Aquatic Botany, 3 (1977) 267—272

UPTAKE AND TRANSPORT OF IRON AND PHOSPHATE BY *VALLISNERIA SPIRALIS* L.

S.-R. GENTNER

Biology and Health Physics Division, Atomic Energy of Canada Limited, Chalk River Nuclear Laboratories, Chalk River, Ontario KOJ IJO (Canada)

(Received 20 July 1976; revised 20 December 1976)

ABSTRACT

Gentner, S.-R., 1977. Uptake and transport of iron and phosphate by *Vallisneria spiralis* L. *Aquat. Bot.*, 3: 267—272.

Using $^{59}Fe^{3+}$ and $^{32}PO_4^{3-}$ as tracers, the uptake and transport of iron and phosphate from a water environment by roots and shoots of the vascular aquatic plant *Vallisneria spiralis* L. were studied. Phosphate is taken up equally by roots and shoots, while roots take up more iron than shoots. Transport within the plant occurs principally in the shoot to root direction. Excretion of both iron and phosphate from the plant was detected.

INTRODUCTION

There has long been disagreement as to whether the absorption of mineral elements and other substances occurs in the roots or in the shoots of submersed aquatic plants (Sculthorpe, 1967). Until recently, arguments were not based on direct experiment. In 1963, Funderburk and Lawrence, using *Heteranthera dubia* (Jacq.) MacMill., detected uptake in both roots and shoots and some transport of ^{14}C-labelled herbicides; the extent of uptake and transport depended on the kind of herbicide. In 1964, Frank and Hodgson showed that roots of *Potamogeton pectinatus* L. are functional, since they transported ^{14}C-labelled 2, 3, 6-trichlorophenylacetic acid. Working with *Myriophyllum brasiliense* Cambess., Bristow and Whitcombe (1971) detected root uptake and transport but little uptake of $^{32}PO_4$ by the shoot. Demerte (1971), using $^{59}Fe^{3+}$, $^{45}Ca^{2+}$ and $^{32}PO_4^{3-}$ as tracers, found variable uptake in root and shoot of *Myriophyllum exalbescens* Fernald.

This paper describes experiments on the uptake and transport within the plant of $^{59}Fe^{3+}$ (supplied as $^{59}FeCl_3$ in HCl) and $^{32}PO_4^{3-}$ (supplied as H_3PO_4 in HCl) by *Vallisneria spiralis* L.

MATERIALS AND METHODS

Vallisneria spiralis L., a commercially available submersed aquatic plant, was chosen because it is easy to grow, reproduces asexually by runners, has well-developed roots and shoots, and is sufficiently sturdy to withstand handling during the course of the experiment. Prior to the experiments, plants were grown in polyethylene tanks holding 75—125 l of dechlorinated tap water (source: Ottawa River) which was changed monthly; rooting medium was 2—5 mm mesh gravel. A growth bench (Controlled Environments Model GB48) kept the water temperatures at 21 ± 0.5° C. A light intensity of 300 foot-candles at the water surface was maintained for an eight-hour day. These conditions yielded healthy, growing plants with shoots of 0.55 ± 0.15 g and roots of 0.13 ± 0.05 g wet weight.

For tracer experiments the plant was positioned so that the shoot was in the wide portion of a funnel and the roots hung through the stem into a beaker. Eicosane (Frank and Hodgson, 1964) was used to form a plug in the stem of the funnel to prevent leakage. 400 ml of filtered tap water was poured into the funnel, and 300 ml into the beaker.

To simulate the light conditions of a soil environment, the root compartment was darkened using opaque black plastic. The shoot compartment was covered with a plastic petri dish with small holes drilled in it; this prevented both excessive evaporation and heat build-up. Layers of cheesecloth were placed on the cover to reduce the light received by the plants to approximately 300 foot-candles for an eight-hour day. Water temperatures were 21 ± 0.5° C.

Experiments took place in a Controlled Environments Growth Chamber, Model F8H. Total stable iron in the filtered tap water was 0.1 μg/ml; phosphate concentration was 0.03 μg/ml, and pH was 7.2—7.6.

Plants were set up as described above and equilibrated with freshly filtered tap water for 24 hours. After 24 hours, the water in either the upper or lower compartment was replaced with tap water containing the radioisotopes, while that in the other compartment was changed using tap water without added radioisotopes. Leakages found in the plugs were also repaired at this time. Water samples for counting were taken immediately after the radioisotopes were added and 24 hours later when the experiment was terminated. Also at termination, water samples taken from the compartment containing no added radioisotopes were counted to detect leaks and/or excretion. The shoots were severed from the plug, as were the roots. After being rinsed for 30 s in distilled water, they were then rinsed for 30 s in 200 ml of a solution of 1% Neodisher T Alkaline Detergent (Mielewerk GmbH) and 0.1 M HCl to remove adsorbed tracer ions. A final distilled water rinse was made to remove traces of detergent. No tissue damage was apparent after this treatment. The plants were then kept for one hour between layers of blotting paper. Wet weights were taken, and the plants were then dried at 90° C for 24 h for dry weights. After weighing, roots and shoots were wet-ashed using concentrated HNO_3

and 30% H_2O_2 and made up to a 10 ml volume; 9 ml were placed in a scintillation vial for gamma counting of ^{59}Fe in a Nuclear Chicago "Tobor" counter (8% efficiency for ^{59}Fe) and 1 ml was dried on a planchet for ^{32}P counting. A Nuclear Chicago automatic beta counter was used for ^{32}P. An aluminum shield of 134 mg/cm^2 was installed between the sample and the counter window to prevent beta particles of less than 0.45 MeV (i.e. the ^{59}Fe beta emission) from being counted. The counter efficiency for ^{32}P using this system was 15%.

RESULTS AND DISCUSSION

Six experiments were carried out at different times under as uniform conditions as possible: the only known variation was the concentration of radioisotopes in the water medium. In each experiment, the radioisotopes were added to the shoot compartments of six plants and to the root compartments of another six plants.

Table I shows data on direct uptake from the water by either the root or the shoot. For each experiment, mean uptake in cpm $\times 10^3$/g wet weight of shoot or root ± standard deviation, mean activity in cpm $\times 10^3$/ml of water, and mean concentration factor (24 h)

$$\left(\overline{x}\ \mathrm{CF} = \frac{\Sigma \dfrac{\text{cpm/g wet weight of plant}}{\text{cpm /ml } H_2O}}{n} \right)$$

are shown for each experiment.

The results of the standard "Student" t-test comparing uptake of phosphate by shoots and roots in each experiment show that there is no significant difference between them ($p > 0.10$). The results for iron, however, show three experiments to be significantly different ($p < 0.05$) with mean root uptake greater than shoot.

Data were further analyzed by plotting water concentration against root and shoot uptake using the method of least squares to obtain the regression equations. Regression coefficients (r) greater than 0.67 ($n = 36$) were obtained for all equations showing the relationship between water concentration and plant uptake to be linear ($p < 0.001$). Comparison of the concentration factors (i.e. the slope of the regression equation) obtained for iron uptake by roots and shoots shows that roots took up significantly more iron than shoots ($p < 0.001$). When phosphate uptakes were compared there was no significant difference between roots and shoots ($p > 0.5$). When the relative amounts of iron and phosphate taken up were compared, it was found that more iron was taken up by both roots and shoots than was phosphate ($p < 0.001$).

Closely related to uptake is transport, i.e. movement into that part of the plant not exposed to tracer. The transport factor is defined as

TABLE I

Direct uptake of ^{59}Fe and $^{32}PO_4$ by *Vallisneria spiralis* L.

Exp. no.		$^{59}Fe^{3+}$				$^{32}PO_4^{3-}$			
		n	$\frac{\bar{x}_{cpm} \cdot 10^3}{ml\ H_2O}$ ± S.D.	$\frac{\bar{x}_{cpm} \cdot 10^3}{g\ wet\ weight}$ ± S.D.	CF*	n	$\frac{\bar{x}_{cpm} \cdot 10^3}{ml\ H_2O}$ ± S.D.	$\frac{\bar{x}_{cpm} \cdot 10^3}{g\ wet\ weight}$ ± S.D.	CF*
1	Shoot	6	0.030	0.9 ± 0.2	31	6	2.07	48.6 ± 10.5	23
	Root	5	0.033	3.3 ± 1.8	101	6	2.09	59.4 ± 19.9	29
2	Shoot	6	0.036	1.1 ± 0.4	33	6	0.71	11.4 ± 2.4	16
	Root	6	0.037	3.7 ± 0.9	104	6	0.69	10.1 ± 1.8	15
3	Shoot	6	0.159	11.3 ± 3.6	73	6	0.46	10.3 ± 6.2	24
	Root	6	0.135	18.3 ± 8.8	152	6	0.48	14.2 ± 6.2	29
4	Shoot	6	0.508	48.5 ± 10.1	96	6	0.59	16.0 ± 3.3	27
	Root	6	0.480	49.1 ± 10.3	102	6	0.57	16.7 ± 2.4	28
5	Shoot	5	0.631	14.7 ± 8.4	23	6	0.39	10.0 ± 4.5	26
	Root	6	0.637	38.9 ± 19.0	62	6	0.49	15.9 ± 6.1	33
6	Shoot	5	0.359	14.5 ± 7.3	41	6	0.55	24.9 ± 14.9	50
	Root	6	0.349	21.8 ± 7.9	61.5	6	0.8	15.9 ± 7.9	23

$$CF^* = \frac{\Sigma \left(\frac{\text{cpm/g wet weight plant}}{\text{cpm/ml water}} \right)}{n}$$

$$TF = \frac{\text{cpm/g wet weight of plant in non-labelled compartment}}{\text{cpm/g wet weight in labelled compartment}}$$

Transport of either ^{59}Fe, $^{32}PO_4$ or both isotopes occurred in 55 of the 61 plants measured (Table II). The large variance shown for transport factors is because those plants showing 0 (or unmeasurable) transport were included in the calculation of the means. Of the 61 plants, 17 showed no transport of $^{32}PO_4$ and 28 showed no transport of ^{59}Fe.

Iron transport from shoot to root is approximately 40 times more than from root to shoot. This difference is significant at $p < 0.001$. For phosphate transport there is an apparent difference between root and shoot with transport in the shoot to root direction being greater, but because of the extreme variability of the data, this was found to be statistically not significant ($p > 0.1$). Movement of iron, and possibly phosphate, from shoot to root may indicate storage in roots.

When the shoot to root transport factors for $^{59}Fe^{3+}$ and $^{32}PO_4^{3-}$ are compared, phosphate transport is greater than that of iron; in the reverse direction, i.e. from root to shoot, phosphate transport is also greater, being about 100 times that of iron.

There is evidence that after transport within the plant, both iron and phosphate are excreted into the non-labelled compartment. Root excretion of phosphate took place in 62.5% of the plants where shoot to root transport occurred; for iron the figure is 50%. Iron was excreted more readily than phosphate from the shoots in those cases where root–shoot transport occurred (Fe 27%; PO_4 3.6%).

The interaction between uptake, transport and excretion is complex, since movement seems to occur in each direction, varying only quantitatively. A simplified approach has been taken here to understand the movement of iron and phosphate. Iron is obviously less mobile than phosphate, which is to be expected, since iron is one of the most immobile of all elements in terrestrial plants (Meyer et al., 1960). Of special note, however, is the fact that iron is

TABLE II

Transport of $^{59}Fe^{3+}$ and $^{32}PO_4^{3-}$ by roots and shoots of *Vallisneria spiralis* L.

Direction of transport	Transport factor $\times 10^{-3} \pm$ S.D.	
	$^{59}Fe^{3+}$	$^{32}PO_4^{3-}$
Root to shoot $n = 36$	0.39 ± 0.81	43 ± 69
Shoot to root $n = 25$[a]	22 ± 30	152 ± 353

[a]Data not considered where leaks occurred.

excreted more readily from the shoots than phosphate.

The results of these experiments only begin to describe the movement of iron and phosphate in aquatic plants. Further research might study the quantitative loss of ions from labelled plants, the effects of iron and phosphate starvation on uptake, the rates of uptake, and the effects of different light—temperature regimes, concentrations of elements, duration of experiments, and ages of plants.

ACKNOWLEDGEMENTS

I wish to thank J.M. Judd for his suggestions during the course of this research and for his invaluable help in preparing this manuscript. I also wish to thank I.L. Ophel for suggesting this line of research.

REFERENCES

Bristow, J.M. and Whitcombe, M., 1971. The role of roots in the nutrition of aquatic vascular plants. Am. J. Bot., 58 (1): 8—13.

Demarte, J.A., 1971. Studies on absorption and translocation of ^{32}P, ^{59}Fe and ^{45}Ca by *Myriophyllum exalbescens* Fernald. Contract AT (30-1) - 3018, USAEC. Ph.D. Thesis, 200 pp.

Frank, P.A. and Hodgson, R.H., 1964. A technique for studying absorption and translocation in submersed plants. Weeds, 12 (2): 80—82.

Funderburk, H.H., Jr. and Lawrence, J.M., 1963. Absorption and translocation of radioactive herbicides in submersed and emersed aquatic weeds. Weed Res., 3(4): 304—311.

Meyer, B.S., Anderson, D.B. and Böhning, R.H., 1960. Introduction to Plant Physiology. Van Nostrand, Princeton, New Jersey, 541 pp.

Sculthorpe, C.D., 1967. The Biology of Aquatic Vascular Plants. Edward Arnold Publishers, London, 610 pp.