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Translocation of ^{14}C in *Macrocystis pyrifera* (Giant Kelp)[1]

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ABSTRACT

The pattern of import and export of ^{14}C-labeled assimilates in *Macrocystis pyrifera* (L.) C. A. Agardh in southern California was studied by labeling single blades on fronds, *in situ*, with [^{14}C]$NaHCO_3$ for 24 hours. The pattern was found to be similar to that known in dicotyledons: actively growing tissue imported and did not export. As a blade reached maturity it began to export, at first only acropetally to the apex which formed it, later also down the frond to sporophylls and frond initials at the base of the frond, and into the apical regions of juvenile fronds; finally there was a phase of declining export, late in the life of the blade, when transport was only downward. Young fronds imported from older fronds until they were approximately 3 meters long, by which time they had developed mature, upward exporting blades. No translocation was found from a younger frond to an older frond, nor was there transport upward from a blade on a frond lacking the apical region.

It has long been supposed that brown algae of the order Laminariales are able to transport photoassimilated carbon compounds within their thalli, through the network of sieve elements. Early studies concentrated on the anatomy of the sieve plates, although it was recognized that whereas in vascular plants the sieve elements are part of a tissue (phloem), sieve elements in kelps are not closely associated with companion cells or parenchyma (2). Only in the last few years have details of kelp sieve element ultrastructure been published (16–18, 20). These studies have underlined the differences between phloem and kelp sieve elements already expected from the vast evolutionary separation of the two plant groups. Investigation of translocation in kelps has also lagged far behind vascular plant studies. Deduced evidence for translocation in *Macrocystis* (1, 14) was not replaced by direct study with tracers until 1963 (11). Parker (12) subsequently demonstrated movement of ^{14}C and fluorescein dye both upward and downward in the stipe of *M. pyrifera* (L.) C.A. Agardh from a source blade, and into other blades including the apical scimitar.[3] It has recently been shown in *Nereocytis leutkeana* (Mert.) P. and R. (7) and *Laminaria hyperborea* (Gunn.) Fosl. (21) that the sieve elements are the conduits for translocation in kelps. In these morphologically simpler kelps it has been established that the distal (mature) region of the lamina exports to the meristematic region at the junction of the stipe and the lamina(e), and to haptera (5, 7, 15). The *Macrocystis* thallus, however, is not such a simple, linear arrangement of source and sinks, but consists of many fronds each with several meristematic regions separated by mature regions. Parker's experiments (11, 12) did not show translocation into young fronds, nor did they detail which blades are sources of assimilates and which are sinks. The objective of the present work was to provide this information, which is a prerequisite to more detailed studies on translocation in *Macrocystis*.

MATERIALS AND METHODS

Work *in Situ*. Experiments were carried out underwater on 52 plants near Arch Rock, Corona del Mar, Calif. The depth of water in the study area was 8 to 10 m at low tide. A single blade somewhere on a frond was selected as the experimental blade, hereafter referred to as the labeled blade. A clear polyethylene bag, with a serum cap held in the side by a short piece of plastic tubing, was sealed around the base of the pneumatocyst with a rubber band, enclosing about 2 liters of the surrounding sea water. Radioactive sodium bicarbonate (about 250 μCi of [^{14}C]$NaHCO_3$) was injected into the bag through the serum cap and mixed with the sea water by agitating the bag. The incubation period was normally 24 hr.

Sampling and Analysis. At the end of the experiment the labeled frond and the young fronds associated with it were cut from the plant and brought to the surface (small plants were brought up whole), where the labeled blade was cut from the stipe with the bag of radioactive sea water still intact around it. The fronds were transported to the laboratory (a trip of about 30 min), where they were measured. A record was made of the relative positions of the fronds and the blades. The fronds (usually in pieces) were stretched out on lines to dry. Blades were then picked off and put into numbered bags. All blades on short fronds were sampled for ^{14}C content, but on longer fronds only the basal laminae (including sporophylls and frond initials), apical laminae (apical scimitar and immature free blades), and a few blades on each side of the labeled blade were kept for sampling. The dried laminae were sent to Simon Fraser University for analysis.

Analysis of total ^{14}C content was conducted on a single, weighed sample of about 25 mg dry wt from the proximal region of each lamina, where autoradiography showed that most activity accumulated (Fig. 1). Nonuniformity of activity in sink blades, combined with the very large number of blades to be sampled, precluded a quantitative study. Because the analysis was qualitative, loss of radiocarbon from blades during drying and storage was considered inconsequential, even though some volatile compounds undoubtedly escaped (23). The dry tissue sample was placed in a scintillation vial, rewetted with 0.1 to 0.2 ml of water, and digested for liquid scintillation counting by the Mahin-Lofberg perchloric acid-hydrogen peroxide method (3). Raw data were corrected for quenching by an external standard counting efficiency curve. Since the background of the vials and cocktail could not be precounted, 25 cpm background was assumed and subtracted from the raw counts, but in view of the uncertainty of the background, and on the basis of values obtained in several blank trails, I chose 100 dpm/10 mg dry wt as the lowest level of "significant" activity.

The data are summarized in qualitative diagrams. The tables of data from which these diagrams were prepared have been lodged in the Depository of Unpublished Data, National Science Library,

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[3] The apical scimitar is the blade at the tip of the frond in which new blades are formed (see Fig. 1).

National Research Council of Canada, Ottawa, Ontario K1A OS2, whence they are available for a nominal charge.

Autoradiographs of plant material were prepared by exposing x-ray film sheets for up to 12 days to tissue dried onto herbarium sheets.

Naming of Fronds. I devised a system for naming fronds to show their ontogenetic relationships (*cf.* Figs. 3 and 4). The first pair of fronds produced by the young plant is called *1°*. Each of these fronds in turn produces one (rarely two) new frond from the frond initial, the lowest blade on the frond; each of the new fronds is called *2°*. The *2°* fronds, and all successive fronds, produce two new fronds each: the ones from the first frond initial (lowest blade on the frond) are termed *3°*, *4°*, *5°*, etc. Those from the second frond initial (immediately above the first) are termed *2′*, *3′*, *4′*, etc. Thus *2°* → *3°* and (later → *2′*. In cases where the branching cannot be followed back to the *1°* fronds or their remains, the fronds are called *x°* (the oldest under study), *x+1°* and *x′*, *x+2°*, and *x+1′*, etc. The two fronds produced by *2′* are called *2″* and 2^{x}. (It was not necessary to name higher order branches because the original branch could not be traced, and *x°* was used.)

RESULTS

Autoradiography (Fig. 1) showed that import of ^{14}C is only by the proximal, meristematic region of the blades. There was an increase in area accumulating radioactivity as the blades matured, and a gradation in activity from the pneumatocyst toward the distal part of the lamina.

The results of the translocation experiments (Figs. 2–4) showed that immature blades imported but did not export. As the distance of a blade from the apex approached 1 m export began (Fig. 2). The blade was still not fully grown at this stage, and import continued. There was a transition region from about 0.75 m to 1.1 m from the apex where export began. Downward export began when the blade was about 3.6 m from the apex, the transition region in this case being rather wider than the first: from about 3.5 to 4.5 m from the apex. The two *2°* fronds in Figure 3 show the difference in export direction with blade position. Upward export ceased approximately 6 m from the apex; the remainder of the sterile blades exported only down the frond.

Apical scimitars and frond initials did not export. Some export was found from sporophylls on very old fronds, but it was very low; sporophylls were often sinks. Sporophylls and frond initials imported from sterile blades closer to the apex which were also exporting to young fronds, and/or from the lower blades of the next oldest frond. No activity was found in the haptera (*cf.* Fig. 3).

Downward export was directed to young fronds, rather than to older blades below the labeled blade. Any young frond close to the frond with the labeled blade received assimilates. For example, in the group of fronds on the left of Figure 3 both *2′* and *4°* contained activity from *2°*, and a small amount of activity was also found in *3°*. However, *3°* was quite long and the immature blades would have been supported chiefly by mature blades on

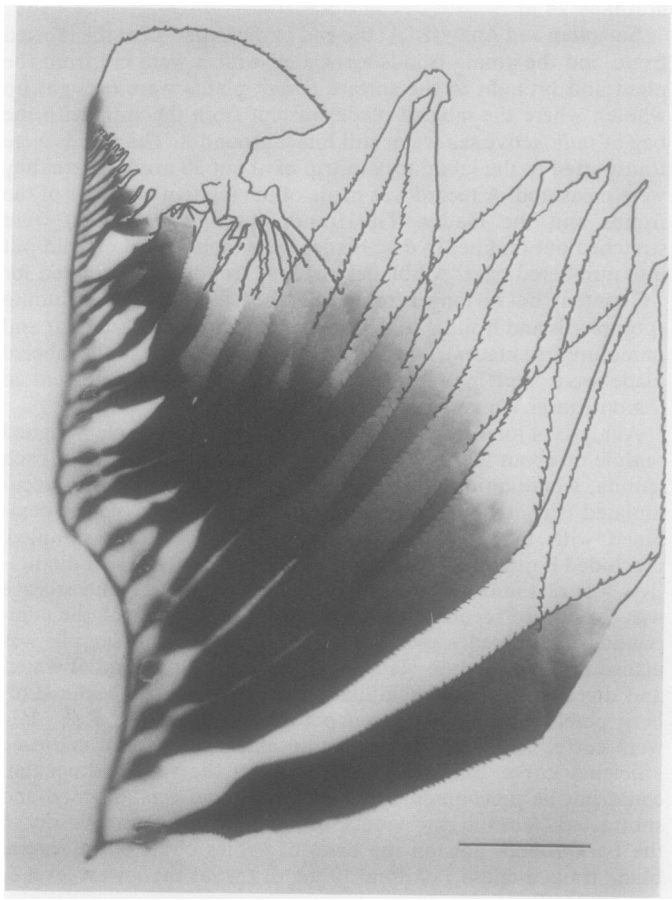

FIG. 1. Autoradiograph of the apical scimitar of a mature frond, on the same frond as the labeled blade which was 1.65 m from the apex. Outlines of the nonradioactive parts of the blades have been drawn in. Scale = 50 mm.

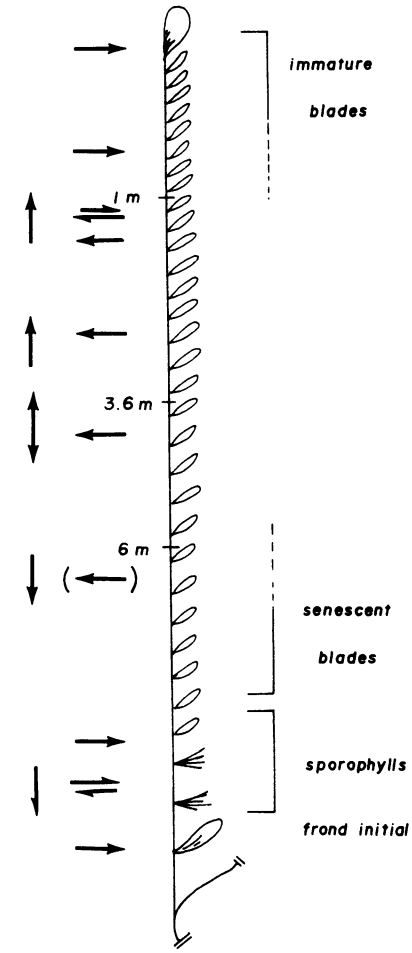

FIG. 2. Diagram, not to scale, to summarize import/export and export direction in mature fronds. Downward transport takes assimilates out of the frond to younger fronds, usually without labeling blades below the source except sporophylls and frond initials (see Fig. 4) Key: ↑ upward export; ↓ downward export; → import, no export; ← predominantly or exclusively export; ⇄ import + export; (←) some export. Approximate positions of the transition regions are marked in meters from the apex.

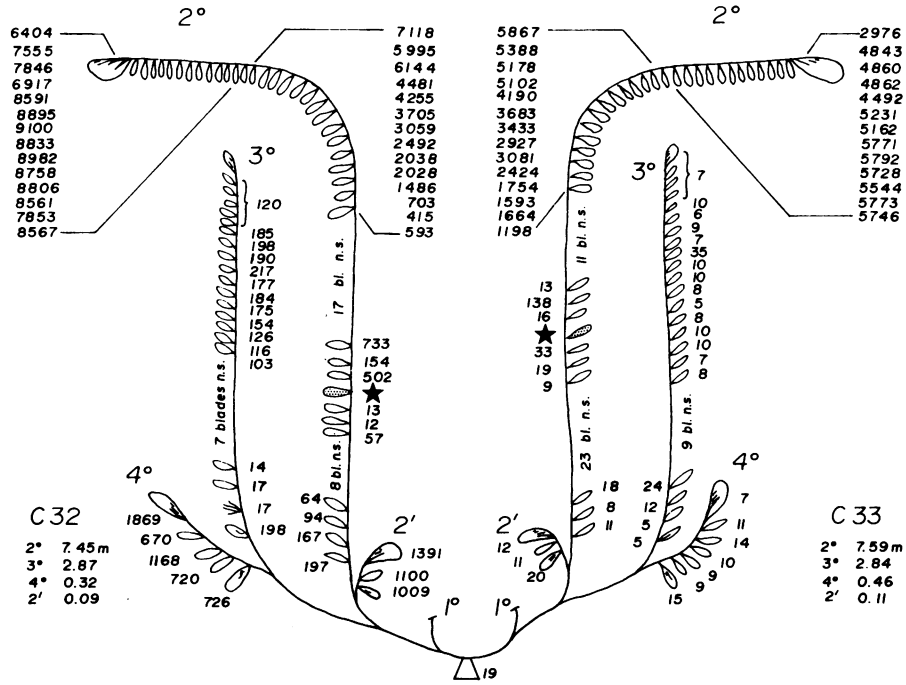

FIG. 3. Two experiments to show the change from only-upward to upward-and-downward transport. The two halves of this plant were almost identical in frond lengths. The labeled blade was on 2° in each case. The labeled blade on the right, 1.98 m from the apex, exported only to the apex of 2°; the labeled blade on the left, 3.64 m from the apex, exported upward to the apex of 2°, and downward to 2' and 4°, and, to a small extent, to 3°. (In other experiments it was shown that activity does not cross from one half of a plant to the other.) Data in dpm/10 mg dry wt. Labeled blades shown stippled and marked*. The plant is drawn schematically for convenience of data presentation.

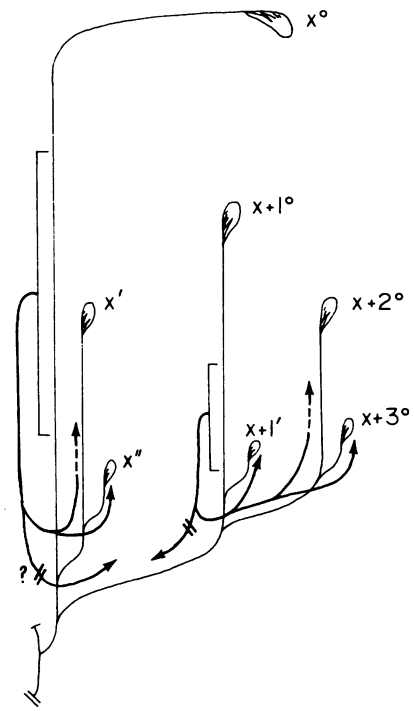

FIG. 4. Diagram, not to scale, of export from source blades on mature fronds which are exporting partly or wholly downward. The apical scimitar is shown at the tip of each frond, but other blades are not shown. Arrows show the directions of translocation; the double lines across two of the arrows indicate that translocation does not occur in that direction. In this general diagram the $x^{+}1°$ frond would be similar to the 2° frond on the left half of Figure 3. Further details in text.

the same frond. Activity did not pass from one side of the plant to the other.

Figure 4 shows the general case for export to juvenile fronds. Assimilates would be moving from $x°$ to x'', and to x' if this was not long enough to support itself (*cf.* Fig. 3); $x°$ would probably no longer support the °–series fronds, since $x+1°$ would be mature and supporting $x+1'$ and $x+3°$; $x+2°$ would be about the same length and maturity as x'. When downward transport begins from $x+2°$ it may support $x+1'$ (as well as $x+3°$ and $x+4°$).

Several of the fronds labeled lacked the apical blades. There was no upward translocation in these fronds. Similarly, young fronds which lacked the apex did not import.

DISCUSSION

In *Macrocystis*, assimilates from the many mature source blades between the immature blades and the sporophylls on a frond are partitioned between the nearby apical growing points (strong sinks) and the sporophylls, frond initials, and probably the holdfast (weak sinks). Translocation in this species thus follows the mature-source to meristematic-sink pattern described for *Laminaria* spp. and other small kelps (5, 7, 15, 17, 18), but a new dimension is added since as many as four apices may receive assimilates from any given source blade, depending on (*a*) the position of the source in relation to the apex of its frond, and (*b*) the lengths of the nearby juvenile fronds. The translocation pattern of a blade can be correlated with its distance from the apex which formed it, which is an index of its maturity. Similarly, import into a juvenile frond depends on its maturity: fronds about 3 m long have mature, upward exporting blades, and no longer import from older fronds. There is no upward translocation in fronds which lack the apical region; Parker (12) also reported lack of acropetal transport in fronds from which he cut the apical scimitar.

Sargent and Lantrip (14) provided the first evidence on the direction of flow of assimilates in *Macrocystis*. They compared the organic production with the rates of growth of different blades, and calculated that the youngest blades had a net import of C while older blades had a net export. Their data show the transition region as being at blade 13 or about blade 25 from the apical meristem (their Fig. 4 and Table 4, respectively); according to

their dry wt data, blades were mature from about blade 50. My data show export beginning at about 1 m from the apex, which is somewhat later than is suggested by Sargent and Lantrip[4]; the discrepency may be due to the latter having studied only three plants, and to their methods: they measured O_2 production by small disks of tissue cut from the blades and incubated for 12 hr in glass bottles, and converted the daily O_2 production to C production assuming that only carbohydrate was formed. By removing the tissue from the blade they would have created wound effects and destroyed the natural source-sink relationships, and it is now known that about half of the translocated organic matter in kelps is amino acids (13, 17). Sargent and Lantrip concluded that mature fronds had an over-all excess production of organic matter, and suggested that this excess would be translocated to the holdfast for storage, or to other fronds for growth. It appears from my results that the latter is the more important, at least in the absence of marked seasonal fluctuations in growth rate.

Parker (12) labeled blades 1.8 to 2.8 m from the apex, and found ^{14}C in stipe and blades both acropetal and basipetal to the labeled blade. In one case he found substantial activity in the apical scimitar, and there was in general more acropetal transport. His labeled blades were in the region I found to have only upward translocation. Inspection of his tables shows that basipetal movement was restricted to a few cm from the source, and is probably due to diffusion; I consider that his data do not conflict with mine.

On the basis of evidence from other kelps (5, 6, 15, 17, 18) I expect that the distal region of the immature blades, which autoradiography showed to be nonimporting, would be supplying assimilates to the proximal region. However, I have not conducted experiments to verify this, and it may be that it only supports its own growth.

My experiments do not take into account C fixed by the blade prior to the experiment. If old laminae export stored C (as, for example, tobacco leaves have been found to do [19]), there could be substantial undetected translocation to younger blades or fronds, and this material would also dilute any newly fixed C.

Growth of *M. pyrifera* in southern California varies little throughout the year (8), so that one does not anticipate seasonal changes in the translocation pattern as was found in *M. integrifolia* (4), or a change from growth to storage (such as Lüning *et al.* found in *Laminaria hyperborea* [6]). Nevertheless, the experiments on *M. pyrifera*, which were all conduced in October and November, may not represent a year-round pattern of translocation. At that time the nitrate and nitrite concentrations in the surface waters at Arch Rock were very low (10). North noted that, "The kelp bed at [Arch Rock] displayed a quite noticeable canopy deterioration at this time [21 October 1974], presumably from adverse water temperatures during summer and early fall" (9, p. 8). It could be that subsurface blades (which were labeled in my experiments) were exporting more to their own apices at this time, at the expense of the juvenile fronds, in order to supplement the apical N supply, the translocate containing a high proportion of amino acids (13). As far as I can judge from my qualitative experiments, the quantity of assimilates received by the juvenile fronds was low. The young fronds are growing in deep, poorly lit water, and are likely dependent for their growth on assimilates from the parent frond (14). If the adult frond apices in my experiments were indeed unusually strong sinks, the translocation pattern at other times of year could be expected to differ from the one I found in the following ways: (*a*) if more ^{14}C were imported by the young fronds it might become detectable within the 24-hr experimental period; (*b*) upward export might begin later, and would probably end sooner; (*c*) downward transport would probably begin sooner.

The pattern of import and export from maturing laminae of *Macrocystis* shows great similarity to the pattern well known in many dicotyledons (22): immature leaves import only, but when half to three-quarters unfurled begin export, first upward, then downward out of the shoot to storage or younger meristems. The massive support of flowers and fruit in angiosperms contrasts with the generally low import of ^{14}C by sporophylls of *Macrocystis*, but production of flowers and fruit occurs late in the life of the plant (or in the particular growing season), and often involves relatively massive structures. In contrast, sporophylls are among the first blades formed (4), they are already large when split off the apical scimitar, grow slowly, and are pigmented. Sargent and Lantrip (14) calculated that a rather small amount of import would account for their growth.

Macrocystis also differs somewhat from dicotyledons in the change from import to export. Because the distal part of the developing lamina is part of the already formed apical scimitar, there is little growth in it (the proximal part of the lamina is meristematic), and no import into the distal region of either the apical scimitar or the free blades. Perhaps the greatest difference in translocation pattern between *Macrocystis* and land plants is that in the latter shoot growth and root growth are of the same order, and a large proportion of the leaves' assimilates go to the roots. In *Macrocystis* hapteron growth is relatively slow, in terms of biomass added (14), and import is correspondingly low. (The haptera are weakly pigmented, but it has not yet been shown whether a significant amount of the growth comes from their own photosynthesis.)

My study provides a qualitative description of the translocation pattern in the giant kelp. With this background more detailed, and quantitative experiments can begin, leading ultimately to an understanding of the mechanism of translocation in brown algae.

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[4] Sargent and Lantrip (14) do not give the distances of these blades from the apices, and it is not clear from their text from precisely which point in the apical scimitar they began counting blades. They measured photosynthesis of the first free blade, which was number 8, 15, and 16, respectively, in their three experiments. Assuming that the transition region was thus at the 1st to 10th free blade, and based on my records of fronds, this would be (very approximately) 0.3 to 0.5 m from the apex. Using the age of the blades plus the average growth rate for the first meter of stipe given by Sargent and Lantrip, the 13th blade would have been at most 0.8 m from the apex.

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