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# Leaf Development and Phloem Transport in *Cucurbita pepo*: Transition from Import to Export

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*Summary.* The capacity of a growing leaf blade of *Cucurbita pepo* L. to import $^{14}C$-labelled photoassimilate is lost in a basipetal direction. Import into the lamina tip stops when the blade is 10% expanded. Development of the leaf progresses linearly with time and the lamina base stops importing when the blade is 45% expanded. Export capacity also develops basipetally and follows immediately the loss of import capacity, at least in the lamina base. The small amount of material initially exported from the leaf tip is redistributed to the still-importing leaf base, delaying export from the lamina until the blade is 35% expanded. Loss of import capacity by the petiole is both basipetal and dorsoventral. The proximal, adaxial portion of the petiole is the last region to cease importing $^{14}C$. Leaves of *Beta vulgaris* L. and *Nicotiana tabacum* L. also lose import capacity in a basipetal direction.

## Introduction

During early growth, from initiation to unfolding, leaves depend upon more mature regions of the plant for their nutrition. As the leaf expands its dependence upon older leaves, as measured by the influx of $^{14}C$ labelled translocate, rapidly diminishes and it soon begins to export material derived from its own photosynthetic assimilation of carbon (Milthorpe and Moorby, 1969). An individual leaf may be considered an asset to the carbon economy of the whole plant when it becomes a net supplier of photosynthate. A more complete understanding of the transition of the leaf from an importing to an exporting organ is therefore fundamental to the study of plant nutrition and productivity. Further, a knowledge of the conditions which immediately precede export from the leaf may give insight into the mechanisms of vein loading and translocation.

An intriguing feature of this period of leaf development is the demonstrated ability of a single leaf, when approximately 50% fully expanded, to begin the export of assimilate before import has completely ceased (Jones *et al.*, 1959; Thrower, 1962; Webb and Gorham, 1964). It has been suggested (Jones and Eagles, 1962) that this apparent bidirectional transport is a consequence of basipetal maturation of the leaf blade, with

export proceeding from the more mature leaf tip while import continues into the relatively immature leaf base. The following experiments were designed to test this hypothesis, primarily with expanding leaves of *Cucurbita pepo*.

## Materials and Methods

*Growth of Plants.* Seeds of the Early Prolific Straight-neck squash, *Cucurbita pepo* L. var. *melopepo* f. *torticollis* Bailey, were germinated in Perlite in a growth chamber with a daylength of 18 h at 23–26°. Light was supplied by fluorescent and incandescent lamps with an intensity, at the level of leaf 5, of 400 microeinsteins $m^{-2}$ $sec^{-1}$ between 400 and 700 nm as measured by a LI-190S quantum sensor (Lambda Instruments, Lincoln, Nebraska). When the plants were 7 days old they were planted in individual pots in Perlite and placed in a greenhouse at a controlled temperature of ca. 23°. The daylength was extended to 18 h with supplementary illumination from incandescent lamps (80 microeinsteins $m^{-2}$ $sec^{-1}$). The plants were fed daily with a modified Hoagland's solution.

*Nicotiana tabacum* L. and *Beta vulgaris* L. plants were grown in soil in the greenhouse under the same conditions of daylength and temperature.

*Choice of Leaves.* Leaves were numbered consecutively beginning with the primary leaf. Leaf 5 of *C. pepo* was chosen for specific study. In experiments where transport of $^{14}C$ to leaf 5 was studied leaf 3 was used as the source leaf, *i. e.* supplied $^{14}CO_2$. Preliminary experiments had shown that throughout the developmental period studied leaf 3 was translocating maximally.

*Leaf Age.* The age of *C. pepo* plants was determined by the plastochron index (PI) developed by Erickson and Michelini (1957). The PI establishes the age of a plant on a morphological rather than a temporal basis. In this way the development of plants with different rates of growth may be closely compared (Michelini, 1958; Maksymowych and Erickson, 1960; Dickmann, 1971; Larson and Isebrands, 1971).

The length of emergent leaves at the shoot apex is used as the morphological scale of development, the plant being $n$ plastochrons in age when the length of serial leaf $n$ is equal to a standard value. Between integral plastochron units PI is determined using the formula of Erickson and Michelini (1957). In the present study a petiole length of 30 mm was chosen as the standard after consideration of the rates of initiation and growth of emergent leaves.

The PI may be used to establish the age of whole plants or, as in the present study, to determine the age of a single leaf. In the latter case the leaf age is best expressed by the leaf plastochron index (LPI) which is obtained simply by subtracting the serial number of the leaf under study from the PI. When the petiole of leaf 5 is 30 mm long the PI is 5 and the LPI of leaf 5 is 0.

An analysis of the growth of a number of individual plants indicated that the LPI is linearly related to time over the developmental period studied. Plastochron intervals of individual plants ranged between 2.00 and 2.28 days per plastochron with a mean value of 2.12 days per plastochron.

*$^{14}CO_2$ Labelling.* Plants selected for experimentation were brought to a laboratory fume hood and illuminated with water-filtered incandescent lights at 100 microeinsteins $m^{-2}$ $sec^{-1}$ at the level of leaf 5. Following a 30-min equilibration period the leaf blade to be labelled was enclosed in a polyethylene bag sealed around the top of the petiole with a wire tag.

$^{14}CO_2$ was generated on the piston of a 50-ml syringe by the addition of an excess of 5N $H_2SO_4$ to 100 μCi $Na_2{}^{14}CO_3$ (95% $^{14}C$) and introduced to the leaf by inserting the needle of the syringe into the bag and pumping the piston for 15 s. Photosynthesis in $^{14}CO_2$ was allowed to continue for 5 min and the polyethylene bag was removed, During the labelling period the petiole of the fed leaf was wrapped in aluminum foil and the rest of the plant was covered with a polyethylene bag to prevent photosynthetic incorporation of $^{14}CO_2$ which may have escaped during the fixation period.

*$^{14}C$ Analysis.* At the end of the experimental period plant parts were rapidly excised and immediately frozen in liquid nitrogen, ground with a pestle and mortar, and the fine powder extracted with 80% ethanol at 70° for 2 h. The insoluble powder was removed by filtration, washed with 80% ethanol, and air-dried. Triplicate 50-μl aliquots of the ethanol extract were counted with a Packard Tri-Carb scintillation counter in a mixture containing 5 g/l of 2,5-diphenyloxazole (PPO) in toluene containing 15% methanol. The $^{14}C$ content of dried, insoluble samples was determined by combusting the powder and collecting and counting the evolved $^{14}CO_2$ by the technique of Watson and Williams (1970). All scintillation samples were corrected for quenching with an external $\gamma$ source. Results are expressed as the combined values of soluble and insoluble $^{14}C$.

*Autoradiography of Whole Leaves.* Leaves were cut from the plant and immediately placed between pre-cooled glass plates at —38°. Freezing of leaves appeared to take place within seconds. The frozen leaves were transferred to pre-cooled Kodak No-Screen X-ray films and exposed for 2 or 3 days at —38° before developing. Unlabelled leaves did not produce any noticeable darkening of X-ray sheets.

*Translocation Period.* Preliminary experiments with *C. pepo* indicated that within 2 h of supplying a 5-min pulse of $^{14}CO_2$ to leaf 3 the $^{14}C$ imported into leaf 5 had reached 90% of its maximum level. Therefore 2 h was chosen as a suitable translocation period. Following previous work, translocation periods of 2 h for *Beta vulgaris* (Geiger and Swanson, 1965), and 6 h for *Nicotiana tabacum* (Jones *et al.*, 1959) were used.

## Results

### *1. Cessation of $^{14}C$ Import into the Leaf Blade of* C. pepo

36 plants were selected in which the lamina of leaf 5 was between 5 and 60% expanded. Leaf 3 was offered $^{14}CO_2$ and following 2 h of translocation the lamina of leaf 5 was frozen and autoradiographed. Photographs of representative autoradiographs are shown in Fig. 1.

When very young, the entire leaf imports $^{14}C$ (Fig. 1a). As the leaf grows the capacity to import is lost progressively, beginning with the leaf tip (Fig. 1b), and continuing basipetally along the leaf length (Fig. 1c, d). Autoradiographs of leaves undergoing this development show a sharp line of demarcation, at right angles to the midrib, between importing (blackened) and non-importing (non-blackened) regions. Basipetal loss of import capacity also begins at the tips of the leaf lobes (Fig. 1c).

In a few experiments the petiole of an importing leaf was steam-girdled, or a cold block at 0° was applied (Webb, 1967). These treatments,

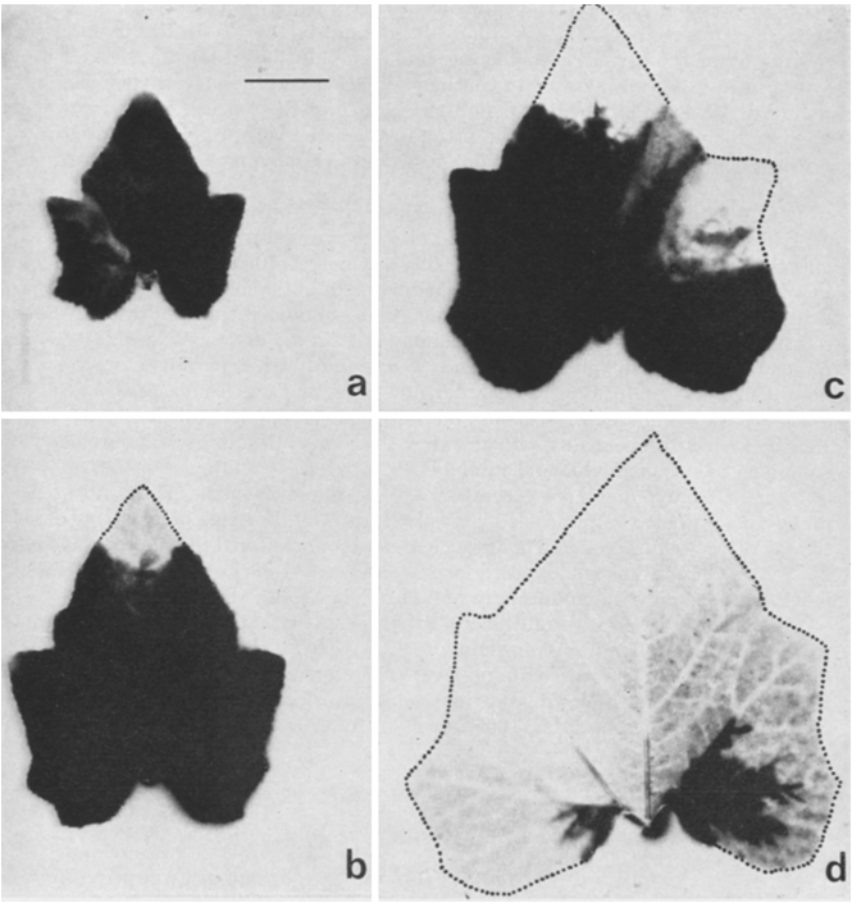


Fig. 1a—d. Autoradiographs of lamina 5 of four *Cucurbita pepo* plants which imported $^{14}C$ from leaf 3 for 2 h. Leaf ages were: a, LPI 0.2; b, LPI 0.4; c, LPI 0.8; d, LPI 1.1. Scale = 2 cm; all photographs are the same magnification

which inhibit phloem transport, stopped the flow of $^{14}C$ into the importing leaf except for a trace quantity of label which became evenly distributed throughout the lamina. This trace quantity of $^{14}C$ is also seen in the distal, non-importing region of partially importing leaves (Fig. 1b, d).

The degree of development of individual leaves was estimated by measuring the distance on the autoradiographs from the junction of the lamina and petiole to the line which demarcates the importing from the non-importing regions of the blade, and expressing this distance as a percentage of the total lamina length. This function is termed the percentage of leaf blade importing, and is plotted against LPI in Fig. 2.

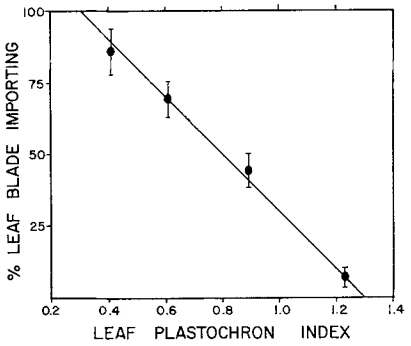


Fig. 2. Development of leaf 5 of *Cucurbita pepo* expressed as the percentage of the total lamina length still importing $^{14}C$. Vertical bars = twice the standard error of the mean

The progressive basipetal loss of import capacity is linearly related to LPI and therefore to time (Fig. 2). Loss of import capacity at the tip begins at LPI 0.3 or 10% leaf expansion (Fig. 3), and by LPI 1.3 (45% leaf expansion) the lamina no longer imports $^{14}C$. The time required to complete this phase of development is 1.0 plastochrons or approximately 2 days.

Under these growth conditions one leaf blade of *C. pepo* undergoes basipetal loss of import capacity at a time. In experiments where leaves 4, 5 and 6 were autoradiographed and leaf 5 was undergoing basipetal loss of import capacity the whole of the blade of leaf 6 was importing while import to the lamina of leaf 4 had completely stopped.

A quantitative estimate of the ability of leaf 5 to import photoassimilate from leaf 3 was made from labelling experiments in which the total $^{14}C$ content of the lamina and petiole of leaf 5 and the rest of the plant was measured following 2 h of translocation of $^{14}C$ from leaf 3. The results are shown in Fig. 4 where the amount of $^{14}C$ in leaf 5 is expressed as a percentage of the total $^{14}C$ translocated from the lamina of leaf 3 in 2 h.

The maximal rate of import into the lamina of leaf 5 occurs at approximately LPI 0.6, well after the leaf tip has lost the capacity to import. This apparent paradox may be due to increasing import by the relatively large importing leaf base. Import to the lamina ceases at LPI 1.3, corroborating the results obtained from whole leaf autoradiography.

### *2. Cessation of $^{14}C$ Import into the Petiole of* C. pepo

Import of $^{14}C$ into the petiole of leaf 5, expressed as a percentage of the $^{14}C$ translocated from leaf 3, increases to a maximum at approximately

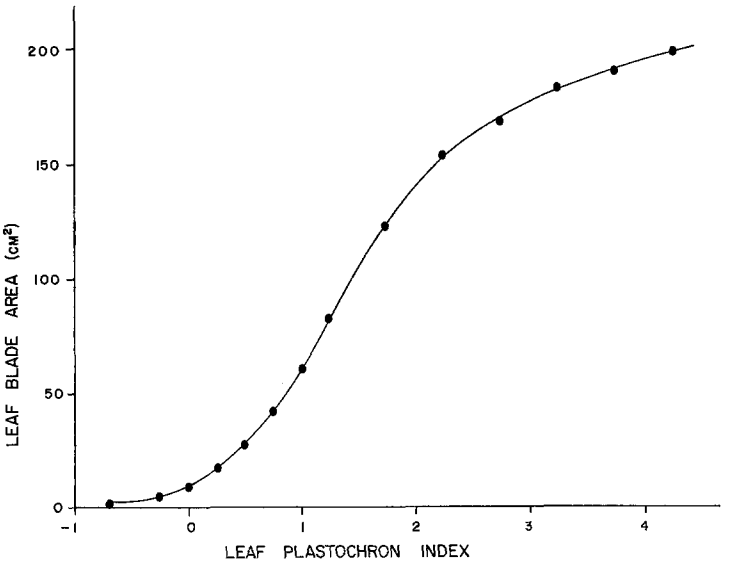


Fig. 3. Area of lamina 5 of *Cucurbita pepo* plotted against leaf age

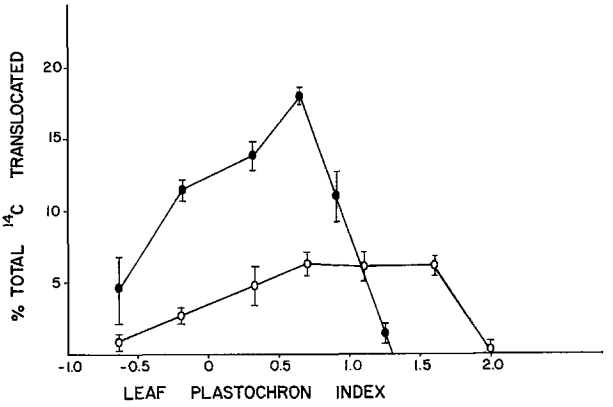


Fig. 4. $^{14}C$ activity in the lamina (●) and petiole (○) of leaf 5 of *Cucurbita pepo* expressed as a percentage of the total $^{14}C$ translocated from the lamina of leaf 3 in 2 h. Vertical bars = twice the standard error of the mean

the same time as import to the blade ceases (LPI 1.3), and drops rapidly to zero at LPI 2.0 (Fig. 4).

Autoradiographs of whole leaves between the ages of LPI 1.3 and 2.0 suggest that the loss of import capacity of the petiole is also progressive and basipetal (autoradiographs not shown). In addition, the loss of import

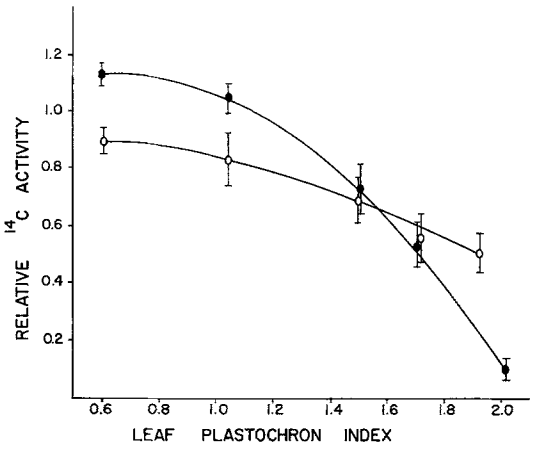


Fig. 5. Relative $^{14}C$ activity in the distal (●) and abaxial (○) halves of importing petioles of leaf 5 of *Cucurbita pepo* compared to the activity in the proximal and adaxial halves respectively. Vertical bars represent twice the standard error of the mean

capacity appears to begin at the abaxial side of the hollow petiole before the adaxial side.

In a quantitative experiment with 30 plants leaf 3 was fed $^{14}CO_2$ and after 2 h the petiole of leaf 5 was excised. The petiole was divided in half transversely and again longitudinally into adaxial and abaxial halves. Each of the quadrants was weighed and the $^{14}C$ content analyzed. The results were pooled in such a way that the amount of $^{14}C$ per unit fresh weight in the distal half of the petiole could be calculated relative to the $^{14}C$ in the proximal half. Similarly the amount of $^{14}C$ in the abaxial half was calculated relative to that in the adaxial half.

The results, plotted against leaf age in Fig. 5, indicate that at approximately LPI 1.2 the amount of $^{14}C$ per unit fresh weight imported to the distal half of the petiole relative to the proximal half begins to decrease, *i. e.* the capacity to import $^{14}C$ is lost in a basipetal direction. At the same time there is a similar though less pronounced dorsoventral loss of import capacity beginning at the abaxial side of the petiole.

### *3. Cessation of $^{14}C$ Import into Leaves of* Beta vulgaris *and* Nicotiana tabacum

Autoradiographic experiments similar to those described for *Cucurbita pepo* were conducted with *Beta vulgaris* and *Nicotiana tabacum* to establish the pattern of development in these species. Mature leaves of

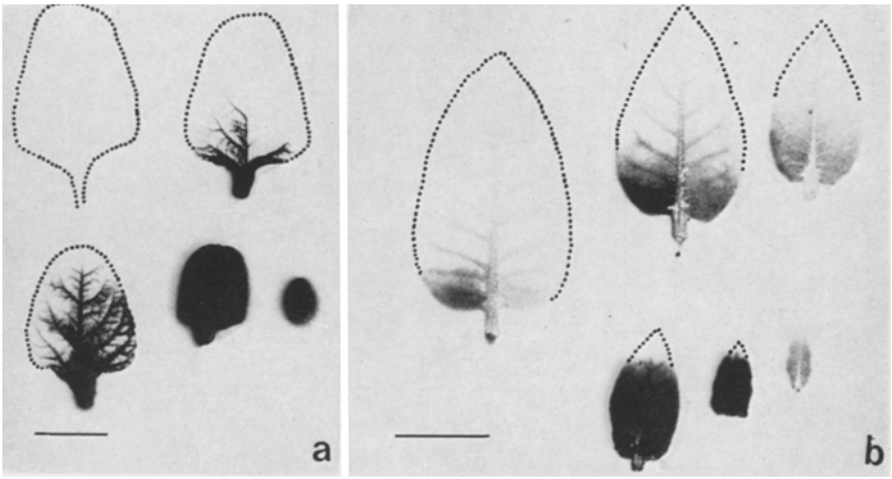


Fig. 6a and b. Autoradiographs of importing leaves of *Beta vulgaris* (a), *Nicotiana tabacum* (b). In both a and b all leaves are from the same plant. Scales = 3 cm

sugarbeet (Joy, 1964) and tobacco (Jones *et al.*, 1959), unlike squash (Webb and Gorham, 1964), translocate primarily along the same orthostichy. Therefore three successive mature leaves of these species were offered $^{14}CO_2$. Leaves 8, 9 and 10 of a 10-week-old sugarbeet plant were labelled and leaves 11 to 15 were frozen and autoradiographed following 2 h of translocation (Fig. 6a). In tobacco, leaves 9, 10 and 11 of an 8-week-old plant were labelled and following 6 h of translocation leaves 13 to 18 were frozen and autoradiographed (Fig. 6b).

Basipetal loss of import capacity in sugarbeet and tobacco leaves is demonstrated by these autoradiographs. The pattern of development in sugarbeet and tobacco leaves differs from that of squash, at least under our experimental conditions, in that many leaves on the same plant, rather than one, are undergoing basipetal loss of import capacity simultaneously.

### *4. Development of Export Capacity of* C. pepo *Leaves*

The development of export capacity of maturing cucurbit leaves was determined by exposing the lamina of leaf 5 to $^{14}CO_2$ and analyzing the $^{14}C$ content of the fed leaf blade and the rest of the plant after a further 2 h. In Fig. 7 the results, from a total of 40 plants, are expressed as the percentage of total fixed $^{14}C$ found outside the fed leaf blade with increasing leaf age.

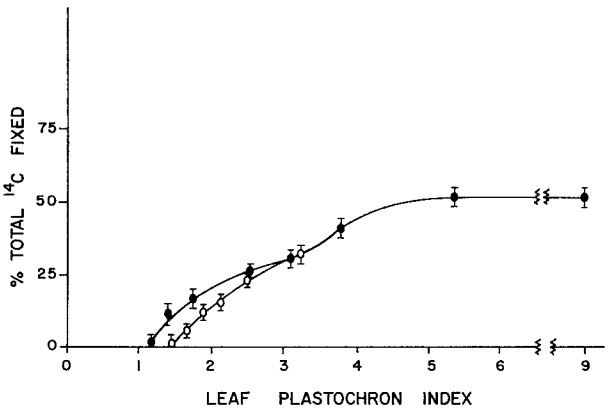


Fig. 7. The amount of $^{14}C$ translocated from the lamina of leaf 5 of *Cucurbita pepo* in 2 h expressed as a percentage of the total $^{14}C$ fixed. Either the entire lamina (●) or just the extreme base of the lamina (○) was allowed to fix $^{14}CO_2$. Vertical bars = twice the standard error of the mean

Transport from the leaf blade begins at LPI 1.1 (35% leaf expansion) and the amount translocated rises sharply before leveling off and again rising at approximately LPI 3.5 (90% leaf expansion) to a maximum of 50% of the total $^{14}C$ fixed.

Does the capacity to export develop basipetally in a pattern similar to the loss of import ability? If export capacity develops basipetally, covering all but the extreme proximal portion of the lamina, to prevent photosynthetic assimilation of $^{14}CO_2$, should retard the export of $^{14}C$ until the "front" of development reaches the base of the leaf blade.

To investigate this possibility lamina 5 of 20 plants was covered, except for the extreme base, with aluminum foil secured to the leaf surface with petroleum jelly. In Fig. 7 it can be seen that export of $^{14}C$ from these partially covered leaf blades does not begin until LPI 1.3. This lag strongly suggests that export ability must develop basipetally following closely in pattern the loss of import capacity.

### *5. Translocation within the Lamina*

Examination of Fig. 2 and 7 indicates that export from the leaf blade begins at LPI 1.1 although import by the leaf tip ceases much earlier, at LPI 0.3. This suggests a lag period of approximately 0.8 plastochrons (2 days) between the end of the import period and the beginning of export at the leaf tip. At the leaf base, on the other hand, export immediately follows loss of import capacity (LPI 1.3).

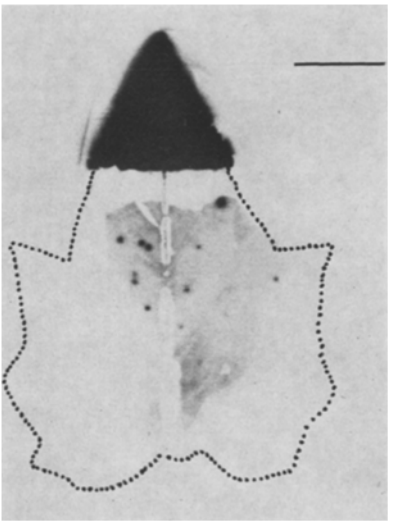

Fig. 8. Autoradiograph of lamina 5 of *Cucurbita pepo*. Although the leaf blade was too young to export (LPI 0.7), $^{14}C$ activity was present in the midrib and lamina outside the fed tip-region 2 h after $^{14}CO_2$ was administered. Petroleum jelly, used to secure the aluminum foil to the leaf, has prevented exposure of the X-ray film to the strip of lamina adjacent to the heavily labelled tip. Scale = 1.5 cm

However, during the period between the cessation of import at the leaf tip and the beginning of export from the lamina, the base of the lamina is still importing. During this period material may be exported from the leaf tip only to be assimilated by the importing lamina base, thereby delaying export from the leaf blade.

Two experiments were designed to discover possible transport from the distal to the proximal region of non-exporting leaf blades. In the first experiment $^{14}CO_2$ was applied locally to the leaf tip by covering the base of the leaf with aluminum foil held securely to the leaf surface with petroleum jelly and leaving only the tip of the leaf exposed. $^{14}CO_2$ was presented to the leaf for 1 min; 2 min later the aluminum foil was removed. 2 h later the leaf blade was excised, frozen, and autoradiographed for 11 days.

Autoradiographs of leaves as young as LPI 0.7 showed significant $^{14}C$ label in the midrib and lamina of the basal region indicating export from the leaf tip (Fig. 8). In control experiments the portion of the lamina which had not been fed $^{14}CO_2$ was covered with crushed ice during the 2 h translocation period. In these experiments no transport of $^{14}C$ could be detected, indicating that the movement of $^{14}C$ occurs in the phloem (Webb, 1970).

In a second experiment it was reasoned that removal of basal, importing mesophyll tissue might induce precocious transport out of otherwise non-exporting leaves. Ten plants were chosen in which the age of leaf 5 was between LPI 0.2 and 1.0. All the basal mesophyll tissue and the major lateral veins were removed from leaf 5 with a razor blade, leaving only the tip of the leaf distal to the upper leaf lobes intact and attached to the petiole by the undisturbed midrib. These leaves were offered $^{14}CO_2$ for 5 min and after 2 h the amount of $^{14}C$ exported from the leaf blades was measured.

Leaves as young as LPI 0.8 exported significant though small amounts of $^{14}C$. Six leaves between the ages of LPI 0.8 and 1.0 exported $9.3 \pm 3.4\%$ of the total $^{14}C$ fixed. Unaltered leaves of identical age did not export more than 0.7% of the total $^{14}C$ fixed. These experiments clearly indicate that transport from the tip to the base of the lamina occurs prior to export from the leaf blade.

## Discussion

The maturation of dicotyledonous leaves progresses basipetally except for the acropetal development of the major venation (Esau, 1960). Our investigations have shown that the functional development of a leaf from an importing to an exporting organ progresses in a basipetal fashion. The suggestion of Jones and Eagles (1962) that bidirectional transport within a single leaf is a consequence of export from the tip and import by the base has been substantiated.

The tip of leaf 5 of *Cucurbita pepo* stops importing $^{14}C$ labelled photoassimilate from mature leaves when the lamina is approximately 10% expanded. Progressive basipetal loss of import capacity is linearly related to time and continues through the length of the lamina and petiole. Import capacity is also lost basipetally in leaves of *Beta vulgaris* and *Nicotiana tabacum*.

In addition to basipetal loss of import capacity the abaxial side of the petiole begins to lose the ability to import before the adaxial side. Similarly, vascular differentiation begins at the abaxial side of young petioles of *Luffa cylindrica* (*Cucurbitaceae*) and extends toward the adaxial side (Shah and Jacob, 1969).

Transport from mature leaves into importing leaves of materials labelled by $^{14}CO_2$ assimilation occurs primarily in the phloem although trace quantities of $^{14}C$ pass through blocks to phloem transport and are distributed evenly throughout the leaf blade. These materials undoubtedly travel in the transpiration stream.

Just prior to loss of import capacity by the lamina base the leaf blade begins the export of $^{14}C$ labelled photosynthate. The delay of export

caused by covering all but the extreme basal region of the lamina strongly suggests that export capacity develops basipetally.

Export from the lamina base begins at the same time that import ceases (45% leaf expansion). This suggests that in all regions of the lamina loss of import capacity is immediately followed by the beginning of export. The fact that cessation of import by the leaf tip is not immediately followed by export from the lamina is explained by the redistribution of translocate from the leaf tip to the still-importing leaf base. This creates a situation wherein an increasingly larger source (the distal end of the lamina) exports to a steadily diminishing sink (the proximal end of the lamina). Eventually the supply to the lamina base exceeds the demand and the excess is translocated out of the leaf blade (35% leaf expansion). Shortly thereafter the entire leaf blade exports (45% leaf expansion).

It should be noted that although import to the leaf tip ceases at 10% leaf expansion we are not able to demonstrate export from the tip and subsequent redistribution within the lamina until 20% leaf expansion (LPI 0.7). Initial export from the leaf tip is either too low to be detected by our methods or does not immediately follow the loss of import capacity.

The demonstration of intra-laminar phloem transport does not agree with the results of Larson *et al.* (1972) who could find no evidence of redistribution within the lamina of eastern cottonwood leaves. The difference in our results may be due to the transient nature of intra-laminar transport. In our experiments with cucurbit leaves redistribution could only be detected in leaves at LPI 0.7. Redistribution probably ceases when import to the base of the lamina stops at LPI 1.3. This is a period of only 0.6 plastochrons. Larson *et al.* (1972) studied leaves over an 8-plastochron period in increments of 1 plastochron. If the developmental patterns of these two plants are comparable intra-laminar transport could easily have been overlooked.

It must be emphasized that our results are not in conflict with the commonly held view that exporting leaves will not under ordinary circumstances either import from other mature leaves (Canny, 1962) or redistribute photosynthate within the leaf blade (Nakata and Leopold, 1967). The redistribution which we have demonstrated is a consequence of basipetal development of the export function of the phloem; it is transient in nature and is not expected to continue in a fully exporting leaf.

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## References

Canny, M. J.: The mechanism of translocation. Ann. Bot. **26**, 603–617 (1962).

Dickmann, D. I.: Chlorophyll, ribulose-1,5-diphosphate carboxylase, and Hill reaction activity in developing leaves of *Populus deltoides*. Plant Physiol. **48**, 143–145 (1971).

Erickson, R. O., Michelini, F. J.: The plastochron index. Amer. J. Bot. **44**, 297–305 (1957).

Esau, K.: Anatomy of seed plants. New York: John Wiley & Sons 1960.

Geiger, D. R., Swanson, C. H.: Sucrose translocation in the sugar beet. Plant Physiol. **40**, 685–690 (1965).

Jones, H., Eagles, J. E.: Translocation of $^{14}$carbon within and between leaves. Ann. Bot., N.S. **26**, 505–510 (1962).

Jones, J., Martin, R. V., Porter, H. K.: Translocation of $^{14}$carbon in tobacco following assimilation of $^{14}$carbon dioxide by a single leaf. Ann. Bot., N.S. **23**, 493–508 (1959).

Joy, K. W.: Translocation in sugar beet. I. Assimilation of $^{14}CO_2$ and distribution of materials from leaves. J. exp. Bot. **15**, 485–494 (1964).

Larson, P. R., Isebrands, J. G.: The plastochron index as applied to developmental studies of Cottonwood. Canad. J. Forest Research **1**, 1–11 (1971).

Larson, P. R., Isebrands, J. G., Dickson, R. E.: Fixation patterns of $^{14}$C within developing leaves of eastern cottonwood. Planta (Berl.) **107**, 301–314 (1972).

Maksymowych, R., Erickson, R. O.: Development of the lamina in *Xanthium italicum* represented by the plastochron index. Amer. J. Bot. **47**, 451–459 (1960).

Michelini, F. J.: The plastochron index in developmental studies of *Xanthium italicum* Moretti. Amer. J. Bot. **45**, 525–533 (1958).

Milthorpe, F. L., Moorby, J.: Vascular transport and its significance in plant growth. Ann. Rev. Plant Physiol. **20**, 117–138 (1969).

Nakata, S., Leopold, A. C.: Radioautographic study of translocation in bean leaves. Amer. J. Bot. **54**, 769–772 (1967).

Shah, J. J., Jacob, R.: Development and structure of phloem in the petiole of *Luffa cylindrica*. Amer. J. Bot. *56*, 821–831 (1969).

Thrower, S. L.: Translocation of labelled assimilates in the soybean. II. The pattern of translocation in intact and defoliated plants. Aust. J. biol. Sci. **15**, 629–649 (1962).

Watson, G. R., Williams, J. P.: Rapid method for wet combustion and scintillation counting of $^{14}$C-labelled organic materials. Ann. Biochem. **33**, 356–365 (1970).

Webb, J. A.: Translocation of sugars in *Cucurbita melopepo*. IV. Effects of temperature change. Plant Physiol. **42**, 881–885 (1967).

Webb, J. A.: The translocation of sugars in *Cucurbita melopepo*. V. The effect of leaf blade temperature on assimilation and transport. Canad. J. Bot. **48**, 935 to 942 (1970).

Webb, J. A., Gorham, P. R.: Translocation of photosynthetically assimilated $C^{14}$ in straight-necked squash. Plant Physiol. **39**, 663–672 (1964).