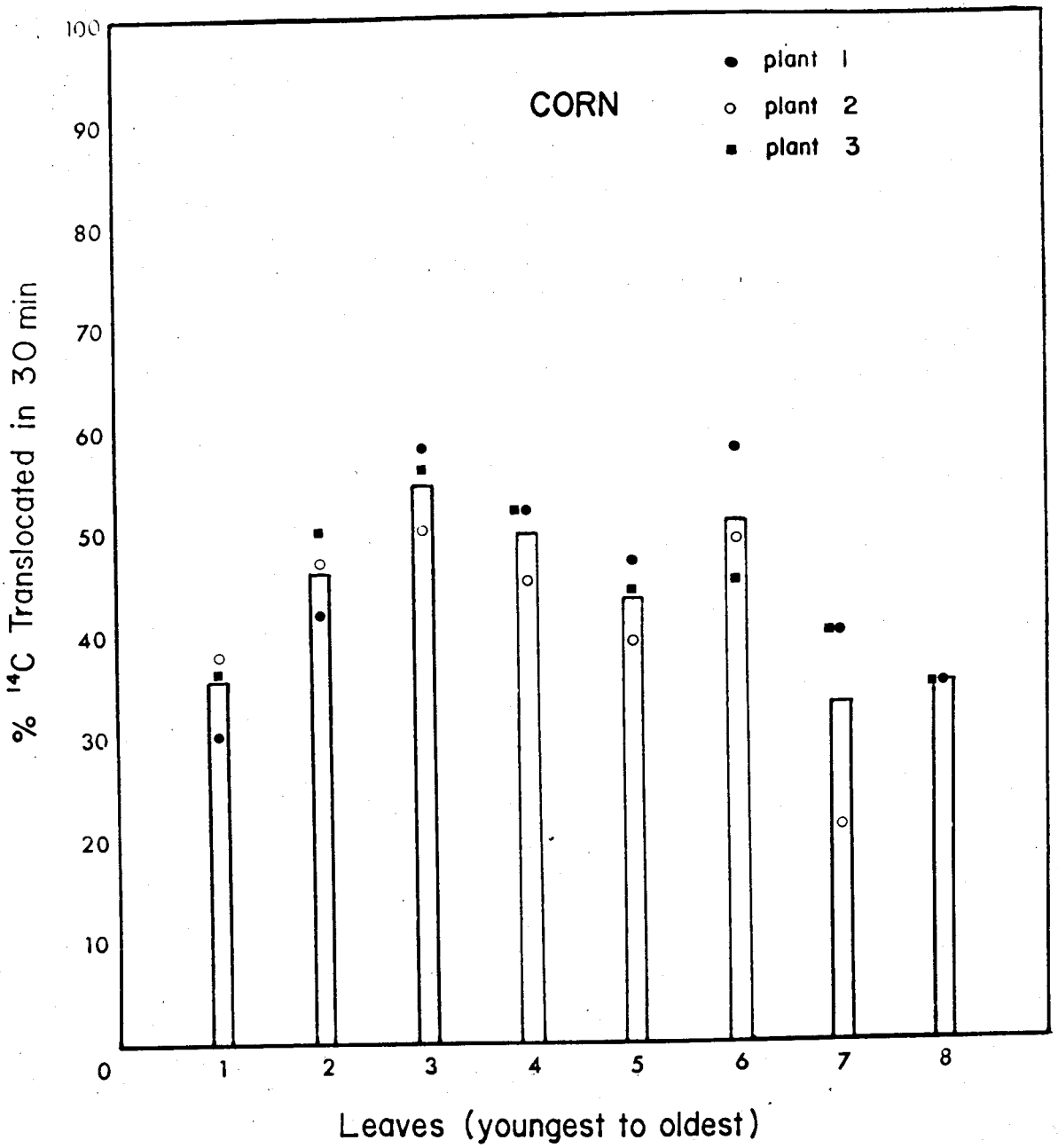
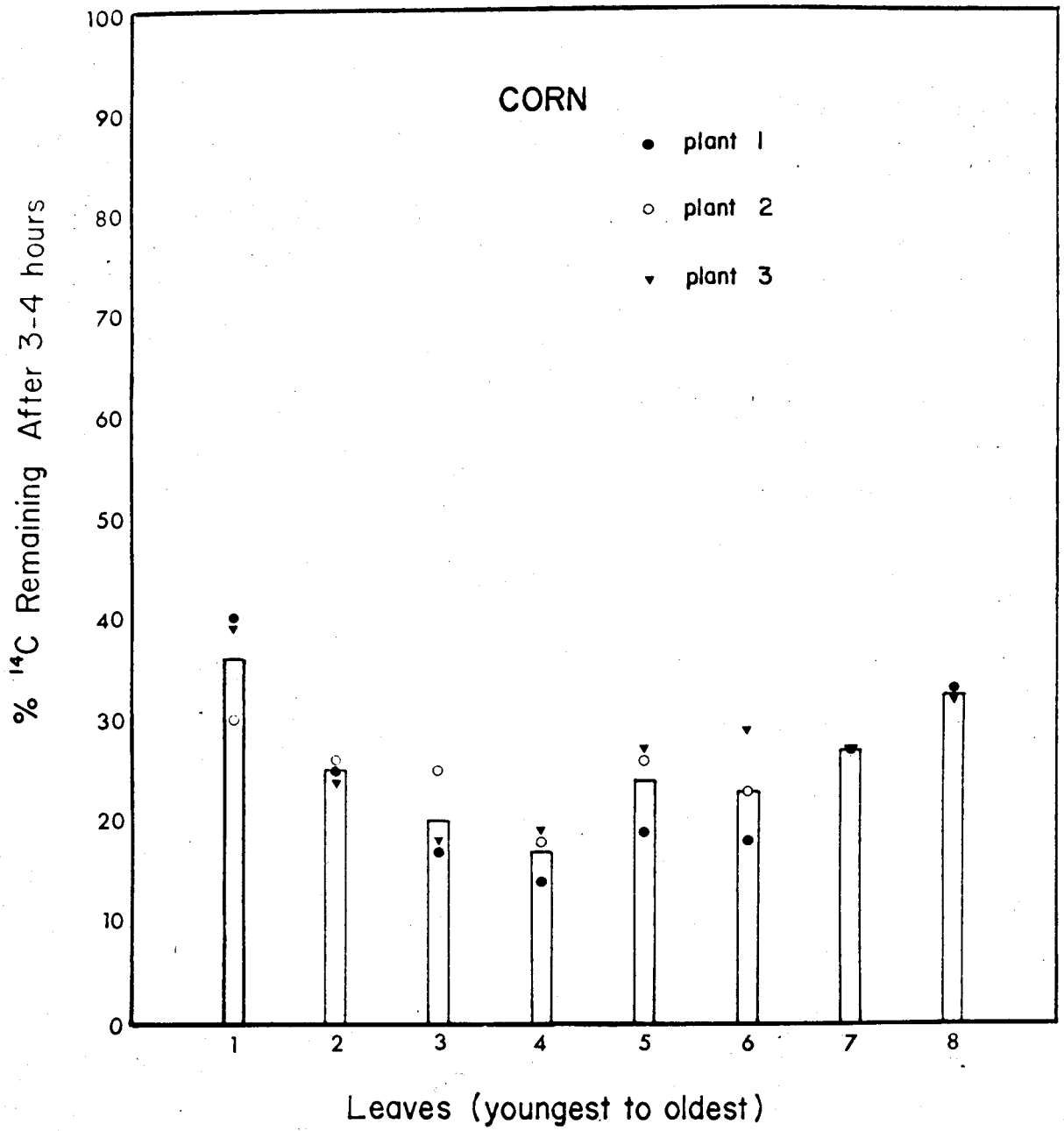

CORN
plant 1
plant 2
plant 3
% ¹⁴C Translocated in 30 min
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
Leaves (youngest to oldest)

Fig. 19. Amount of ^{14}C left in the fed areas of corn leaves of different ages on 2 month old plants, 4 hours after feeding.


CORN
plant 1
plant 2
plant 3
% ¹⁴C Remaining After 3-4 hours
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
Leaves (youngest to oldest)

When the amount of ^{14}C remaining in the fed area (expressed as a percentage of the initial amount) is plotted against time the points fall along a curve which has the form of a decay curve. When the same results are plotted on a semi-log plot a point of inflection in the curve becomes apparent. From these 2 plots several pieces of information can be gained about translocation from the leaf. The semi-log plot provides a measure of the relative rate of loss of ^{14}C which is in reality the relative rate of loss of sucrose. The rate of loss is made up of 2 components: a rapid rate and a slow rate. During the rapid rate of loss most of the sucrose is translocated from the fed area. The slow rate must also represent a loss of sucrose-^{14}C from the leaf. Although other compounds such as, glucose, fructose and malic acid are found in the fed area there is no evidence that these are translocated. The amount of sucrose-^{14}C left in the fed area at the time the rate of loss of ^{14}C changes cannot account for the amount subsequently lost. The amount of ^{14}C in the insoluble fraction is decreasing during the slow rate of loss. It appears that the slow rate of translocation involves the sucrose still present in the fed area, but also ^{14}C that is being converted from other compounds into sucrose.

The rate of loss of ^{14}C from the fed area, as a percentage of the initial amount, was found to be independent of the size of the pulse (Table VII). As a result, the slope of the rate of loss curve and the time at which the point of inflection in the curve occurs do not change as long as the conditions are kept constant.

This phenomenon provides 2 important pieces of information. First, the pulse size need not be carefully controlled in the feeding and the slope of the line can be used to provide a measure of the effect of different factors on the rate of translocation from the leaf. Secondly, this phenomenon indicates several things about the process of translocation from the leaf. It shows that after the pulse label the ^{14}C is not simply diluted out by the photosynthate that follows as is suggested by the logarithmic nature of the rate of loss curve. If the translocation from the leaf were a dilution or flushing process by new photosynthate the length of time required for the sucrose-^{14}C to be exported would be dependent on the pulse size. Another important conclusion that can be drawn from the fact that the rate of loss is independent of the pulse size is that the sucrose-^{14}C mixes evenly with the endogenous sucrose in the leaf.

The amount of endogenous sucrose in the leaf is unknown. The measure of the amount of ^{14}C in the leaf is only a relative measurement. The counting efficiency of the ^{14}C in the leaf by means of the G-M detector was not calculated. Because of these 2 unknowns the specific activity of the translocated sucrose is not known. Therefore, the measure of the rate of loss of ^{14}C from the leaf only provides a measure of the relative rate of translocation of sucrose from the leaf, and does not give a quantitative measure of the translocation rate.

The linear plot provides a measure of the percentage of the assimilates that are held back. This can be determined from the point at which the curve levels off. In other words it provides

a measure of the availability of the assimilate for translocation. In corn about 90% of the assimilated ^{14}C is translocated from the leaf. From this measurement and a measure of the rate of photosynthesis we can calculate the actual rate of translocation from the leaf. In corn the rate of CO_2 fixation at 2600 ft-c and 26° was found to be 560 ugm of CO_2 dm^{-2} min^{-1} (Table III). This gives a rate of translocation from the leaf of $560 \times \frac{90}{100} \times \frac{342}{12 \times 44} = 327$ ugm of sucrose dm^{-2} min^{-1}. This rate is about 2 and one-half times higher than the one calculated for sugar beet (Geiger & Swanson 1965b) using an entirely different method.

As was mentioned earlier the rate of loss of ^{14}C from the fed area consists of 2 components. The first component, the rapid rate of loss, involves the translocation of about 75% of the assimilated ^{14}C. This 75% of the ^{14}C must be more readily available for translocation than the remaining ^{14}C, even though some of it is still in sucrose. This indicates that the sucrose in the leaf is in at least 2 separate pools or that the sucrose in the leaf is compartmentalzed. The sucrose which is moved out rapidly and which is readily available for translocation is said to be in the translocation pool, the remainder is said to be in the storage pool. The term pool is used here to denote a quantity of material and has no cytological meaning.

At 26° the sucrose in the translocation pool is moved out of the assimilating area in 80 minutes or in other words at 26°, 80 minutes are required for the sucrose in the translocation pool to be completely replaced by newly synthesized sucrose. This time 80 minutes at 26° will be called the translocation pool turn-

over time where the expression 'turnover time' is used as 'the time required for sucrose to move through the pool'. The turnover time of about 80 minutes is almost identical to the turnover time of 84 ± 8.6 minutes calculated for sugar beet (Geiger & Swanson 1965b).

During the turnover time of about 80 minutes 75% of the assimilated ^{14}C went into and out of the translocation pool. This gives a rate of $560 \times \frac{75}{100} \times \frac{342}{12 \times 44} = 272$ µgm of sucrose dm^{-2} min^{-1} moving into and out of the pool. For the duration of the experiment the rates of assimilation and translocation did not change and therefore, the rates of movement into and out of the pool must have been constant. From the rates of movement from the pool and the time required for the pulse to move through the pool a pool size of $272 \times 80 = 21760$ µgm of sucrose dm^{-2} of leaf area is calculated.

A change in temperature affected all 3 characteristics of translocation. Fig. 16 shows that the initial high rate of translocation decreases as the temperature decreases from 26 to 7°. This indicates that the rate of translocation of sucrose is under physiological control and is not simply a diffusion process. A decrease in the temperature from 26 to 7° increases the turnover time of the translocation pool from about 80 to 100 minutes, and decreases the percentage of the assimilates that go into the pool, or conversely the amount of assimilates remaining in the fed area is increased. The one process which was not affected by temperature was the mobilization of the assimilates that did not go directly to the translocation pool. This is indicated by the parallel nature

of the lines on the log rate plot. This lack of sensitivity to temperature of this process could be due to the fact that any enzymes that may be involved are not affected by a change in temperature, or that this process is mostly a diffusion process involving the sucrose not in the translocation pool. This slow rate of translocation decreases logarithmically which does suggest a diffusion process. The amount of ^{14}C left in sucrose and other compounds at the lower temperatures was not investigated and therefore the amount of enzymatic conversion involved is not known.

There were no significant differences between the effects of temperature on translocation in the Dent and Flint hybrids and therefore any difference in resistance to low temperatures between these hybrids is not in the 3 characteristics of translocation studied.

Another factor that was studied in relation to translocation was light. In corn there was little effect of darkening the leaf blade on the translocation from the fed area. The small differences that were noted could in part be due to an incomplete incorporation of most of the ^{14}C into sucrose or due to a slight drc in temperature that may have resulted from darkening the leaf. From these experiments there was no evidence that translocation was ligh dependent as has been suggested by Hartt (1965c), Hartt and Kortschak (1967) and Hartt et al. (1964) from work with sugarcane. Indications from my results with corn are that light was only involved in the formation of the photosynthate. Bilenko et al. (1964) on the other hand found a direct effect of light on translocation to the roots

in corn, but they did not measure the actual translocation from the leaves, but only translocation from the whole top.

The method of measuring the amount of ^{14}C left in the fed area of a leaf can also be used to study the effect of internal conditions, on translocation from the leaf. Both the initial rate of loss of ^{14}C and the total amount of the assimilates translocated changed with the physiological maturity of the leaf. Not enough data was obtained to determine whether there was any effect on the turnover time of the translocation pool. In corn, young expanding leaves do not translocate as much of their assimilates nor is the rate of loss as rapid as that from leaves that are about fully expanded. Leaves that were between 3/4 and fully expanded had the highest relative rate of translocation and translocated the greatest percentage of the assimilated carbon. In the older leaves the relative rate decreased with age and the amount of carbon retained in the leaf increased. These results indicate that leaves that are about fully expanded are the most efficient in translocation and also that the leaves operate at maximum efficiency for only a relatively short time.

Both temperature and stage of maturity affect the relative rate of translocation. This drop in rate from the maximum was in all cases accompanied by an increase in the amount of the assimilates remaining in the leaf. This suggests that the effect of temperature and age is primarily on translocation out of the assimilating cells and that the assimilation rate is not affected as much. The results from Part I show that the rate of sucrose production was relatively

unaffected by a change in temperature, and thus sucrose production would not be a major factor in the temperature and age effects on translocation.

From the studies on photosynthesis and translocation it appears that some part of the translocation process is controlling the loss of ^{14}C from the leaf and not photosynthesis.

A Comparative Study of Translocation

Introduction.

Species differ widely in their ability to mobilize and translocate their assimilates. Sugarcane is able to translocate about 80% of an initial pulse of assimilated ^{14}C in 4 hours (Hartt and Kortschak 1967), sugar beet translocates about 60% in 3 hours (Mortimer 1965), soybean about 30% in 12 hours (Clauss et al.1964), tobacco about 22% in 5 1/2 hours (M. Shiroya et al.1961) and pine seedlings about 15% in 7 hours (T. Shiroya et al.1962). Corn is able to translocate about 80% in 2 1/2 hours (Fig. 11A).

The method of measuring translocation by determining the loss of a pulse of photosynthetically assimilated ^{14}C from a leaf was used in a comparative study of the leaves of 8 species.

Experiments and Results.

Plant species were chosen for which seed could be readily obtained and which could be readily grown in the greenhouse.

Table VIII. Developmental Stage of the Plants.

Millet	8 to 12 leaf stage, mature blades 20 to 30 cm long
Sorghum	6 to 8 leaf stage, mature leaves 20 to 30 cm long
Sunflower	6 to 8 leaves fully expanded
Radish	Rosette stage with 3 fully expanded leaves
Castor bean	3 fully expanded leaves
Soybean	1 to 2 trifoliate leaves fully expanded
Nicotiana	12 to 15 leaves, beginning to flower
Tomato	5 fully expanded leaves

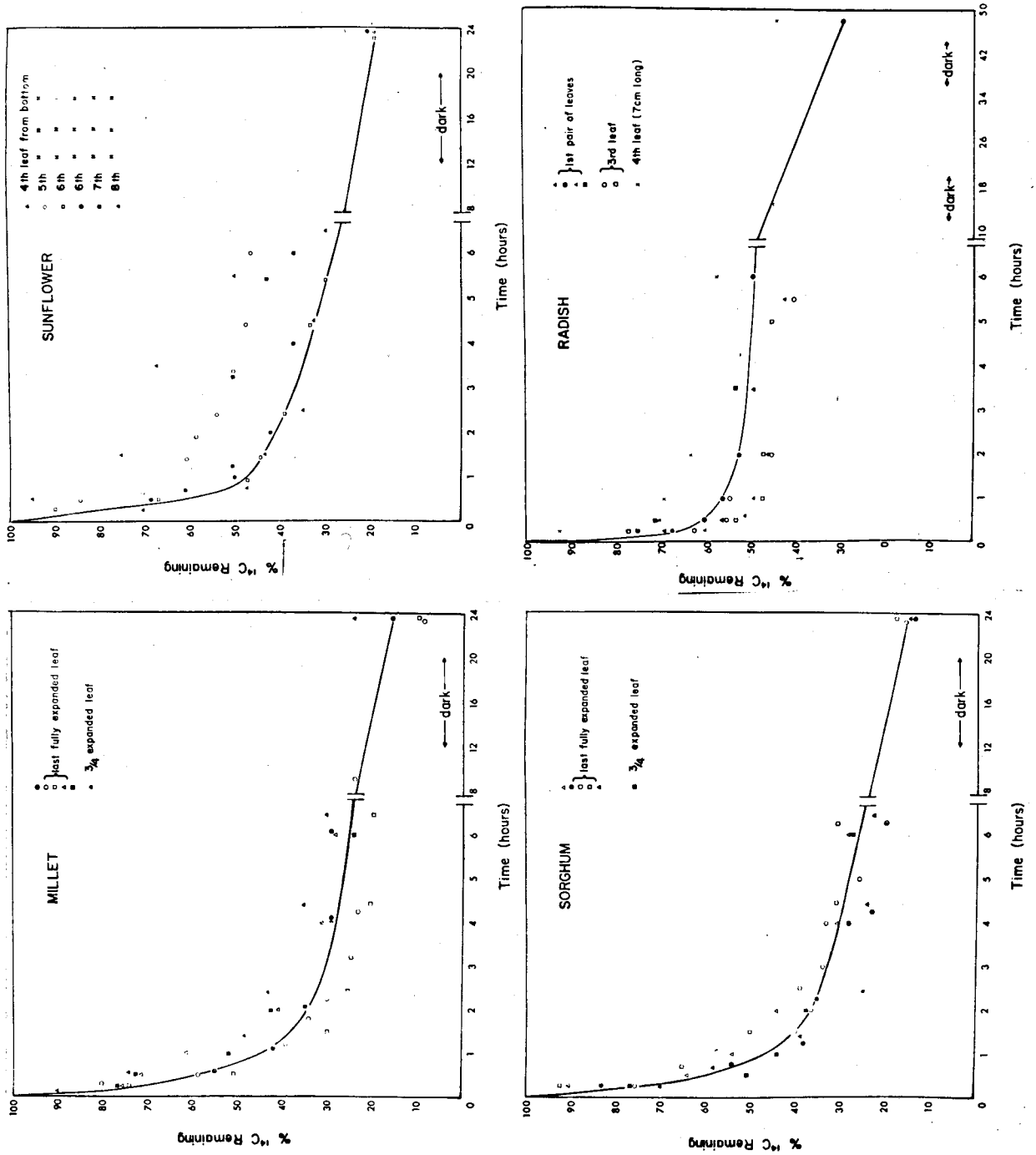
A section of leaf, 3 mm wide, on each plant was fed $^{14}CO_2$ for 3 minutes. The loss of radioactivity from the fed area was measured at various time intervals after feeding. When little additional radioactivity was being moved from the fed areas they were extracted in boiling 80% ethanol. For most species this was done 6 hours after feeding, except for radish and castor bean which were done after 1 hour. The percentage of activity in the ethanol-soluble and insoluble fractions was determined and the compounds in the ethanol-soluble fraction analyzed by paper chromatography and autoradiography.

For the tomato plants the loss of ^{14}C from the leaves was measured as well as the amount translocated after darkening either the fed leaf, the whole plant except the fed leaf, or the whole plant including the fed leaf.

For most of the species a study of translocation in leaves at different stages of development was included. A minimum of 2 replicates was done for each stage of development.

Comparison of 3 parameters of translocation in different species. The amount of ^{14}C left in the fed areas of the leaves of different species is summarized in Fig. 20. Curves are drawn for the mean values of leaves just after full expansion had been reached. The rates of loss of ^{14}C decreased logarithmically for all the species studied as is indicated when the log percentage ^{14}C remaining is plotted against time (Fig. 21). As was found in corn the rates of loss were made up of 2 components,

Fig. 20. Amount of ^{14}C left in the fed area of leaves of different species at various times after feeding.


SUNFLOWER
4th leaf from bottom
5th " " "
6th " " "
6th " " "
7th " " "
8th " " "
dark
% 14C Remaining
Time (hours)
RADISH
1st pair of leaves
3rd leaf
4th leaf (7cm long)
dark
dark
% 14C Remaining
Time (hours)
MILLET
last fully expanded leaf
3/4 expanded leaf
dark
% 14C Remaining
Time (hours)
SORGHUM
last fully expanded leaf
3/4 expanded leaf
dark
% 14C Remaining
Time (hours)

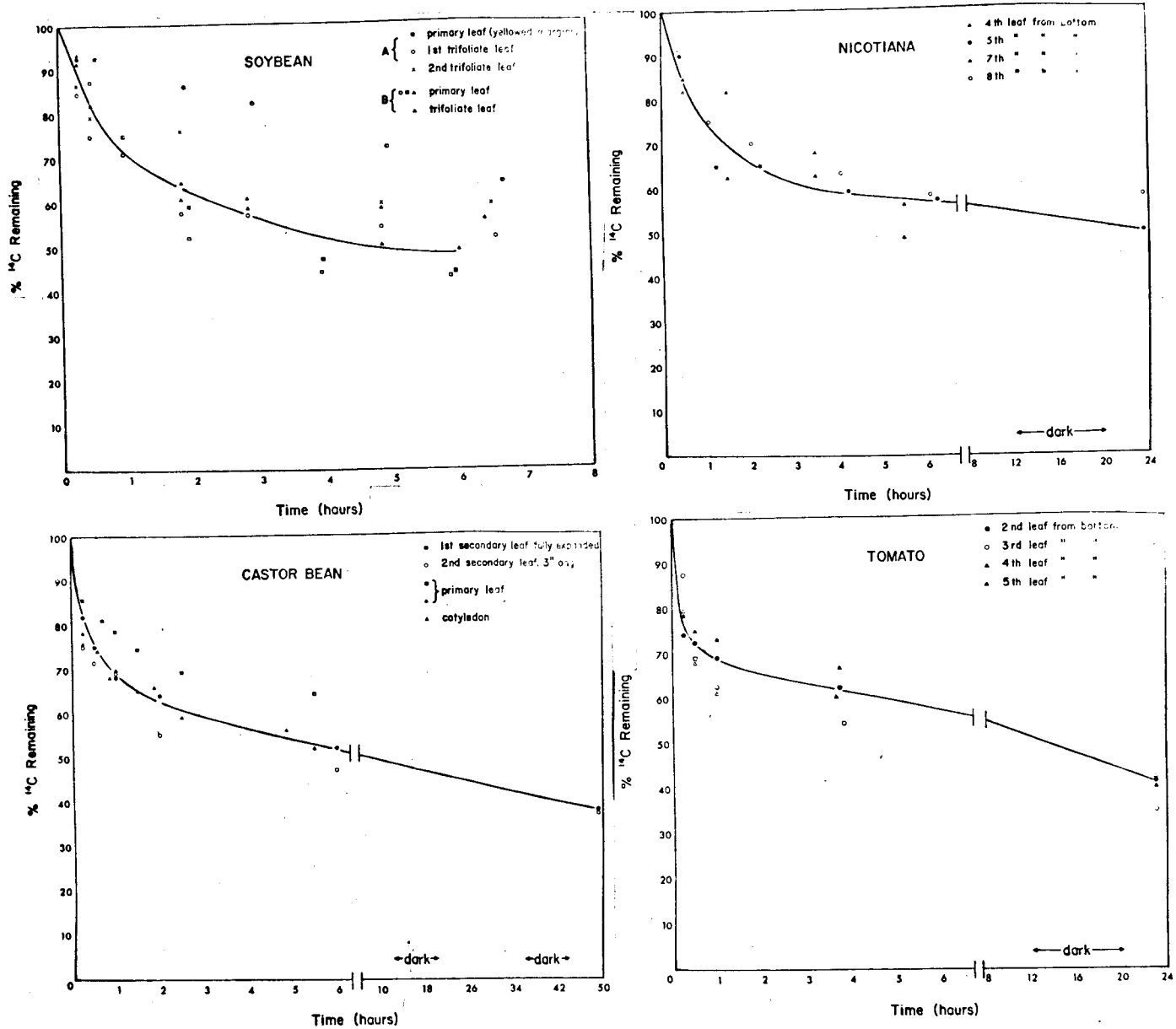
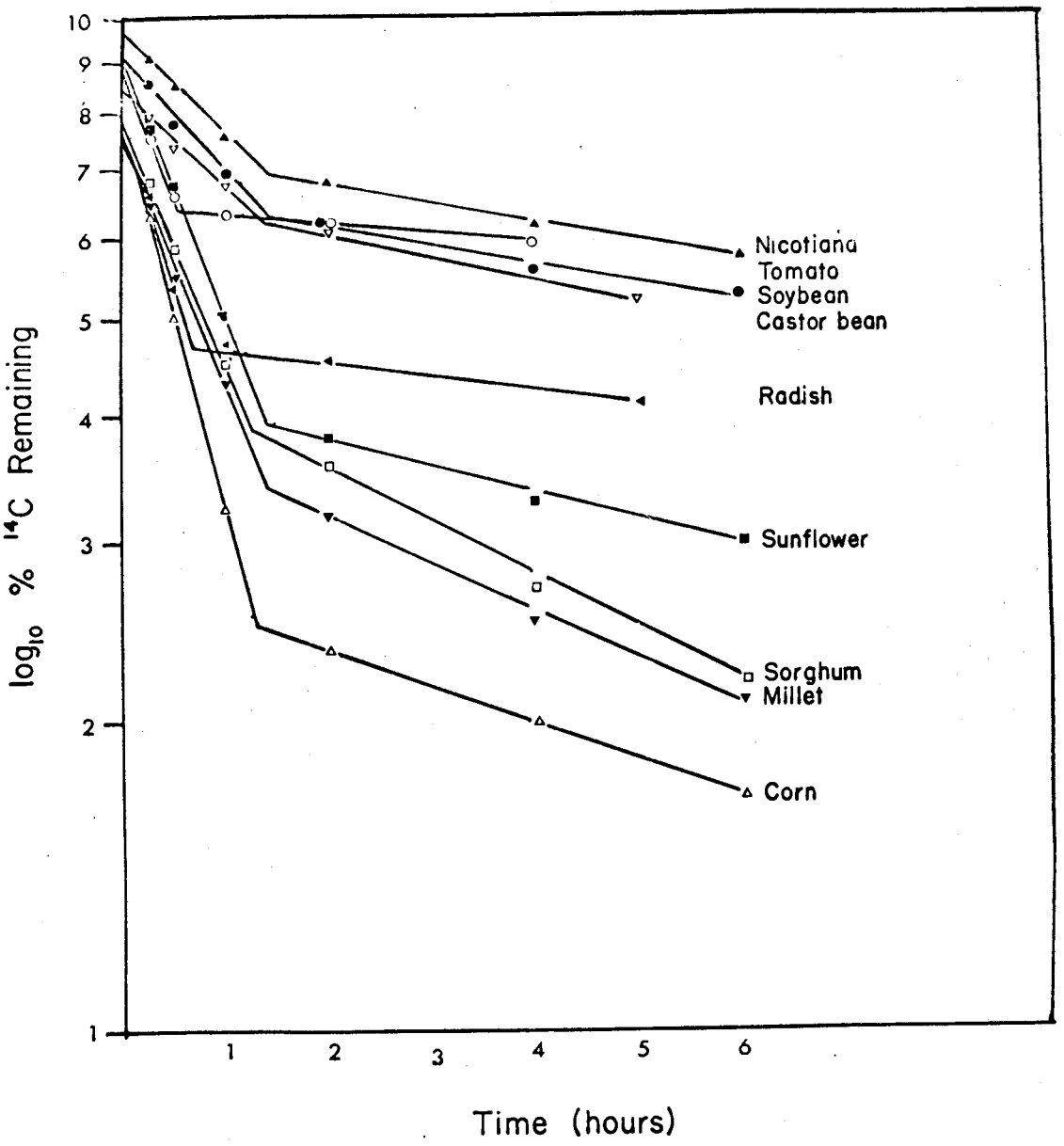

SOYBEAN
A
primary leaf (yellowed margins)
1st trifoliate leaf
2nd trifoliate leaf
B
primary leaf
trifoliate leaf
% ¹⁴C Remaining
Time (hours)
NICOTIANA
4th leaf from bottom
5th " " "
7th " " "
8th " " "
dark
% ¹⁴C Remaining
Time (hours)
CASTOR BEAN
1st secondary leaf fully expanded
2nd secondary leaf, 3" long
primary leaf
cotyledon
dark
dark
% ¹⁴C Remaining
Time (hours)
TOMATO
2nd leaf from bottom
3rd leaf " "
4th leaf " "
5th leaf " "
dark
% ¹⁴C Remaining
Time (hours)

Fig. 21. Amount of ^{14}C left in the fed area of leaves of different species at various times after feeding.


10
9
8
7
6
5
4
3
2
1
log10 % 14C Remaining
1
2
3
4
5
6
Time (hours)
Nicotiana
Tomato
Soybean
Castor bean
Radish
Sunflower
Sorghum
Millet
Corn

a rapid rate of loss lasting for about 80 minutes followed by a slower rate. The rapid rate of loss varied considerably from species to species, and in all the species was slower than the rate found for corn. After corn, radish had the next most rapid rate, followed by millet, sorghum, sunflower and tomato. Soybean, castor bean and nicotiana had much slower rates of loss during the first 80 minutes. The subsequent slower rate of loss did not follow the pattern from species to species as the rapid rate, and did not vary as much as the more rapid rate.

The amount of the assimilates retained by the leaves of different species after 24 hours varied from about 12% in corn to about 50% in nicotiana. Millet and sorghum were close to corn in retaining about 15%. Most of the other species retained between 30 to 40% of their assimilates.

The time at which the breaking point occurred in the curve (Fig. 21) divided the plants into two groups. This breaking point is the turnover time of the translocation pool and was the same in most of the species studied in that it occurred from 75 to 85 minutes after the pulse label. This is the same time found for corn and sugar beet (Geiger and Swanson 1965). Radish and tomato had much shorter turnover times from 35 to 40 minutes after feeding or about half the time required by the other species.

A period of dark had no effect on the mobilization of the assimilates except for the two species with the short turnover time of the translocation pool. Whether there is an interrelationship between the turnover time and the effect of dark on the

remobilization of the assimilates or whether the occurrence of the 2 in the same species is coincidence requires further investigation.

In all the species investigated the stage of the development of the leaf affected both the rate of translocation as well as the total amount of the assimilates that was translocated. In all the species the leaves that had just reached the stage of full expansion were the most active in translocation both as far as the rates of translocation and the amounts translocated are concerned. Leaves both younger and older were not as active in translocation.

Comparison of the distribution of ^{14}C in the fed areas of different species after translocation. The distribution of the remaining ^{14}C in the fed areas of the different species is summarized in Fig. 22 and Table IX. The species differed greatly in the compounds in which the ^{14}C was being held in the leaves. In most species the percentage of the ^{14}C in the ethanol-insoluble fraction was between 55 and 70%. Two exceptions were radish with about 40% and castor bean with about 20%. The compounds in the ethanol-soluble fraction in which the ^{14}C was held varied from species to species. Castor bean was the only species with large amounts of sucrose, but it contained only traces of free glucose and fructose whereas the other species stored only small amounts of sucrose but substantial amounts of free glucose and fructose. Radish and nicotiana retained large amounts of the ^{14}C in malic acid. Soybean retained large amounts in a number of different amino acids. The other species had varying amounts among different amino acids and

Fig. 22. Distribution of ^{14}C in the fed area of leaves of different species 6 hours after feeding in soybean, millet, corn, sunflower, nicotiana and sorghum and 1 hour and 5 or 10 minutes after feeding in castor bean and radish.

S sucrose

G glucose

F fructose

MA malic acid

Asp asparagine

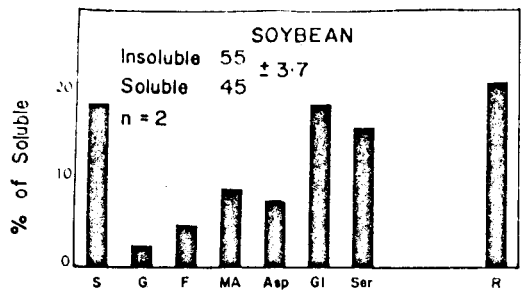
Gl glutamic acid

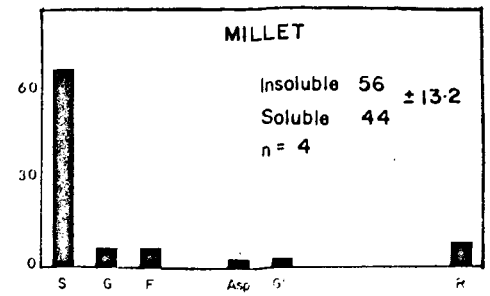
Ser serine

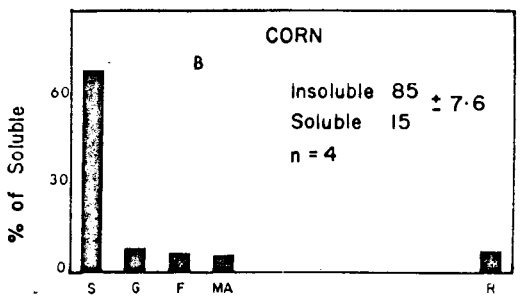
Gly glycine

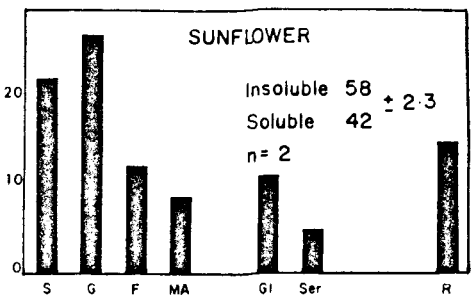
AspA aspartic acid

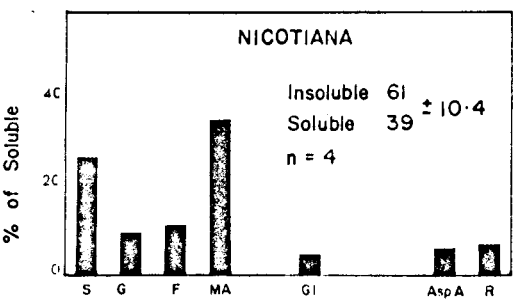
R remainder

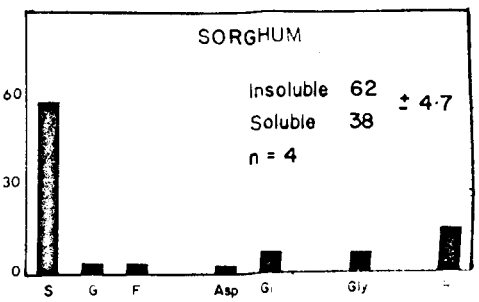

SOYBEAN
Insoluble 55 ± 3·7
Soluble 45
n = 2
% of Soluble
S G F MA Asp Gl Ser R

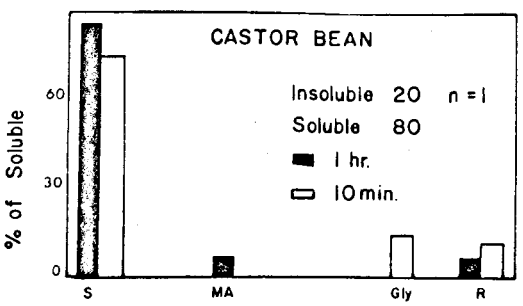

MILLET
Insoluble 56 ± 13·2
Soluble 44
n = 4
S G F Asp R


CORN
B
Insoluble 85 ± 7·6
Soluble 15
n = 4
% of Soluble
S G F MA R


SUNFLOWER
Insoluble 58 ± 2·3
Soluble 42
n = 2
S G F MA Gl Ser R


NICOTIANA
Insoluble 61 ± 10·4
Soluble 39
n = 4
% of Soluble
S G F MA Gl Asp A R


SORGHUM
Insoluble 62 ± 4·7
Soluble 38
n = 4
S G F Asp Gl Gly


CASTOR BEAN
Insoluble 20 n = 1
Soluble 80
1 hr.
10 min.
% of Soluble
S MA Gly R

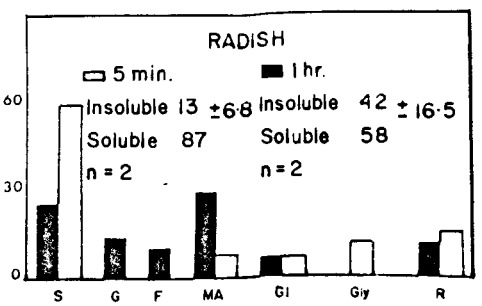

RADISH
5 min.
Insoluble 13 ± 6·8
Soluble 87
n = 2
1 hr.
Insoluble 42 ± 16·5
Soluble 58
n = 2
S G F MA Gl Gly R

Table IX. Comparison of distribution of ^{14}C in the fed areas of leaves of different species after the rate of translocation has diminshed to a slow rate.

Species	% Soluble	% Insoluble	Sucrose % of Soluble	Sucrose % of Total	Sucrose % of Fed Activity
Corn	32	68	32	10.2	2.0
Millet	44	56	68	29	7.4
Sorghum	34	66	51	19	5.0
Sunflower	42	58	22	9.0	4.6
Radish	58	42	25	14	7.0
Soybean	45	55	20	8.8	5.0
Castor bean	81	19	86	69	48
Nicotiana	42	58	22	9.0	4.6

malic acid It is interesting to note that the different species retained in their fed areas from 2 to 48% of the total ^{14}C assimilated in sucrose in spite of the fact that the rapid rate of translocation was over.

Effect of dark on translocation in tomato. The results of the effect of darkening the fed area, the rest of the plant or both on the amount translocated in 15 hours are summarized in Table X. When the entire plants were kept in the dark they translocated about 20% more than when they were kept in the light. When either the fed leaf or the rest of the plant alone were kept in the dark the amount translocated from the leaf was somewhere in between the amounts translocated when the entire plant was kept in the light or when the entire plant was kept in the dark. The plants that were in the dark grew about 2 cm more in height than those in the light over the 15 hour period. In the light the tomato plants stored large amounts of starch in the leaves.

When the translocation curves for all the species were compared it was noticed that 2 species, tomato and radish, had a stimulated translocation in the dark (cf. Fig. 20) period following the 12 hour light period.

Discussion

The method of measuring the loss of ^{14}C from the fed area of a leaf was applicable to other species besides corn. Data was obtained on 3 characteristics of translocation: the rate of loss of ^{14}C, the amount of the assimilated ^{14}C translocated and the turnover time of the translocation pool.

Table X. Effect of dark on the percent ^{14}C translocated in 15 hours from $^{14}CO_2$ fed tomato leaves.

Fed leaf and rest of plant in light	Fed leaf and rest of plant in dark	Fed leaf in light rest of plant in dark	Fed leaf in dark rest of plant in light
38	70	61	69
53	74	68	59
58	74	60	72
55	66	62	62
51	64		
51	71		
48	72		
Mean 51 ± 5.9	70 ± 3.6	63 ± 3.1	66 ± 5.2

The rate of loss of ^{14}C from the fed areas of leaves of different species was different. The rates varied from the rapid rate in corn to rates 2 to 3 times slower in nicotiana and soybean. The stage of development of the leaf must also be considered. In most species the rate of translocation from leaves at different stages of development varied as much as the rates from different species.

The turnover time of the translocation pool was the same in all species except in tomato and radish. Tomato is known to translocate sucrose (Went & Hull 1949) and it also stores large quantities of the assimilated ^{14}C in starch (Hofstra, not reported) in the leaves. The sucrose in the leaf is being used for both translocation and starch formation and this may be the explanation for the shorter turnover time of the main sucrose pool. The other species were not examined for the amount of ^{14}C that may have been stored in starch, but most of the species used are known not to store much starch in the leaves.

The amount of the assimilated ^{14}C retained by the leaf also varied with the species. Almost all the species held back most of the ^{14}C in the ethanol-insoluble compounds with smaller amounts in other compounds that are not translocated. All the species still contained significant amounts of the ^{14}C in sucrose after the translocation of ^{14}C had virtually ceased. In all the

species, except castor bean, the amount of ^{14}C in sucrose was between 2 and 8% of the fed ^{14}C (Table IX). Thus sucrose does not appear to be a major storage compound in the leaves of most species, and yet small amounts are held in the leaves. Castor bean is different in that it contains large quantities of sucrose (48% of the fed ^{14}C) which apparently were not readily available for translocation. This species provides interesting material for a further study.

Summary: Control of Translocation.

Translocation is the process whereby assimilated carbon is transported from the leaves to organs where the carbon is utilized or accumulated. This process makes possible the storage of large quantities of organic carbon in specialized organs in the plant. On the other hand the storage of carbon in organs different from the leaves is completely dependent on translocation from the leaves. Thus translocation through the vascular tissue serves to supply sinks with the necessary assimilates. In this research work several pieces of information have come to light which suggest where translocation may be limited and controlled in the plant.

It seems probable that the organ most directly dependent on translocation (the sink) is the one having most control over the process. In corn it was found that in young fast growing plants, up to 90% of the assimilated ^{14}C was translocated from the fed areas of the leaves. Most of the translocated ^{14}C was found in the growing regions of the plant. Although older leaves translocated at a slower rate than younger leaves, they translocated the same total amount of the assimilated ^{14}C over a 24 hour period. Thus in young active plants there was no appreciable build up of assimilate in any of the leaves. These plants were not tested to see whether a decrease in the activity of the sink would decrease the translocation of assimilates from the leaves. Evidence from other work suggests that a decrease in the activity of the sink

does decrease translocation, causing a build up of assimilates in the leaves and stems (Burr et al.1957, Lupton 1966, Nelson and Gorham 1959, T. Shiroya et al.1962a, and Starck 1964).

Just where the sink affects translocation and how control is exerted over this process is still poorly understood. There are many sites along the translocation pathway where the sink might affect translocation. One of the possible sites is at the sink itself. Münch (1930) suggested that translocation through the phloem was due to the removal of translocate from the phloem at the sink, setting up a concentration gradient of the translocate along which it would move. The work with corn shows that concentration gradients do exist along the phloem, but these are due to a local accumulation of the translocate which was mostly reversible. These gradients were independent of the sinks. Other workers have found that sucrose in the plant will move against concentration gradients, and that this movement is polar (Loomis 1945, Leonard 1938, 1939, and Phillis and Mason 1933). Translocation through the phloem is not by a diffusion along a gradient, but rather the process is under metabolic control as shown by its temperature sensitivity (Bohning et al. 1953, Hartt 1965, Hewitt and Curtis 1948, Swanson and Bohning 1951, Vernon and Aronoff 1952, Webb and Gorham 1965, and Whittle 1964).

Studies on the effect of temperature on the translocation of ^{14}C from the fed area of the leaf indicate that movement from the leaf is very sensitive to a lowering of the temperature. It was more sensitive that the movement in the phloem. Translocation

from the leaf was affected linearly between 7 and 26°, whereas translocation through the phloem was insensitive to a change in temperature between 15 and 30° (Webb 1967). These results suggest that the transfer of assimilates from the assimilating cells may become limiting very quickly under sub-optimal conditions. Because of this sensitivity to temperature it seems probable that the transfer of the translocate to the phloem may be the process in translocation where translocation is controlled.

There is other evidence to suggest that the control of translocation may be in the transfer of the assimilates across the membrane to the phloem. The membranes appear to control the compounds that are translocated. Although many water-soluble compounds were present in the leaves, only sucrose was translocated. In all the species the membranes were a barrier to some of the sucrose in the cells. Only the sucrose in the translocation pool was translocated. In castor bean large quantities of sucrose were retained in the leaf. In most of the other species a high percentage of the assimilates was held in the cells in other compounds. Species such as corn and sunflower did not retain such a high percentage. One might speculate that this high retention is due to the fact that the transfer of assimilates out of the cells is limiting as has been suggested in soybean (Nelson et al. 1961).

The studies on translocation in corn show that translocation out of the fed area of the leaf is more sensitive to a change in temperature than CO_2 fixation. At low temperature the

incorporation of ^{14}C was only slightly retarded, whereas the rate of translocation was greatly reduced. The results of the study of the effect of temperature on translocation showed that at the lower temperatures a higher percentage of the assimilate was retained in the leaf, indicating that the rate of translocation had been reduced more than the rate of photosynthesis. At lower temperatures translocation becomes limiting and assimilates accumulate in the leaf.

All the studies on translocation reported here show that light has no effect on translocation, except to form the translocate. In corn there was no significant difference between the rate of translocation in the light and in the dark. In the other species studied the rate of loss of ^{14}C was no slower in the dark than it was in the light. In 2 species (tomato and radish) the rate of loss of ^{14}C increased in the dark. These results indicate that the control of translocation is not affected by light.

Some evidence is beginning to accumulate on the mechanism through which translocation may be controlled in the plant. As has been mentioned earlier a comparison of the work of Hew (1965) and Lupton (1966) suggests that the sinks may be influencing translocation through hormones. It is a well established fact that actively growing regions are high in plant hormones. Auxin is known to be translocated in plants (Skoog 1938, and Went 1937). In corn the rate of translocation decreased as the leaves became older, as would be expected if auxins were influencing the rate of

translocation. Older leaves are known to become low in hormones as they reach senescence. Auxins are known to stimulate respiration (Bonner 1933) and also increase the movement of material through the membranes (Ursino 1964). Vernon and Aronoff (1952) found that 2,4-dichlorophenoxyacetic acid (2,4-D) decreased the rate of translocation from the site of photosynthesis to the translocation stream. Since 2,4-D acts like an auxin and becomes inhibitory at very low concentration, these results again indicate a sensitivity of translocation out of the leaf to auxin.

The experiments reported here suggest that translocation from the leaves may be the limiting factor in translocation. That the sink is able to alter or control translocation through hormones was not established in this work. However, the test system that was established for translocation out of the leaves gives a ready way of testing the effect of the activity of the sink on translocation. The test system can be used to study the effect of inhibiting or stimulating the activity of the sink on translocation from the leaf.

These studies suggest that translocation may be controlled at the membranes between the assimilating cells and the phloem. The studies here could readily be expanded to an intensive study of the control of translocation in higher plants.

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