

# THE TRANSLOCATION OF RADIOACTIVE PHOSPHORUS BY THE AQUATIC VASCULAR PLANT *NAJAS MINOR*<sup>1</sup>

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**Abstract.** The translocation of phosphorus by the aquatic vascular plant *Najas minor* All. was examined using radiophosphorus (<sup>32</sup>P). Whole plants showed a lack of phosphorus translocation either from the root to the shoot or in the reciprocal direction. Autoradiographic experiments indicated that the lack of translocation may be due to a very slow movement of this nutrient in both the root and the shoot. Phosphorus was calculated to move 0.6 mm/hr in roots and 1.2 mm/hr in foliage presumably through cells rather than in the vascular system.

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The translocation of nutrients by aquatic vascular plants ranges from plants which do not possess roots and obtain all of the required nutrients from the water, to plants which translocate a large quantity of required nutrients from their roots and will not grow well unless they are rooted in the sediments (Denny, 1972). The translocation of phosphorus by plants which translocate significant quantities of this nutrient, has been widely investigated (McRoy and Barsdate, 1970; Bristow and Whitcomb, 1971; DeMarte and Hartman, 1974). Little research, however, has been done on rooted aquatic vascular plants which do not translocate phosphorus. The work of Waisel and Shapira (1971) indicated that certain rooted aquatic vascular plants may translocate phosphorus, notably members of the Najadaceae. They found that phosphorus translocation in *Myriophyllum spicatum* was considerably less, in their experiments, than that reported by Bristow and Whitcomb (1971) for the same species. Variations in technique could account for the different results reported. These variations, however, might account for Waisel and Shapira's (1971) observation of a lack of translocation in several species of the Najadaceae. We were especially interested in *Najas minor* because it is a species which is spreading throughout

Ohio and adjacent states and replacing other species formerly abundant in this region (Wentz and Stuckey, 1971).

## MATERIALS AND METHODS

*Najas minor* All. foliage was collected from Squire Pond at the Case Western Reserve University Biology Field Station, Squire Valleeve Farm, Hunting Valley, Ohio. Foliar cuttings were planted in tanks in the field station greenhouse. Each tank contained 10 cm of pond sediments covered by 1000 ℓ of aged tap water to a depth of 45 cm. The cuttings were allowed to grow until they had established dense beds of mature plants.

Plants were removed from the tanks, washed in sterile demineralized water, and placed into sterile medium prior to their use for individual experiments. The experimental medium was one-tenth strength Hoagland's medium (Hoagland and Arnon, 1950) with the phosphate concentration adjusted to 10 μg PO<sub>4</sub>/ℓ, buffered with 10<sup>-3</sup>M Tris HCl (Sigma Chemical) adjusted to a pH of 7.0.

Measurements of translocation of phosphorus in whole plants were carried out in chambers modified from those proposed by Frank and Hodgeson (1964). Chambers consisted of two 500 ml polyethylene bottles joined at the necks with a polyethylene tube. Septa and vent tubes were added to permit access to the lower chamber. The upper chamber was open to the atmosphere. Aluminum foil was placed around the lower chamber to exclude light.

Plants with roots were selected, rinsed and placed in the chamber. The stem-root junction was sealed with petrolatum and both chambers were filled with sterile medium. Ten μCi of <sup>32</sup>P as H<sub>3</sub><sup>32</sup>PO<sub>4</sub> (New England Nuclear) were added to either the upper or lower chamber, depending on the experiment. All plants were incubated at 20° C under two 40 watt cool white fluorescent lights. Incident radiation at the surface was 100 μ watts/cm<sup>2</sup>.

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At the end of 8 hours the plants were removed, sectioned at the stem-root junction, rinsed and dried for counting. In several experiments, plants were sectioned at 1 cm intervals prior to drying and counting, to permit an evaluation of the position of the isotope in the organs.

Dried plant material was ground to a powder, weighed onto 2.5 cm ringed aluminum planchets, fixed with a 10% glycerine solution and dried to a thin film. Planchets were counted on a Tracer-Lab Compu/matic II gas-flow Geiger-Muller counter to 10,000 counts. All results were corrected for background and decay.

Translocation in single organs was examined with an autoradiographic assay. Either a root or leaf attached to a plant was placed into a 1 mm (ID) capillary tube 3 cm in length and sealed from the remainder of the plant with petrolatum.  $H_3^{32}PO_4$  (1 to 5  $\mu$  Ci) was injected into the tube with a hypodermic syringe (25 gauge needle). Plants and isolated leaves or roots were placed into sterile medium and incubated under the same conditions as previous experiments. Plants were harvested at 2 hour intervals over a 12 hour period, dried between 2 pieces of blotting paper, and the position of the petrolatum seal marked. Dried plants were placed between 2 fresh pieces of blotting paper, their positions marked, then placed against a 2 $\frac{1}{4}$ " x 3 $\frac{1}{4}$ " plate of KODAK Tri-X Pan Professional film. The plates were placed into light-proof envelopes and exposed for 7 days, then developed. Developed plates were measured between the petrolatum seal position and furthest point of advance of tracer into the unlabeled portion of the plant as a measure of translocation velocity.

### RESULTS

The data show that there is no significant translocation of phosphorus by *Najas minor* either from the roots to the foliage or in the reciprocal direction. Plants in which the root was labeled with  $^{32}PO_4$  translocated virtually no phosphorus into either portions of the root in unlabeled medium or into the foliage (table 1). Replicates 6 and 7, in which the entire root was labeled, showed only a small (less than twice background) amount of tracer movement from the root to the foliage. This may have resulted from leakage through the petrolatum seal, or from carry-over during removal of plants at the end of an experiment.

The second set of experiments in which the foliage was placed in labeled medium showed similar results. No significant quantity of the tracer was found in the unlabeled portions of the plants (table 2).

Figure 1 shows the activity of the plant at 1 cm intervals when either the

TABLE 1  
*The  $^{32}P$  activity in various organs of *Najas minor**

Plant	Counts/Minute/mg Dry Weight		
	Root in Labeled Medium*	Root in Unlabeled Medium	Foliage
1	433	14	0
2	2808	11	0
3	1575	0	0
4	1672	0	0
5	6770	10	0
6	4203	—	17
7	2302	—	20

\*After 8 hours in the light when the entire root (plants 6-7) or a portion of the root (plants 1-5) was placed in labeled medium. The remainder of the plant was contained in a chamber with unlabeled medium.

root or the foliage was labeled with  $^{32}P$ . There was no movement of tracer from the roots to the foliage or in the reciprocal direction. The tracer activity of the foliage was reasonably uniform from the top of the plant to the stem junction in

TABLE 2  
*The  $^{32}P$  activity in roots and foliage of *Najas minor**

Plant	Counts/Minute/mg Dry Weight*	
	Foliage	Root
1	3741	0
2	4996	0
3	1034	11

\*After 8 hours in the light when the foliage of the plant was placed in labeled medium (upper chamber labeled) and the root in the lower chamber contained in unlabeled medium.

experiments where the foliage was labeled. A similar result was observed for the proximal portion of the root. The root tip, however, contained a significantly greater quantity of  $^{32}P$ , which may indicate that the root tip is the major site of absorption.

Autoradiographic experiments confirmed the lack of translocation. There was a small but observable spread of tracer through the organs as the duration of the experiment increased (fig. 2).

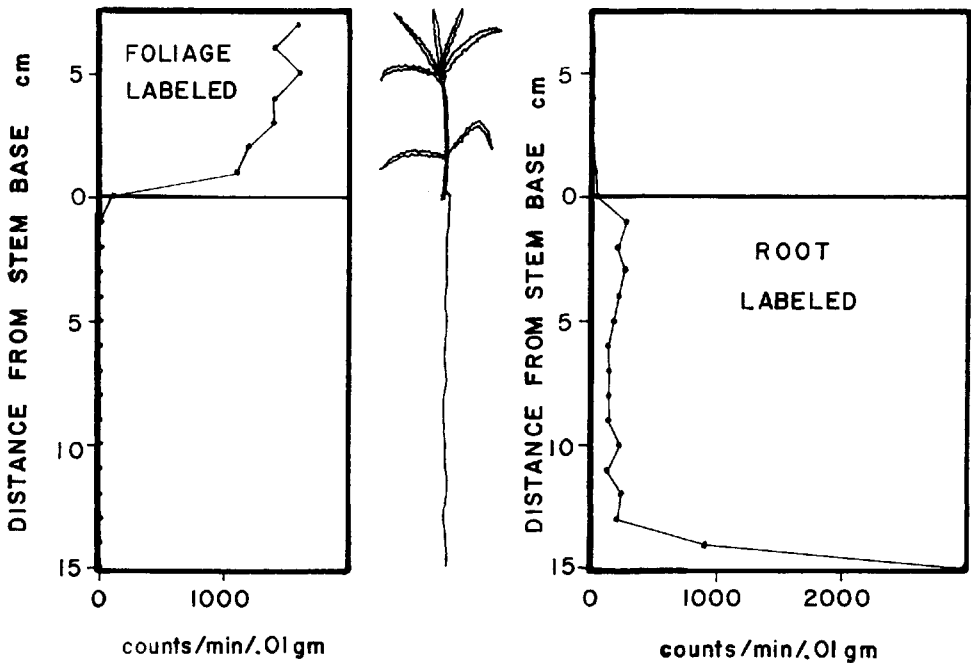


FIGURE 1. The activity of  $^{32}\text{P}$  in counts per minute per 0.01 gm of a *Najas minor* plant sectioned at 1.0 cm intervals. This experiment was separate from those shown in tables 1 and 2 but was run with the same apparatus and external conditions. Graphs represent plants where either the foliage or root was placed in labeled medium.

Plants harvested at 4 and 6 hours showed little spread of the tracer beyond the petrolatum barrier. The root translocation after 6 hour (fig. 2-E) showed some translocation to the stem-root junction, however, this trend was not maintained in later samples (fig. 2-F). The  $^{32}\text{P}$  activity in the foliage was localized in the portion of the leaf placed into labeled medium and in the stem node after 12 hours (fig. 2-C) but no activity was observed beyond the node. The rate of movement of the tracer was calculated as  $0.62 \text{ mm/hr} \pm 0.03$  for the roots and  $1.21 \text{ mm/hr} \pm 0.16$  for the foliage. The rate of tracer movement in the foliage was significantly greater ( $P < .01$ ) than in the roots.

#### DISCUSSION

The aquatic vascular plant *Najas minor* did not translocate phosphorus from the roots to the foliage or in the reciprocal direction. This finding differs from data on previously studied species of aquatic vascular plants (McRoy

and Barsdate, 1970; Bristow and Whitcomb, 1971; DeMarte and Hartman, 1974) where a large portion of the phosphorus absorbed by the roots was translocated to the foliage.

Lack of translocation of phosphorus by *Najas minor* may be a result of the anatomical characteristics of the plant. The vascular system is reduced to xylem lacunae and several strands of phloem (Sculthrope, 1967). *Elodea densa* and *Myriophyllum spicatum*, which also have reduced vascular systems, have been shown to translocate phosphorus (Bristow and Whitcomb, 1971).

Autoradiographic analysis showed a very slow movement of tracer and tracer appeared to be spread throughout the labeled portion of an organ, rather than become localized within a specific transport tissue. This may be expected in plants with reduced vascular systems where movement of material in an organ may be from cell to cell through the parenchyma; similar to that reported for *Chara* (Littlefield and Forsberg, 1965).

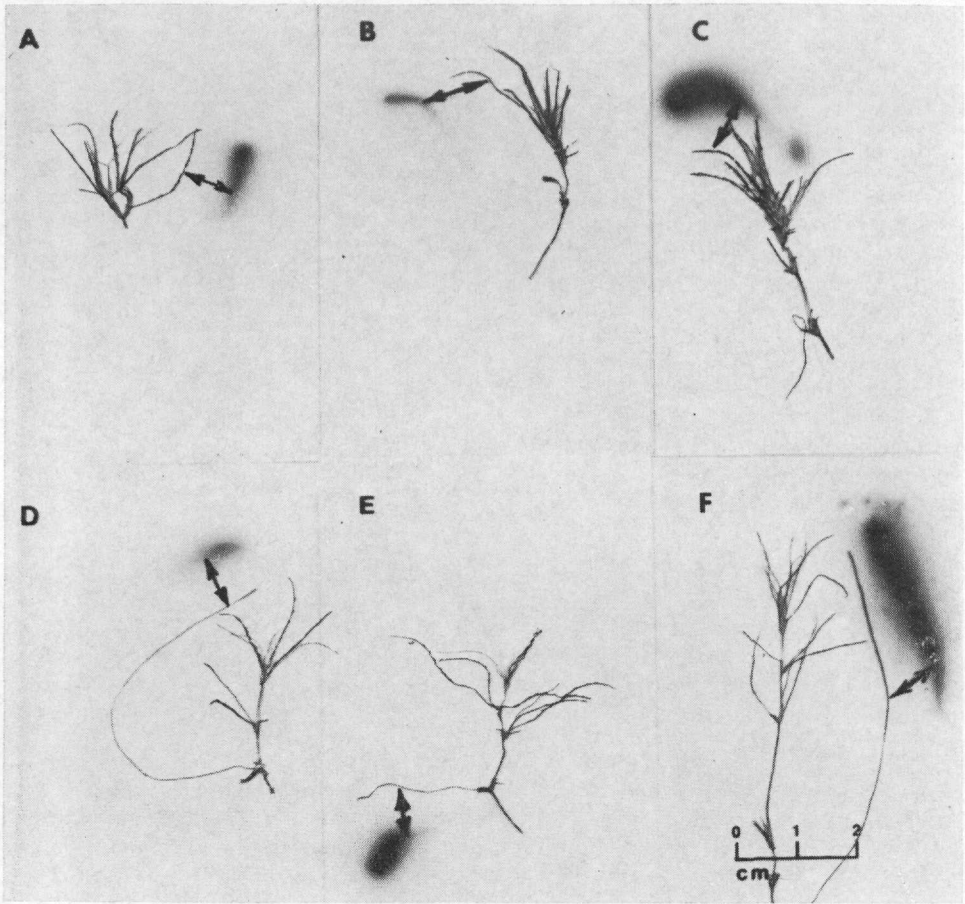


FIGURE 2. Autoradiographic plates showing the movement of  $^{32}\text{P}$  in *Najas minor* when either a single root or leaf was placed in labeled medium for various lengths of time. A-C leaf labeled and D-F root labeled; (A. 4 hr, B. 6 hr, C. 12 hr, D. 4 hr, E. 6 hr, F. 12 hr). The plant from each experiment was placed on the autoradiograph displaced several mm from the position occupied when the plate was exposed. Arrows indicate the position of the petrolatum seal on both the labeled organ and the autoradiograph.

It is possible that only a small fraction of the absorbed nutrient may have been available for translocation. This phenomenon has been observed in barley roots (Russell and Martin, 1953; Loughman, 1960; Crossett and Loughman, 1966). In our experiments with *Najas minor* most of the tracer absorbed remained at the site of absorption.

Some of the phosphorus absorbed during autoradiographic studies over 12 hours appeared to be translocated by the foliage and accumulated in the stem nodes. Some accumulation may have

taken place at the root tip. DeMarte and Hartman (1974) reported an accumulation of absorbed tracer by the nodes and budding regions of *Myriophyllum exalbescens* and concluded that these were sites of active growth. If incoming nutrients were rapidly assimilated by the node into new cellular material, there would be little remaining for further movement to other parts of the plant.

The most important implication of the autoradiographic experiments was that the lack of observed translocation was not merely a result of the petrolatum barrier.

The seal in these and other experiments was identical. The longer autoradiographic experiments demonstrated that the tracer could, indeed, cross the petiole, and the barrier itself was not the reason for the observed lack of translocation.

It is possible that translocation of phosphorus, while lacking in these experiments, might be stimulated by a chemical gradient between the roots and the foliage. Our experiments used identical medium in both root and foliage chambers, which was the same design employed by several previous investigators, who demonstrated a substantial translocation of phosphorus by other species of aquatic vascular plants.

If sediment phosphorus is not readily available *in situ*, as suggested by our work, then the foliage must compete with other biological systems for the phosphorus present in the water column. Under such conditions, the aquatic vascular plant *Najas minor* would tend to be favored in those systems where the input of nutrients is sufficiently sustained to meet foliar requirements for those nutrients. This hypothesis agrees with that of Wentz and Stuckey (1971), who proposed that the warming and enrichment of Ohio waters were responsible for the successful invasion of *Najas minor* into this area. A sustained enrichment of the water column with phosphorus could provide an environment suitable for the growth of the plant which must obtain all of its foliar phosphorus directly from the water.

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