

Relations between Light Level, Sucrose Concentration, and Translocation of Carbon 11 in *Zea mays* Leaves

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ABSTRACT

The mechanism of carbon transport in *Zea mays* leaves was investigated using carbon 11 which is a short lived (half-life 20.4 min) positron-emitting isotope. The gamma radiation produced on annihilation allows *in vivo* or nondestructive measurement of the isotope and the short half-life allows many measurements of translocation to be made on the same leaf within the same day.

Carbon 11 produced by the $^{10}\text{B}(\text{d},\text{n})^{11}\text{C}$ nuclear reaction was converted to $^{11}\text{CO}_2$, fed to a leaf as a short pulse, and assimilated during photosynthesis. The progress of the radioactive pulse along the leaf in the phloem was monitored in several positions simultaneously with counters. The counters were NaI crystals with photomultipliers and the output was amplified, passed to single channel analyzers, and the counts accumulated for 20 seconds every 30 seconds. Corrections were made for the half-life and background radiation by computer, and the results were displayed on a high speed plotter. Information derived from the corrected data included the speed of translocation, the shape of the radioactive carbon pulse, and the influence of light and distance along the leaf on these parameters. The plants were kept under controlled environment conditions during all measurements.

A speed was derived from the time displacement of the midpoint of the front of the pulse, measured at two positions along the leaf. This was an apparent mean speed of translocation because it averaged a variation in speed with distance, variation in speed between or within sieve tubes, and it averaged the mean speed of all of the particles in the pulse.

A wide range of speeds of translocation from 0.25 to 11 cm min^{-1} was observed but most of the variability was due to the variation in light available to the leaf. For example, the speed of translocation was proportional to the light level on either the whole plant or individual leaf. Shading of the leaf established that the light effect was not localized in either the feeding area or in the portion of the leaf on which the measurements were made. It was proposed that the speed was dependent on the proportion of the leaf in the light upstream from the last counter. The speed of translocation was relatively independent of the stage of growth of the plant, age of the leaf, and the time during the diurnal light cycle.

Data obtained on the level of the reducing sugars, starch, and sucrose in the leaf were related to the speed of translocation. A biphasic relationship between speed and sucrose concentration in the leaf was established and the high speeds measured during experiments only occurred when sucrose concentrations in the leaf exceeded 8% of the dry weight.

The shape of the pulse loaded into and translocated in the phloem was estimated from the half-width of the pulse. The half-width was primarily determined by loading phenomena which resulted in an increase in the half-width from 2 minutes when fed to the leaf to more than 40 minutes in the phloem. In many examples, the pulse continued to broaden with distance along the leaf from the fed region. The half-width was independent of the speed but highly dependent on the light level.

Carbon translocation is an integral component of the growth- and yield-determining processes in higher plants. To characterize long distance C transport, it will be necessary to measure the speed and sugar concentration of the solution and the cross-sectional area of the sieve tubes active in translocation. Other data, such as the turgor pressure and the water, ion, and sugar exchange rates of the sieve tubes, are required to establish the mechanism of C transport and explain the cause of the variation in C translocation between species, or between plants, under different environmental conditions.

Routine measurement of some of these translocation parameters has not been easy. Through the development of techniques to produce ^{11}C in this laboratory, we are now able to measure routinely the speed of translocation and the shape of the pulse in the phloem (36). Carbon 11 is a short lived isotope with a half-life of 20.4 min that decays by emission of 0.96 Mev positrons, which on annihilation yield two 511-keV gamma rays. The high energy gamma radiation allows nondestructive monitoring of the isotope *in vivo* and, by use of external detectors, the distribution with time of the isotope throughout the plant can be studied. The short half-life of the isotope restricts its use to about 3 hr in our application, but is advantageous because the same leaf can be reused for many days, and at least six experiments are possible on the same leaf within the same day. The ease and efficiency of detection of the gamma radiation allow the use of small amounts (10^{-13} g) of C, and short counting periods (less than 1 min) can be used which allows dynamic studies of isotope transport. The low levels of radiation and the short half-life of carbon 11 would prevent radiation damage to the plants.

Continuous feeding of carbon 11 to the plant is possible if an accelerator or cyclotron is used on-line. Under many conditions, it will be more practical to use pulse-feeding as practiced in this laboratory. From pulse-feeding, an immediate estimate of the speed of translocation and the half-width pulse can be made. The speed is obtained by determining the time taken for the pulse to pass between two points a known distance apart and the half-width is measured directly from each pulse.

The role of speed in translocation has yet to be defined, although two aspects are of special importance. The relationship between speed and mass flow of sugar will be critical information for understanding the physics of flow in the sieve tubes (2) and, for plant productivity studies, the time delay between C fixation and its utilization in new leaf production will be important to crop growth rate. Canny (2) reports about 30 measurements of the speed of translocation in different species under different conditions and using different techniques. The range was 0.1 to 2.5 cm min^{-1} . There have been few attempts to relate speed to any genetic, physiological, or environmental parameters.

Crafts and Crisp (4) suggest that light may be an important

parameter in translocation because it is required for photosynthesis and, if the driving force for translocation is osmotic, then photosynthesis provides one source of osmotically active substances. For example, the rate of translocation (mass moved per unit time per unit cross-sectional area of the sieve tubes) has been shown to be greater in the light than the dark (13–15, 21, 25, 28, 31). Photosynthesis may stimulate translocation in several ways: (a) increasing ATP (and NADPH) levels which may influence CO₂ fixation, distribution of C between products, sucrose transport, sieve tube loading, and transport in the sieve tubes; and (b) increasing the level of C compounds (primarily sucrose) which would increase the respiration rate and/or change osmotic conditions in the leaf.

The rate of translocation would not be expected to be correlated with the instantaneous rate of photosynthesis, as translocation can occur in the dark and in nonphotosynthetic tissues. Translocation must, at least partly, be independent of a direct effect of the cyclic and the noncyclic photophosphorylation reactions, even though these processes may stimulate some aspects of translocation. It is more likely that a product of the C cycle is involved and it has been proposed that the level of sucrose in the leaf determines the rate of translocation (11). At least as an initial proposition, we predict sucrose concentration will also be likely to be important in influencing the speed of translocation, under conditions of nonlimiting water and nutrients and at optimum temperatures. Light may also influence the rate of evaporation of water from the leaf and thereby influence translocation through an effect on the water relations of the plant.

Experiments reported here establish the relationships between the environmental parameter light, sucrose levels in the leaf, and the speed of translocation. Four aspects were investigated: (a) the quantitative relationship between light level and the speed of translocation; (b) localization within the leaf of the light effect on speed; (c) the relationship between the speed of translocation and the levels of sucrose in the leaf; and (d) the effect of light level and position in the leaf on the shape of the isotope pulse.

The plant species used in experiments was maize. It has a lack of CO₂ evolution in the light and a high affinity for CO₂ (33, 34) which is characteristic of a C₄ plant. Sucrose has been shown to be the major C compound translocated in maize (16).

MATERIALS AND METHODS

Plant Materials and Environmental Conditions. *Zea mays* (cv. Morden) plants were grown from seed in controlled environment cabinets at 28 ± 1 C, 80 ± 10% RH, 12-hr day length and at a light level of 240 w m⁻² (400–700 nm) produced by 400 w HPLR mercury vapor lamps. The plants were grown in perlite and regularly fed a modified Hoagland nutrient solution. A comparison was made of translocation in plants at different stages of growth but most plants were about 4 weeks old and had five fully expanded leaves. All experiments were conducted in a controlled environment cabinet kept at the same conditions as given above. The light level was varied by raising or lowering the lamps. The thermal environment was continuously monitored in eight positions by copper-constantan thermocouples with the output read on a multichannel digital voltmeter. The temperature in all parts of the system was 28 ± 1 C at all times (leaf temperatures 28 ± 2 C), even during periods of transition from light to dark and when parts of the plant were in the dark.

A small leaf chamber enclosed part of the leaf. Temperature control was by a water jacket. Normal air was humidified and passed through this chamber at approximately 5 liters min⁻¹ to maintain 300 ± 20 μl/l CO₂ concentration in the chamber at all times. The area of leaf enclosed by this chamber was about 15 cm². The organization of the equipment has changed considerably over the period of the experiments reported here, and a description of equipment used in the early stages is given elsewhere (36).

Carbon 11 Production and Counting Procedures. The isotope carbon 11 was produced by the ¹⁰B (d,n) ¹¹C reaction using deuterons from a 3-Mev Van der Graaff accelerator (22). A solid enriched ¹⁰B target was bombarded with deuterons and O₂ gas was passed across the face of the target. The O₂ reacted with ¹¹C produced in the nuclear reaction to form ¹¹CO and ¹¹CO₂ which were recovered from the target area by a differential pumping system. The ¹¹CO was converted to ¹¹CO₂ by heating to 600 C in the presence of CuO wire and any H₂O was removed from the air stream by a Dry Ice-alcohol trap. A liquid O₂ trap was used to remove ¹¹CO₂, and contaminant gases, such as excess O₂ and N₂, were exhausted to the atmosphere. This technique has the potential for producing ¹¹CO₂ without contamination from any other gases, including ¹²CO₂ or ¹³CO₂. The ¹¹CO₂ was recovered by warming the liquid O₂ trap to room temperature and the gas was transferred to a syringe ready for injection into the leaf chamber.

The ¹¹CO₂ was fed into a leaf chamber enclosing a 2-cm wide strip of the maize leaf. The leaf was exposed to normal air between isotope feeding periods. The radioactive gas was fed as a pulse of between 3 and 5 min duration and was flushed from the system within 1 min by using normal air. The natural photosynthetic reaction was used to incorporate the ¹¹CO₂ into ¹¹C-labeled sucrose. During translocation experiments in the dark, it was necessary to expose the leaf to light for about 5 min. This influenced the results, but the ratio of light to dark was 1:36 and the light period would only have a small effect on the results. The procedure was the same for all dark measurements, so at least the treatment results can be compared.

The activity of ¹¹C *in vivo* was monitored externally with counters set at 10-cm intervals downstream (*i.e.* toward the leaf base) from the feeding chamber. Three or four counters were used. The relationship between the fed region, sieve tubes, counters, and leaf base is shown diagrammatically (Fig. 1). Each counter was shielded by lead to collimate the counts to a specified segment of the leaf.

The detectors were NaI crystals (5 × 2.5 cm) coupled to a photomultiplier and preamplifier. The output was then passed to an amplifier and single channel analyzer set at 511 kev. The counts were accumulated on blind scalers and at intervals the counts were printed and punched onto paper tape using a teletype. The paper tape was then fed to a computer and the data corrected for background counts and the half-life of the isotope.

The period required to accumulate sufficient counts for accurate analysis depends on numerous physical and physiological aspects of the system. The parameters that can be altered include the activity of the isotope fed to the leaf, the length of the feeding period, the width of the fed areas, the width of the collimator slit, the distance between counters, the sensitivity of the detectors, and the counting period. Physiological attributes are usually under investigation and therefore any limitations in these components have to be accepted. Plant species and environmental conditions will influence the loading characteristics and the speed of translocation, and will necessitate continual adjustment of the settings for efficient counting. For our experiments, the counts were accumulated over 20 sec, with two counting periods/min. To improve the counting statistics at low speeds, and to standardize the data, the mean count rate/min was derived from two 20-sec counting periods. The corrected data were normalized and the output from each counter recorded, using a high speed plotter.

Time zero in all results was the time of injection of the isotope into the feeding chamber. For the feeding and flushing periods, there was high isotope activity in the vicinity of the counters and on some occasions this gave spurious counts during the first 4 min. After flushing, the isotope activity in these regions diminished and its effect on the counters was negligible. Curve fitting or smoothing of the data was unnecessary.

Two parameters were derived from the isotope data: the speed

DIAGRAMMATIC REPRESENTATION OF A MAIZE LEAF

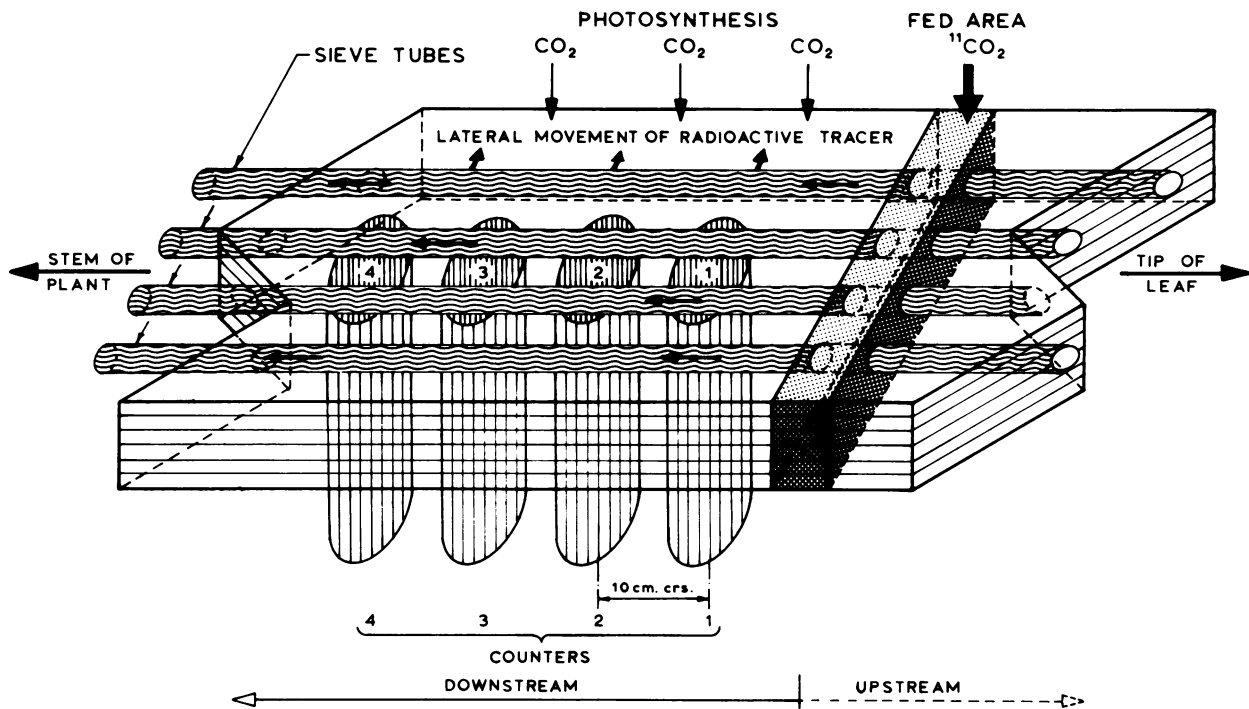


FIG. 1. Diagram of the relationship between the fed region of the maize leaf, the sieve tubes, and the counters.

of translocation, and the half-width of the pulse. Both parameters were obtained by direct measurement from the plotted data. The speed was taken from the time delay in the arrival of the midpoint of the front of the pulse between any two counters. The half-width was the width of the pulse (expressed as time) at half the maximum height of the pulse at each counter.

Carbohydrate Determination. The plants used for carbohydrate measurements were kept under identical conditions, including pretreatment light or dark as previously reported (36). This allowed direct correlation to be made between speeds measured previously with carbohydrate levels reported here.

Small segments or all of the leaf (150–200 mg, fresh wt) was harvested and plunged into 20 ml of boiling 80% ethyl alcohol. Three extractions with 80% boiling ethyl alcohol were made and the combined supernatants kept for soluble sugar estimation and the residues were kept for starch estimations.

For soluble sugar determinations, Chl and lipids in the supernatants were removed by adding chloroform and equal volumes of H_2O . The solution containing sugar was evaporated to dryness on a rotary evaporator. Twenty ml of distilled H_2O was added and an aliquot was taken for analysis of both the reducing and the total soluble sugar. Sucrose was taken as a difference between total soluble sugar and reducing sugar.

Reducing sugar levels were estimated using the neocuproine-HCl method (5). Reducing sugar was measured using *A* at 425 nm and by comparison with a standard curve for reducing sugar obtained using glucose.

Total soluble sugars were determined using the anthrone reagent (32, 37). Absorbance was measured at 625 nm and the total soluble sugar level obtained by comparison with a standard set of glucose samples.

Starch in the leaf tissue was analyzed using MacRae's enzymic method (20). The residue obtained from the leaf tissue after extraction with boiling 80% ethyl alcohol was ground and suspended in distilled H_2O . This suspension was heated for 30 min at 100 C and the volume of the sample was kept constant. This gelatinized the starch. The starch was hydrolyzed with amyloglu-

cosidase at 60 C for 40 hr and resulted in the production of glucose. A layer of paraffin oil prevented oxidation of the glucose.

Glucose was estimated by the glucose oxidase method (17). The tissue extract was incubated with distilled H_2O and glucose oxidase reagent at 37 C for 1 hr. After the addition of 18 N H_2SO_4 , the absorbances were read at 540 nm against a water blank and were compared with results from a standard curve using different amounts of glucose. The starch content of the leaf was calculated from knowing the glucose concentration and the original weight of the starting tissue.

RESULTS

GENERAL FEATURES OF CARBON 11 TRANSLOCATION

Direction of Photosynthate Movement in the *Zea mays* Leaf.

The pulse of $^{11}CO_2$ was fed to a 2-cm wide leaf segment, two-thirds of the distance from the base to the tip of the leaf. The direction of transport of the isotope was monitored by counters (5×2.5 cm) 10 cm either side of the fed region. The isotope predominantly moved toward the leaf base. The detector (5×2.5 cm) upstream from the fed region was replaced with a crystal (7.5×7.5 cm) for several experiments, and, with the higher sensitivity of this detector, it was possible to detect counts in the leaf tip on some occasions. A pulse was observed in several experiments (Fig. 2). The total counts moving acropetally were about 2% of the counts in the pulse moving toward the leaf base over the same period. It is also possible that the acropetal movement was in the xylem. In view of the highly directional nature of C transport in maize leaves under these physiological conditions, the results in this paper will only refer to translocation toward the leaf base.

Shape of the Efflux Curves. A counter beneath the fed region monitored the loss of counts from that leaf segment. There is little loss of CO_2 in the light from photorespiration in maize (34), and therefore the change in counts was primarily due to translocation. Differentiation of this efflux curve yields the shape of the

pulse of isotope loaded into the phloem. Two contrasting efflux curves are illustrated in Figure 3A. In one example, 50% of the ^{14}C fed to the leaf as a 4-min pulse was still in the leaf after 30 min, and in the other example, the same amount was present after 90 min. There was a considerable and variable delay between C fixation and translocation.

One implication of this delay is that there may not be a close relationship between the rate of translocation and the rate of photosynthesis unless there is a constant and irreversible loss of C to the nonsucrose pool and a rapid turnover of the sucrose pool. For example, at the beginning of the light period, photosynthesis may quickly attain its maximum value (within 5 min),

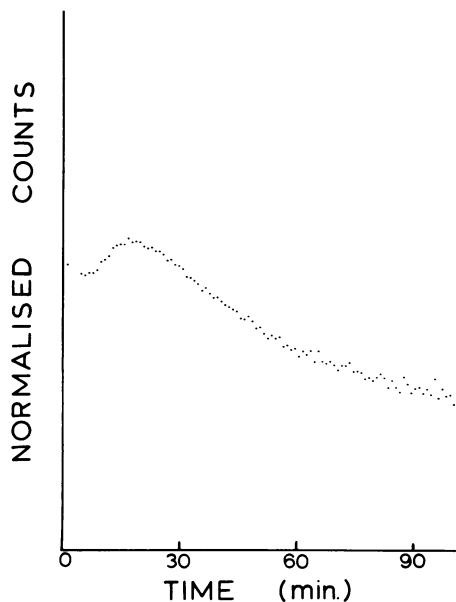


FIG. 2. Evidence for acropetal transport in a *Z. mays* leaf. A NaI crystal (7.5×7.5 cm) 10 cm toward the leaf tip from the fed region monitored a pulse of ^{14}C moving acropetally. Total number of counts in the pulse was less than 2% of the counts moving toward the base. Peak of the pulse at about 20 min indicates a speed of about 0.5 cm min^{-1} .

whereas 50% of the labeled C assimilated during the period may not have been translocated 30 min (or more) later.

In C_4 plants, such as maize, it was anticipated that light activation of some of the photosynthetic enzymes may cause delays to CO_2 fixation during a dark-light transient. This was previously tested using *Atriplex spongiosa*, and the delays, although present, were not significant (33).

The maximum slope of the efflux curve was measured in numerous experiments but it was highly variable and the variability difficult to explain. The plant was transferred from dark (16 hr) to light and kept in 240 w m^{-2} of light for 48 hr, under constant environmental conditions. The same position in the leaf was fed each experiment. The value in the dark was about the mean of the values determined in the light. The low values early in the light period may be associated with the increase in the total carbohydrate of the leaves (see Fig. 11A). There was no direct relationship between the speed of translocation and the maximum slope of the efflux curve, at least in this example, as the speed was constant at $3.5 \pm 0.5 \text{ cm min}^{-1}$.

Pulse Shape and Estimation of an Apparent Mean Speed of Translocation. A feature of all results was that the half-width of the isotope pulse increases from 2 min for the pulse fed to the leaf as $^{14}\text{CO}_2$ to greater than 40 min at the first counter. The broadening occurred in the C metabolism between photosynthesis and translocation. Although the feeding period was kept constant, the half-width of the pulse in the leaf was highly variable. Characteristic shapes of the pulses at three counters along the leaf are shown in Figure 4. Examples of a pulse for a faster (Fig. 4B) and a slower moving stream (Fig. 4C) are also illustrated.

It was not possible to define the speed as being the mean speed for all of the particles in the pulse because the tail was occasionally diffuse and had long delays. For a slow moving stream and at large distances from the fed region, the time taken for the tail to reach the last counter was beyond the time scale of the experiment (e.g. last counter in Fig. 4C). Lateral movement of isotope out of the sieve tube could also influence the measured speed, but the effect is likely to be least in the front of the pulse. As a result of these considerations, the speed was estimated from the midpoint of the front of the pulse. A speed estimated in this way can only be an apparent mean speed of translocation, although it

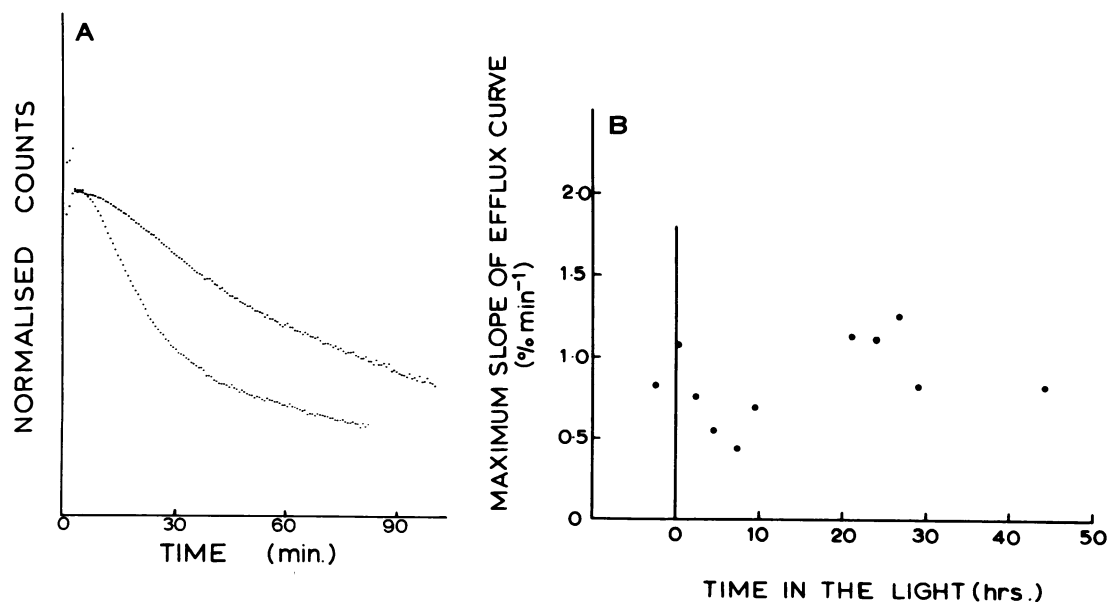


FIG. 3. A: Change in counts in the fed region as a function of time (an efflux curve, ref. 36). Differentiation of these curves indicates the shape of the isotope pulse loaded into the phloem. Data illustrate the variation that can occur and are evidence that the half-width of a pulse fed to the leaf is substantially altered before entering the phloem. B: Variation in the maximum slope of the efflux curve on a leaf under constant environmental conditions and 240 m^{-2} light level. The plant was pretreated with 16 hr darkness.

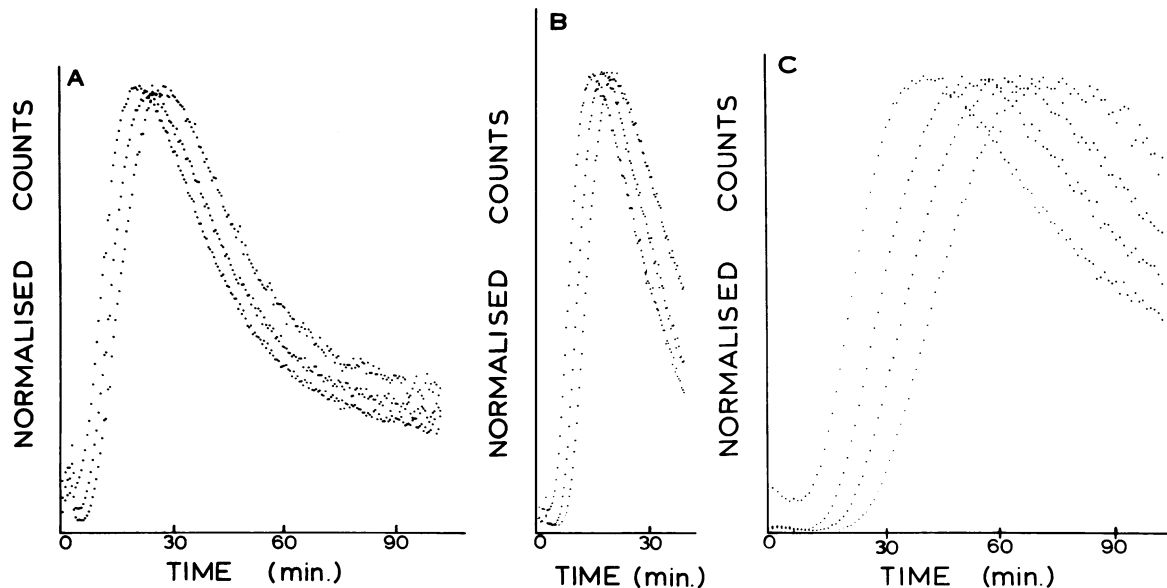


FIG. 4. Three sets of data illustrate the variation in the shape and time displacement of the pulses that occurred during these experiments. Data were corrected for half-life and background and the results are photographed from computer plots. No smoothing of the results was necessary. Each dot in A is for a half-min interval; in B and C, they are for 1-min intervals. For comparison of results, the data have been normalized to the maximum count rate. In all cases, the curves represent in order of increasing time from time zero (time of injection) the counts on counters 1, 2, 3, and 4, respectively. Distance between counters was constant at 10 cm, with the first counter 10 cm from the fed region.

will be referred to in this paper as the speed of translocation. It is not possible to compare directly the results calculated in this way with other work, unless the techniques for analysis are similar.

The shape of the pulse also had implications for the maximum period between measurements. This period was determined by the rate of fall off in the tail of the pulse and the natural decay in isotope activity. The period between measurements need not exceed 3 hr and under many conditions was 2 hr.

Physiological Age of the Plant and the Speed of Translocation. The plants used during the period of these experiments included a wide range of physiological ages and stages of growth. The influence of the physiological state of the plant on speed was investigated by selecting measurements made under similar conditions, *i.e.* over a 20-cm path length, a temperature of 28 ± 2 C, and a light level of 240 ± 40 w m⁻². There may have been some effect on the results of an improvement in the experimental techniques during the period and the variation in the environment between treatments. By standardizing the growing conditions of the plant, the species used, the environment, and arrangement of the leaf during the measurements, it was possible to sort the results according to the stage of growth.

The results in Table I give evidence for a small (less than 10%) increase in speed during the period of stem elongation and cob formation, but this effect is unlikely to be important. During the growth periods investigated, there was apparently little effect on the speed of translocation of potential variations in sink strength due to the stage of growth, in a fully expanded leaf. Other measurements in this paper primarily refer to plants in the intermediate stage of growth, but the results would not be substantially affected by some variation in the physiological age of the plants.

Factors Determining the Speed of Translocation

Light Level. The light level on a whole maize plant was varied and the speed of translocation monitored over 20 cm on one leaf. The light was measured in the position of this leaf and the light level was kept constant along the length of the leaf. The plant was kept at each light level for a 12-hr day and until three consecutive measurements of the speed were constant (to ± 0.2 cm min⁻¹), to ensure that the plant was in equilibrium at each new light level. The same leaf was used for all experiments and a

Table I. The Speed of Translocation and the Physiological Age of the Leaf and Plant

No.	Stage of Growth	Leaf Position	Measurements No.	Range of Speeds cm min ⁻¹	Mean Speed cm min ⁻¹
1	Young (<3 weeks) Vegetative	4th of 7	5	3.5 - 4	3.8 ± 0.2
2	Young Vegetative	5th of 7	6	3.7 - 4	3.9 ± 0.1
3	Intermediate (3-5 weeks) Stem elongation	5th of 7	3	3.6 - 3.9	3.8 ± 0.1
4	Intermediate Stem elongation	4th of 6	3	4.1 - 4.8	4.5 ± 0.3
5	Mature (>5 weeks) Cob formation	5th of 9	5	3.9 - 4.1	4.0 ± 0.1
6	Mature (>5 weeks) Cob formation	6th of 10	10	3.5 - 4.3	3.9 ± 0.2

speed was measured in the dark at the beginning of each day to test that there were no carry-over effects between days or treatments.

The dark values before each light period were 1.1 ± 0.2 cm min⁻¹. These values, shown with the diurnal variation in speed for two light levels (Fig. 5A), indicate there were no major carry-over effects. At low light, the speed reached an equilibrium value soon after the light was turned on, but at high light there was a lag of several hr, usually 3, before the speed was constant. As is evident in Figure 5A, light level influenced the speed of translocation. There was an approximately linear relationship between light level and the speed of translocation (Fig. 5B).

The relationship between light level and speed, extrapolated to zero light, yields a value for the speed in the dark. This value was 1.1 cm min⁻¹, which is similar to the value measured after 12 hr of darkness (Fig. 5A).

Speed of Translocation during Transfer from Light to Dark Conditions. The light level experiments indicated that the time constant for adaptation of the speed of translocation to high light was long (greater than 1 hr). In contrast, photosynthetic responses to a change in light are short (less than 5 min). The time constant for adaptation of the speed of translocation to darkness was investigated. The same experiments identified the site of the light effect on translocation. These investigations only altered the light on the leaf on which the speed was measured. The rest

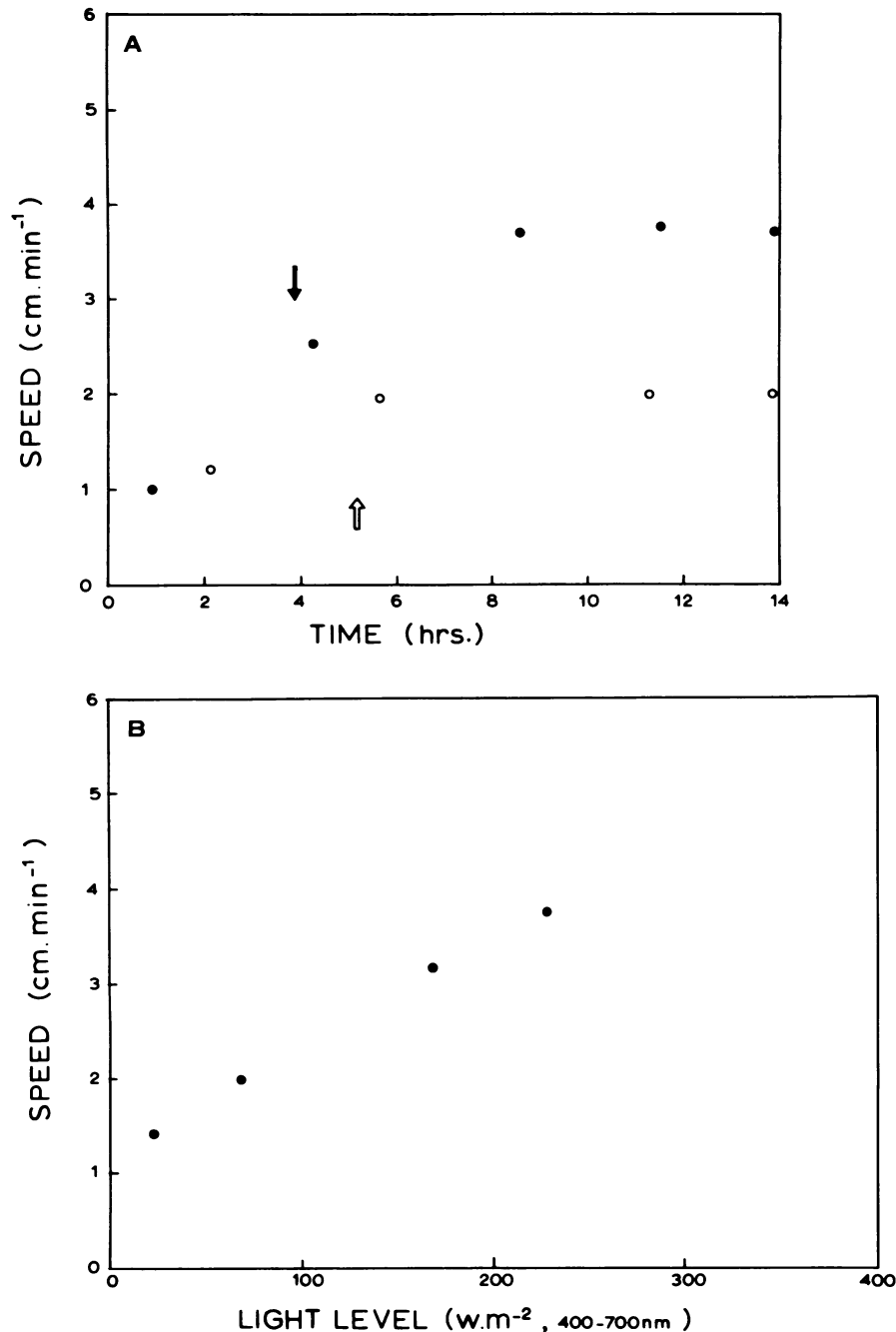


FIG. 5. Influence of light level on speed of translocation. A: Daily course of the speed of translocation in the same leaf at two light levels during a dark-light transition. Arrows indicate light on; (⇒, ○): 70 w m⁻²; (⇒, ●): 230 w m⁻². Speed in the dark was 1.1 ± 0.1 cm min⁻¹ and was constant for three values at each light level. Speed was measured over a 20-cm length of leaf. B: Relationship between light level and speed of translocation in maize. All values were determined on the same leaf under constant environmental conditions, except for light level. Speed was measured over 20 cm and the reading at each light level was the mean of three values which were constant to within 0.2 cm min⁻¹.

of the leaves on the plant and the feeding chamber were kept at high light.

The results of the darkening treatment are given in Figure 6A. Speed was measured at intervals over a 12-hr period, with the first 6 hr at 240 w m⁻² and the remaining time in the dark. Measurements were made at four positions downstream from the feeding chamber and, from these measurements, the changes of speed with distance were derived. The speed of translocation was in equilibrium with the light level before the leaf was darkened. After 6 hr in the dark, the speed had dropped from about 3.8 cm min⁻¹ to 1 cm min⁻¹, with a time constant of about 1.5 hr.

In the light, the speed was faster over the last two 10-cm segments than in the first segment, but in the dark, any influence of distance from the fed area on speed was eliminated.

Localized Shading Treatments and the Speed of Translocation. Shading treatments were applied to identify the site of the effect of light on the speed of translocation. Initially, a 20-cm length of the leaf directly over the counters was shaded, and the leaf area involved was approximately half of the area of the leaf upstream from the last counter. The measurements reported in Figure 6B were made over 20 cm on two different maize leaves. The leaf was allowed to come to equilibrium with the light level for 6 hr before the shading treatment was applied. Shading caused the

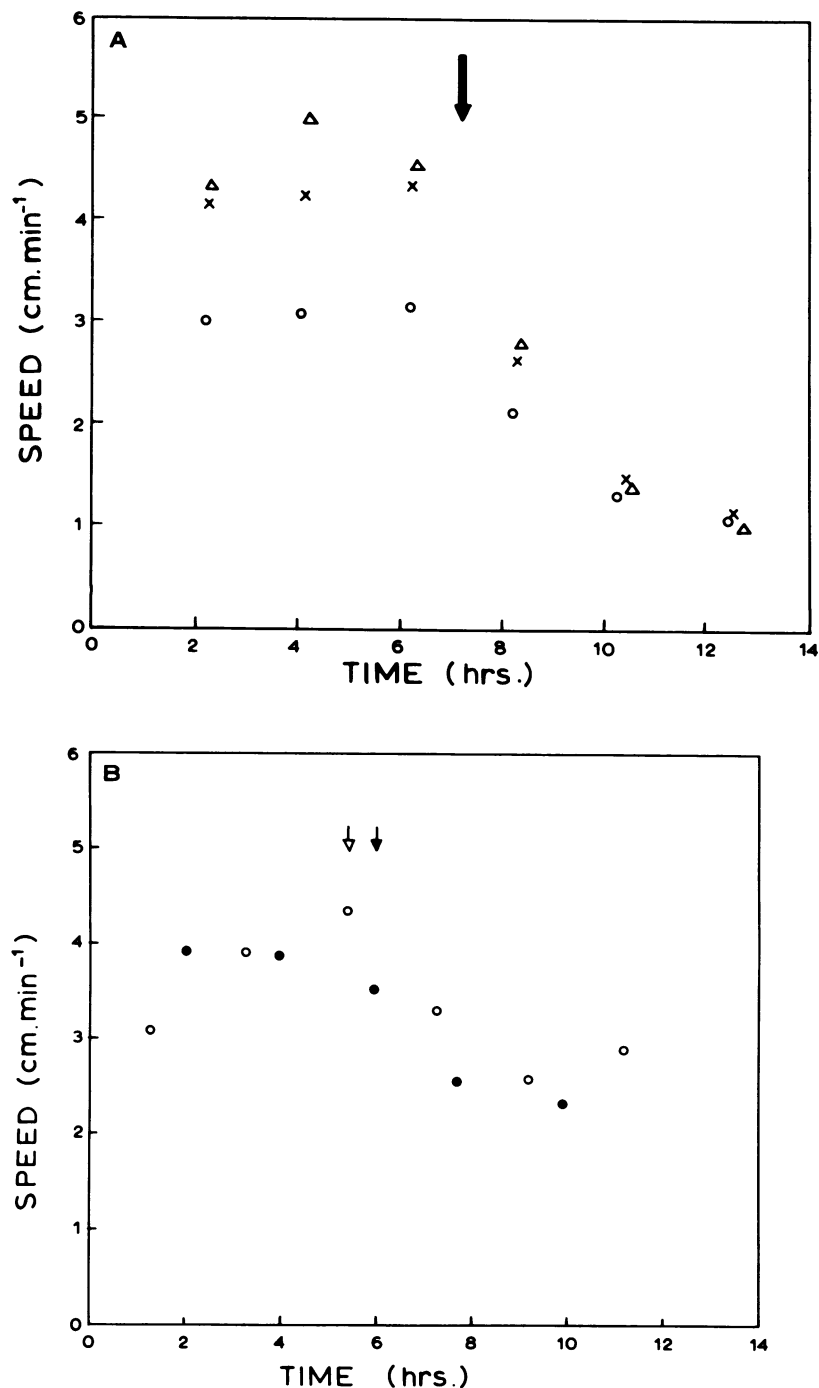


FIG. 6. A: Influence of a light-to-dark transition on the speed of translocation at three positions along the leaf 10 to 20 (○), 20 to 30 (×), and 30 to 40 (△) cm from the fed region. Time zero is lights on and the arrow is lights off. Differences in speed with distance along the leaf are removed after 4 hr dark. The whole plant was kept at 240 w m^{-2} and only the leaf on which the measurements were made was darkened. The fed region was kept in the light. B: Influence of shading 50% of the area of a leaf upstream from the counters on the speed of translocation over 20 cm. Two different leaves were used (○, ●) and (→, →) refer to lights off for each leaf. The rest of the plant was in high light.

speed to decline from 3.9 cm min^{-1} to a new equilibrium value of 2.6 cm min^{-1} . Assuming the speed in the dark would be about 1 cm min^{-1} , shading 50% of the leaf reduced the speed by about 45%. These results suggest that the reduction in speed was approximately proportional to the area of the leaf that was shaded upstream from the last counter.

Comparison of Figure 6, A and B establishes that there is a contribution from outside the shaded area to the components determining the speed. The reduction in speed with shading could be a consequence of a reduction in photosynthesis.

A 10-cm length of leaf between two counters was shaded, and the speed was measured in that segment and in two 10-cm segments immediately upstream and downstream from the shaded portion. Measurements of speed in this example include the effect of distance along the leaf on the speed. The effect of distance on the speed was investigated in high light and before the shading treatment was applied (Fig. 7). There was a large increase in the speed between the first two segments and only a small effect of distance on speed between the last two segments. The shading treatment decreased the speed by less than 20% at

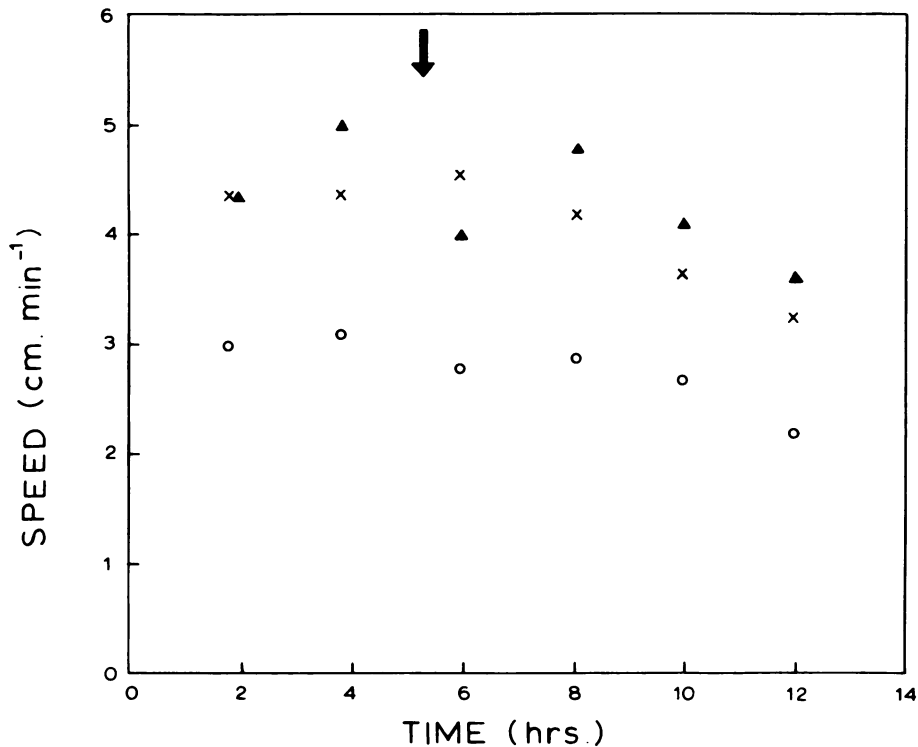


Fig. 7. A: Ten-cm length of leaf between two counters was shaded. Speed over 10 cm was measured immediately upstream (O), and immediately downstream (Δ) from the shaded region and in the shaded region (x). The whole plant was kept at $240 \pm 10 \text{ w m}^{-2}$ throughout the experiment.

all positions, which was in proportion to the area shaded.

The effect of shading different positions in the leaf was investigated in a further series of experiments. The same leaf was used for all measurements. The area shaded was approximately 10% of the area upstream from the counters, and shading was either a 10-cm length upstream or downstream from the fed area or a 10-cm length over the counters. Each treatment occupied a day. The mean speed over 20 cm was the same on 3 consecutive days after 4 hr in high light (Fig. 8A).

Shading upstream from the fed region or shading a 10-cm length over the counters had a small effect on the speed of translocation over a 20-cm segment. In contrast, shading the leaf between the fed region and the first counter resulted in an approximately 40% increase in speed. Analysis of the variation in speed between the first and second 10-cm segments indicated (Fig. 8B) that there was a decline in speed in the second segment, but approximately a doubling of the speed in the segment immediately downstream from the shaded area. One speed, shown in the figure as being in excess of 7 cm min^{-1} , was $11 \pm 1 \text{ cm min}^{-1}$.

On the same leaf, a series of measurements were made in which the feeding chamber was covered. There was no effect of shading this small part of the leaf on the mean speed of translocation, measured over a length of 20 cm downstream from the fed region.

Factors Determining Pulse Shape

Light Level and the Shape of the Isotope Pulse in Phloem. A further characteristic of the solution flowing in the sieve tubes is the shape of the isotope pulse. The parameter used in these experiments to describe the shape is the width at half-maximum height. An indication of the variation that can occur in the shape of the pulse was previously given (Fig. 4, A, B, and C). Many experiments were terminated before the half-widths could be determined and the results in these cases are presented as being greater than 100 (or 120) min.

The half-width of the pulse at any one position in the leaf was relatively constant (that is $\pm 5 \text{ min}$ for a variation of $\pm 10\%$)

when the environment was constant (Fig. 9A). The half-width under normal conditions and at high light was $45 \pm 10 \text{ min}$ for most positions within the leaf. In most examples given here, the half-width of the pulse increased with distance down the leaf, although the extent of the increase was variable and in some examples doubled along a 30-cm segment. In many experiments, the increase in half-width with distance remained constant throughout a 12-hr period when measurements were made at 2-hr intervals (9A).

Shading Effects on Pulse Shape. A 10-cm segment upstream from the feeding chamber was shaded and it had little effect on the shape of the pulse at any counter (Fig. 9A). The influence of shading on the shape of the pulse in other treatments was more pronounced. In another experiment, 10 cm between the first two counters was shaded but this only caused an increase in half-width 4 hr after shading.

In another experiment, the half-width was relatively constant at all positions along the leaf, and shading the leaf between the feeding chamber and the first counter initially removed any differences (Fig. 9B). Subsequently, this treatment caused the half-width at the first counter to be greater than at the second or third counters. After 4 hr of shading, the half-width of the pulse increased at all counters. In contrast to the lack of changes in half-width, this treatment caused substantial variations in the speed of translocation between the first two counters (Fig. 8B).

Shading the feeding chamber while the rest of the leaf was kept in high light resulted in a dramatic increase in half-width from 55 to 100 min within 2 hr (Fig. 9C). Subsequently, all half-widths were greater than 120 min. These effects were relatively independent of the speed of translocation, as the speeds at 2-hr intervals throughout the day were consecutively 3.77, 4.21, 2.8, 3.7, 3, and 3.6 cm min^{-1} over a 20-cm length of the leaf. These results provide evidence that the speed is independent of the shape of the pulse but that the half-width is substantially affected by light level in the fed region. A reverse treatment was also applied, *i.e.* where the whole leaf except the feeding chamber was shaded. The results shown in Figure 9D indicate that this

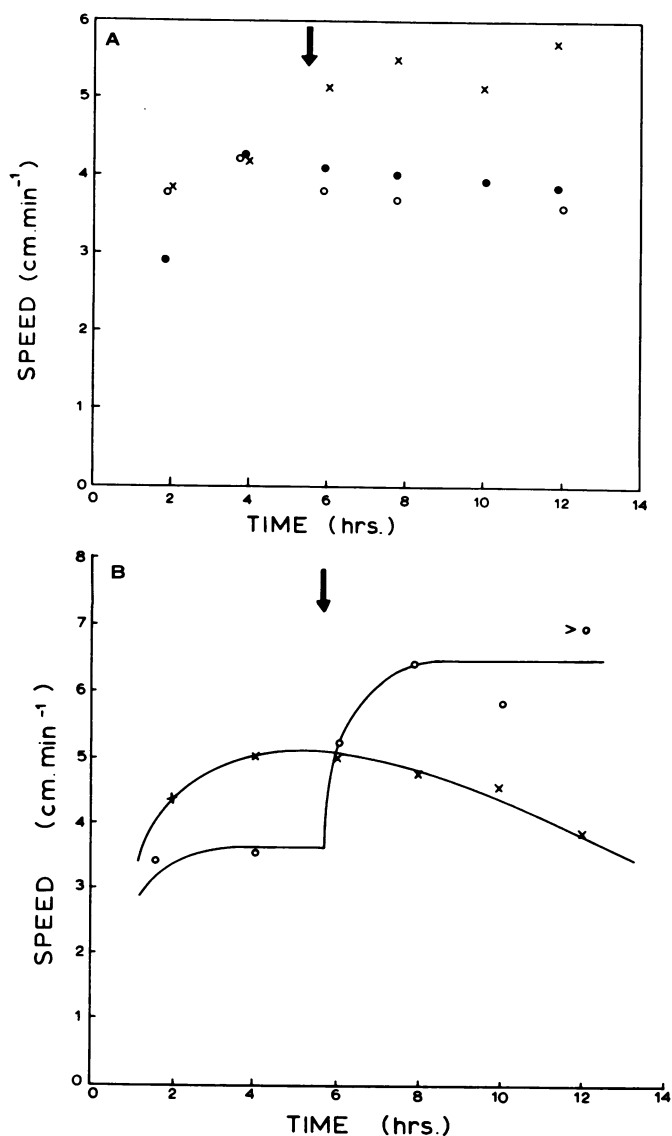


FIG. 8. A: Measurements of the speed of translocation on the same leaf on 3 consecutive days with different shading treatments. The plant was under controlled environment conditions, a light level of $240 \pm 10 \text{ w m}^{-2}$ and light period of 14 hr. On each day, the whole leaf was left in the light for about 5 hr before shading was applied, and the speed over 20 cm after 4 hr of light was the same, $4.2 \pm 0.1 \text{ cm min}^{-1}$ on each day. A 10-cm length of leaf was shaded each day: day 1 (O) immediately upstream from the leaf chamber, day 2 (●) over the counters; and day 3 (×) downstream but adjacent to the leaf chamber. →: time of application of the shade treatments. Shading the fed chamber gave results the same as for shading immediately upstream from the feeding chamber. B: Data on speed for the shading treatment between the fed region and the first counter in A was analyzed to give the variation in speed over two 10-cm segments. (→): time of shading; (O): segment adjacent to the shaded region; (×): speed in the segment furthest downstream. There was much variation in the speed in the first region and the value indicated as > was 11 cm min^{-1} .

treatment caused a slow increase in half-width with time at all counters.

The light level on the whole plant had a significant effect on the half-width of the pulse at the first counter. The half-width of pulse as a function of light level is shown in Figure 10, for an experiment previously reported (Fig. 5, A and B).

Variation in Carbohydrate Levels in Leaves in Prolonged Light and Dark. The behavior of carbohydrates in the leaves were characterized by measuring the level of reducing sugars,

starch, and sucrose. The total carbohydrate level in the leaves continued to increase, even after 40 hr of continuous light and irrespective of the previous treatment (Fig. 11A). This accumulation was as starch after the first 6 hr in the light. The level of sucrose in both examples reached a maximum value within 12 hr in the light, although the time taken to reach this value was considerably longer in a plant pretreated 48 hr in the dark. The level of the reducing sugars was relatively constant throughout the light period.

There was a significant interrelationship between the rate of starch formation and sucrose concentration (Fig. 11A). Irrespective of the dark pretreatment, the rate of starch accumulation showed a marked increase about the time that the sucrose concentration reached a maximum value. This enhanced rate of starch formation did not continue for a long period and the rate of accumulation of starch eventually slowed down. The enhanced rate of starch formation was apparently linked to the maximum sucrose concentration, which also influenced the speed of translocation (Fig. 12, A and B).

On transfer from 48 hr of continuous light to darkness, there was a relatively rapid decline in the sucrose concentration of the leaves. The decline in starch was parallel to the decline in sucrose but there was relatively little change in the level of the reducing sugars (Fig. 11B).

From previously reported data on the speed during prolonged light or on transfer to darkness (21, 35), it was possible to develop relationships between the speed of translocation and the carbohydrate content of the leaves. These measurements were not made on the same leaves, but on plants treated in a complementary manner with respect to the environment (including daylength, light level, temperature, humidity, and pretreatment, and the stage of plant growth). The measurements of speed and carbohydrate levels were obtained from plants transferred from light to darkness. The results indicated that the speed of translocation was high, even when the sucrose concentration was only 1% of the dry weight. Over the range of several concentrations from 1 to 8%, there was a relatively small increase in the speed of translocation.

The level of sucrose was limited by the dark treatment and to obtain higher concentrations of sucrose, it was necessary to investigate the relationship between the speed of translocation and sucrose concentration in the light. This is shown for two different pretreatments in Figure 12, A and B. In both cases, the results confirmed the previous conclusions, *i.e.* that the speed of translocation at low sucrose concentrations of about 1% was relatively high, and between 1 and about 8% there was a plateau region over which the increase in sucrose concentration caused a relatively small increase in speed. It was most significant, however, for both sets of data, that increases in sucrose concentration above about 8% resulted in large increases in speed. This response effectively limited the level of sucrose that could be accumulated in the leaf. It was significant also that this same level of sucrose affected the rate of starch accumulation (Fig. 11A). The combination of the effect of sucrose concentration on both speed and starch formation suggests that there is a self-regulatory process in maize leaves which prevents the sucrose concentration in the leaf from increasing much above 8%.

DISCUSSION

Carbon 11 Technique. These data provide several examples of the reliability of the carbon 11 technique for measuring the speed of translocation and the physiological variability of speed. The standard deviation for the speed was between 0.1 and 0.3 cm min^{-1} for results in Table I, although many different plants were used. The plants were grown under the same controlled environments. In another example (Fig. 5A), the speed was $\pm 0.1 \text{ cm min}^{-1}$ for the same leaf and at a constant light level,

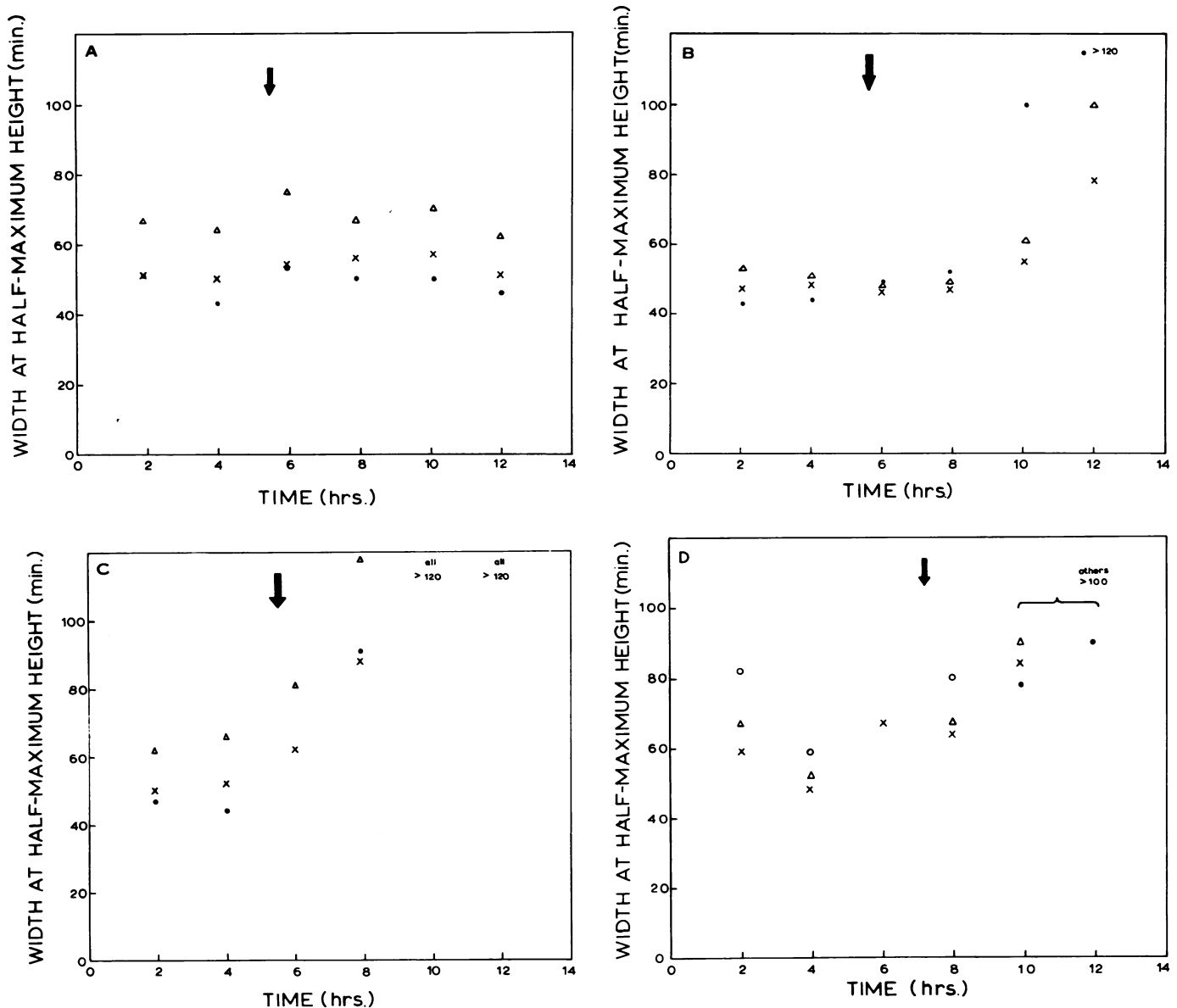


FIG. 9. Half-widths of the pulses as a function of position in the leaf and shading treatments. Arrows indicate the start of the shading treatments. A: Influence of shading a 10-cm length upstream from the feeding area on the half-width of the pulse at three counters, 10 (●), 20 (×), and 30 (Δ) cm downstream from the fed region. B: Half-width of the pulse at 10 (●), 20 (×), and 30 (Δ) cm from the fed chamber when the region between 0 and 10 cm was shaded. C: Influence of shading the feeding chamber on the half-width of the pulse at the counter 10 (●), 20 (×), and 30 (Δ) cm away. After 4 hr of shading, the half-widths were greater than 120 min at all positions. D: Whole leaf was shaded except the feeding chamber. Half-width of the pulse was measured at 10 (×), 20 (Δ) and 30 (○) cm.

throughout a day. The speed was also within ± 0.1 cm min^{-1} in the dark at the start of 4 days of experiments and after a dark period of 12 hr. Further evidence for the repeatability of the data is given in Figure 8. The speed was 4.2 ± 0.1 cm min^{-1} on the same leaf, on 3 consecutive days after 4 hr of high light each day.

A similar degree of reliability was evident in the half-width measurements, although there was more physiological variability, as illustrated by the standard deviation of the measurements at low light (Fig. 10).

Speed of Translocation. Measurements of the speed of translocation made over the last 40 years have been summarized by Crafts and Crisp (4), and Canny (2). They include about 50 estimates of the speed of translocation from numerous species, using a variety of techniques and under many environmental conditions. In this paper, we report about 100 measurements of speed; the same technique and species are used and the influence

of one environmental parameter, light, is investigated. Our experience with the carbon 11 isotope during these experiments, and in those reported previously, has established that ^{11}C can be used in a routine manner to monitor several attributes of the translocation system in higher plants (21, 35, 36).

Speed referred to in this paper was defined as an apparent mean speed of translocation measured from the time delay in arrival of the midpoint at the front of the pulse over a known path length. As previously discussed (36), there are differences in the estimate of speed made from different positions within the pulse, particularly between the front and the tail of the pulse. In many respects, it is only a mean speed; e.g. it reflects all C compounds and different compounds may move at different speeds, and it is a mean of variations in speed within an individual pulse, between sieve tubes within the same bundle and between different vascular bundles. Furthermore, it only refers to the first pulse of material loaded into the phloem following

feeding. There may also be losses of isotope along the path and this in turn would influence the shape of the pulse and therefore the speed.

In view of the variety of techniques previously used to estimate the speed, it is difficult to compare the previous and present results directly, although there is no reason to suggest that they would be substantially different if the same methods for analysis are used. Previous estimates have yielded a wide variety of speeds, but the bulk of the results indicate values between 0.025 and 2.5 cm min^{-1} . Values up to 6 cm min^{-1} have been recorded for cucumber and sugarcane with one unexplained

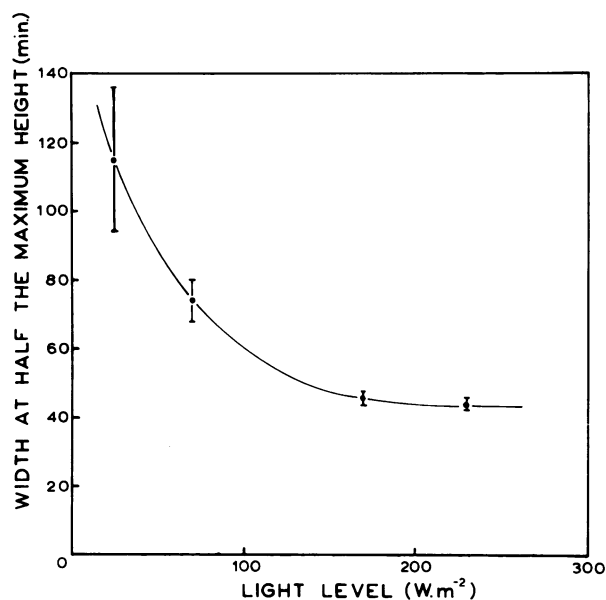


FIG. 10. Relationship between the half-width of the pulse and the light level. Light level on the whole plant was varied. Half-width was measured at the counter 10 cm downstream from the fed region and is the mean of three values at each light level.

exceptional value of 120 cm min^{-1} (4). The speeds measured for maize in the experiments reported here were between 0.25 and 11 cm min^{-1} , and therefore are within the general range of previous estimates. They illustrate the variability that can be expected for any one species and make it difficult to define a speed characteristic of maize.

Environment Effects on the Speed of Translocation. The variability in the speed of translocation in maize was not random and was due to environmental conditions.

A schematic summary of all results is given in Figure 13. There is a significant speed of translocation in the dark under a normal light/dark cycle, but with light the speed increases to a new equilibrium value, depending on the light level, with a half-time of about 30 min. Shading part or all of the leaf reduces the speed with a half-time of about 1.5 hr. Previously, it was shown that the half-time for the initial decay in speed in the dark was 5 hr for a leaf previously treated in 48 hr of continuous light (21). In this paper, a half-time of about 1.5 hr was observed in a plant pretreated with 7 hr of high light. The speed in the dark declined to about 1 cm min^{-1} during a day length of 12 hr of darkness and, with prolonged darkness (48 hr), the speed can be as low as 0.3 cm min^{-1} (21).

Speeds up to 5 cm min^{-1} were observed in parts of the leaf in darkness, which indicates that there is not likely to be a direct effect of light on the translocation process. This does not preclude some effect on the loading of photosynthate into sieve tubes. Shading of the feeding chamber did not alter the speed measured 20 cm downstream, but it did cause a pronounced broadening of the pulse. There was only a 20% increase in half-width of the pulse during the first measurements after the lights were turned off (Fig. 9C). This suggests that light was acting indirectly in determining the pulse shape, which reflects the loading phenomena. Light level influences the loading process as indicated by the reduction of the half-width from 115 ± 21 to 46 ± 2 min when the light level was increased from 25 to 170 W m^{-2} .

Sucrose Concentration and the Speed of Translocation. Products of the C cycle of photosynthesis are intimately involved in translocation because sucrose and other compounds are translo-

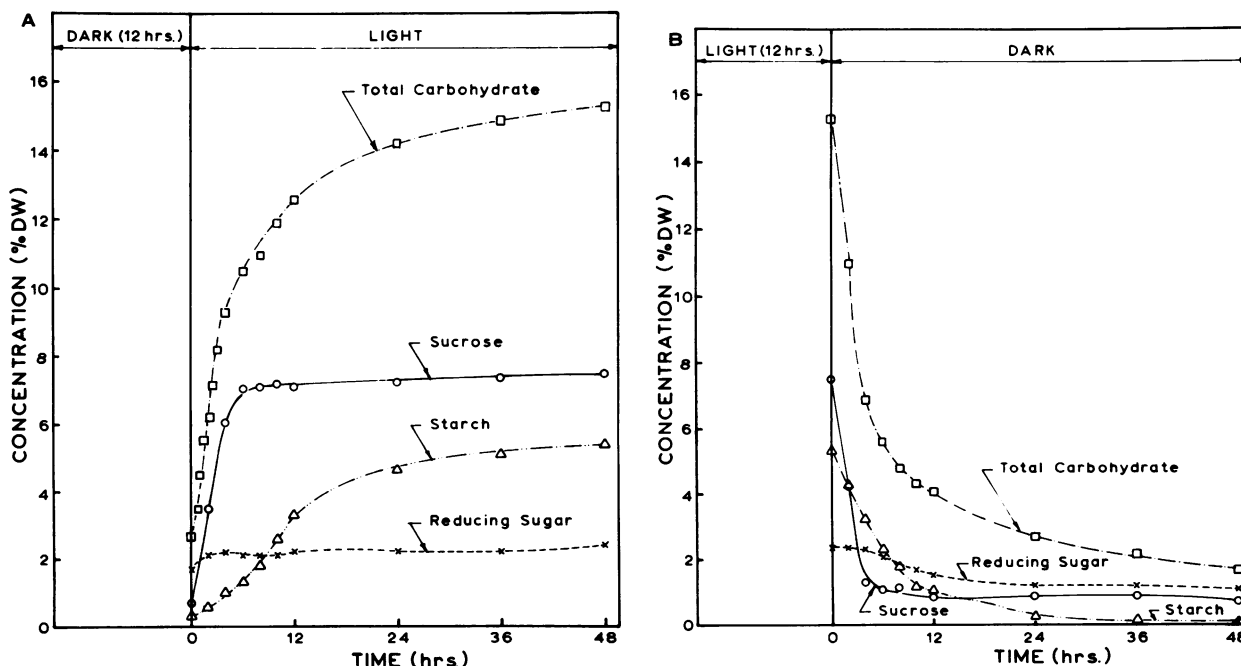


FIG. 11. A: Accumulation of carbohydrates in the light. Time course of changes in the reducing sugar, sucrose, starch, and total carbohydrates on transfer from a normal dark period (12 hr) to continuous light. Concentrations are expressed as per cent dry weight of the leaf. B: Reduction in carbohydrate levels in a maize leaf on transfer from light to dark. Same plant as used in A.

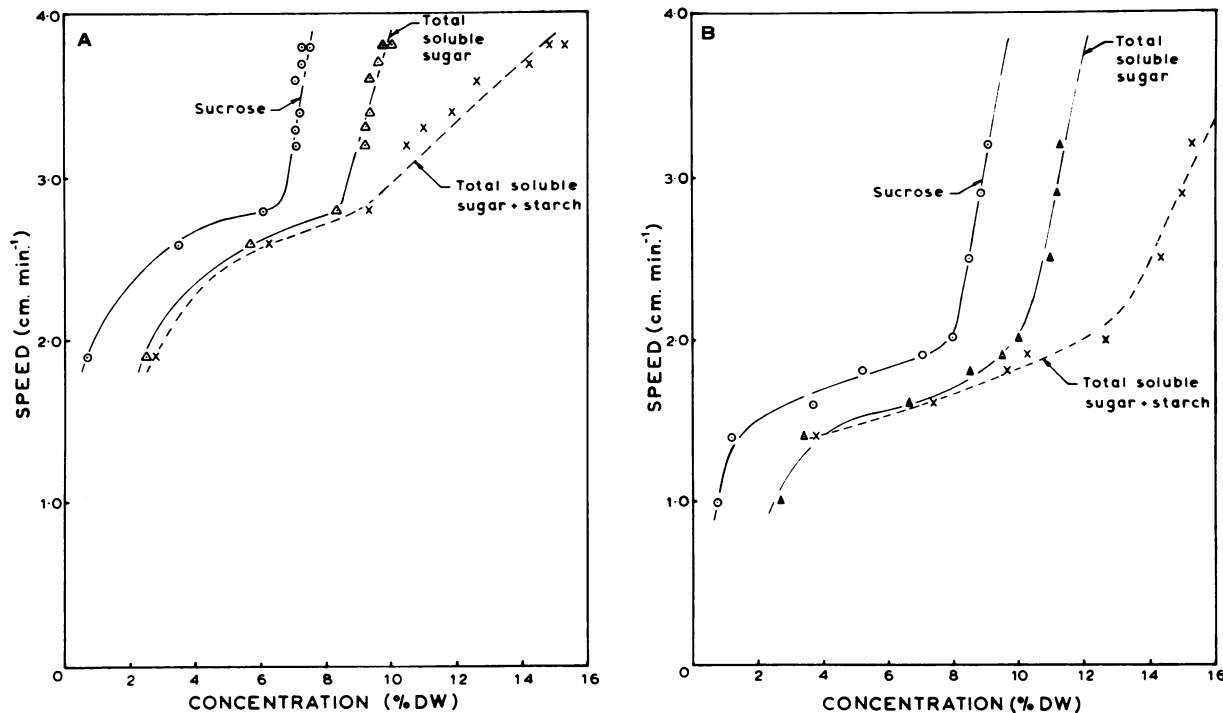


FIG. 12. Relationship between speed and the concentration of sucrose, total soluble sugars, and total soluble sugars plus starch. A: Measurements made in the light in a plant pretreated with a normal dark period (12 hr). B: Plant pretreated with 48 hr of darkness before measurements.

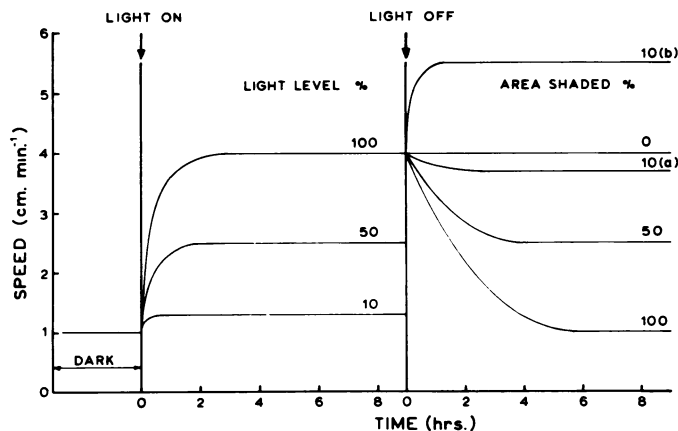


FIG. 13. Diagram of response of speed of translocation to light level and darkness as established in experiments in this paper; 100% light level was 240 w m^{-2} . 10(a): Normal response if 10% of the area upstream from the counters is shaded; 10(b): observed response when an area immediately downstream from the chamber was shaded.

cated. Sucrose may also be involved in the mechanism of translocation because it is osmotically active and it can provide C skeletons for an energy source. In considering a possible control mechanism linking C fixation-translocation-utilization, it is attractive to postulate that it would revolve about sucrose (or some other specific sugar). This would allow adjustment between the C supply and the rate of transfer to sites of utilization.

The preliminary measurements relating the gross sucrose levels in the leaf to the speed of translocation established that a biphasic response was involved. Multiphasic responses relating the rate of uptake to the concentration are familiar in ion uptake studies (18) and have also been shown for sugar uptake as a function of the external sugar solution in castor bean seedlings (19), tobacco cells (3), and in sugar beet leaves (27). These similar relationships may suggest common mechanisms but the cause of the multiphasic responses has yet to be established.

That mesophyll cells, veins, and sieve tubes are capable of accumulating sugar from an external source must be considered a basic feature of the operation of these cells. In photosynthetically active cells, sucrose is generated inside the cell but outside the chloroplast. Leakage or even diffusion of sucrose from cells may occur and the presence of an active uptake process may be significant in maintaining the sucrose within the plasmalemma. This active uptake of sucrose process presumably is significant in preventing leakage of sucrose along the sieve tubes during the translocation process. The small amounts of energy required for this mechanism to operate may be provided by the companion cells.

High speeds of translocation ($>3 \text{ cm min}^{-1}$) were recorded in maize only when the sucrose concentration exceeded 7%. For sucrose concentrations between 1 and 7%, the speed for a normal maize leaf was 2 to 2.7 cm min^{-1} at high light, although this could be reduced to 1.5 to 2 cm min^{-1} for a leaf exposed to 48 hr of darkness before the light treatment. This suggests that a prerequisite for high speeds may be a high sucrose concentration in the leaves. This relationship may be significant in interpreting previous measurements of speed where maximum speeds of about 2.5 cm min^{-1} have been indicated.

Using the C-11 technique with wheat, cotton, and tomato, the speeds were low ($<2 \text{ cm min}^{-1}$) and the sucrose levels were low ($<5\%$). In rice, however, we have measured speeds of 3.35 cm min^{-1} (35) and the concentration of sucrose in the rice leaves was about 8% (Chang, unpublished results).

The sucrose concentrations reported here are for the whole leaf tissue, whereas there are probably wide variations in sucrose concentration between cells within the leaf. The gradients in sucrose concentration within the leaf will be significant for the movement to, and loading of, sucrose into the sieve tubes (3, 27, 29). The multiphasic relationship between sucrose concentration and the speed of translocation may suggest that there is dual mechanism for sucrose accumulation into the sieve tubes.

At low sucrose concentrations (up to 8%), there is active uptake, resulting in a higher sucrose concentration in the sieve tube region than in the bulk tissue. Above a sucrose concentra-

tion of 7 to 8%, the concentration may be higher in the bulk tissue (this may be in the bundle sheath cells) than in the sieve tube zone, resulting in loading due to sucrose concentration gradients. The high sucrose concentrations in leaves may influence metabolic activity through an osmotic or hydrostatic pressure effect, such as osmotically induced biochemical alterations of enzyme activity or membrane permeability (1, 10, 12, 26, 30, 38).

Pulse-Shape Analysis. The shape of the carbon 11 isotope pulse at any position within the leaf is a consequence of numerous factors: (a) spatial resolution of the counters; (b) time course of feeding $^{11}\text{CO}_2$; (c) metabolic steps between $^{11}\text{CO}_2$ and ^{11}C sucrose; (d) mixing of labeled and unlabeled sucrose molecules; (e) transport to the sieve tube region; (f) mechanism of uptake into the sieve tubes; (g) loss of tracer from the solution flowing in the sieve tubes; (h) the mechanism of translocation; (i) the speed distribution between different sieve tubes; and (j) the speed distribution within a sieve tube. For the techniques used in this paper, the first three factors will not substantially alter the shape of the pulse and (g) and (j) have previously been suggested to be unimportant (6, 36).

Events in the fed area (factors d, e and f) have been shown, in this study, to be especially significant in determining the shape of the pulse. Results in which there was no change in shape of the pulse with distance along the leaf can be used to indicate the half-width of the loading pulse. For these results, it was shown that the half-width of 2 min for $^{11}\text{CO}_2$ fed to the leaf was transformed to 45 min between the mesophyll cells and the sieve tubes. The half-width of the pulse at the first counter rapidly broadened from 45 to 120 min (and maybe 170 min, Fig. 10) when the fed region was shaded. Furthermore, the half-width was invariably related to the light level. This can be seen directly by differentiating the efflux curves such as those shown in Figure 3. The contrast in the shape of the efflux curves for those two examples illustrates the variation in loading processes in the fed region.

The shape of the pulse was not always constant with distance along the leaf. This would be expected under nonequilibrium conditions after a perturbation was introduced, but it was also evident under apparently equilibrium conditions. A likely cause was that there was a variation in speed between sieve tubes but the increase in half-width was not always linear with distance. For example, it was approximately linear in Figure 9, B, C, and D, but not in A. Interpretation of these data would be complicated by the photosynthetic activity occurring along the length of the leaf.

These results suggest that the shape of the pulse was primarily determined in the fed region of the leaf, although there may also be an effect due to variation in speeds between sieve tubes. These conclusions are consistent with those previously reported for maize (36) and soybean (6-9).

The results provide evidence of a slow phloem-loading process and a bulk flow mechanism in the sieve tubes. There was substantial evidence for variations in both speed and half-width with distance along the leaf. These would be expected in a system such as the maize leaf where sucrose is being loaded along the length of the system investigated. In the Münch scheme for translocation (23, 24), the driving force for bulk flow is a pressure gradient from source to sink. The high speeds recorded in this study suggest that large pressure gradients are present with low hydraulic conductivity of the maize sieve tubes. Whether the pressure differentials required to sustain the high speeds recorded in maize would occur over longer distances (such as in trees) remains an important aspect of translocation for future investigation.

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