THE UPTAKE OF NITRATE AND AMMONIUM BY SUCCESSIVE ZONES OF THE PEA RADICLE

By V. O. GRASMANIS* and K. P. BARLEY†

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Summary

Measurements were made of the uptake of ammonium and nitrate by successive zones of the radicle of intact seedlings of *Pisum sativum* L. A modified version of the potometer designed by Gregory and Woodford (1939) was used to supply separate zones, and the amounts of nitrogen absorbed were determined with the stable isotope ^{15}N.

The uptake of ammonium or nitrate differed between zones over the 13·5-cm length of radicle examined, the uptake of each form of nitrogen per unit length of radicle being greatest in the apical centimetre and least in the 1·0–4·5-cm zone. Nitrogen was absorbed from 7 to 10 times more rapidly in the ammonium than in the nitrate form, depending on the zone examined.

I. INTRODUCTION

In the past five years wide interest has been shown in a number of mathematical models proposed to describe the uptake of ions from soils by plant roots. It has generally been assumed that within the soil there is negligible flow of nutrient ions in the direction of the long axis of the root. This neglects the elongation of the root, and also overlooks the well known fact that, in experiments with stirred solutions, gradients of salt absorption are commonly found along roots.

Before more realistic theories can be devised, further information is needed on the uptake ability of different zones of the root. The present investigators are interested principally in comparing the uptake of nitrogen in its nitrate and ammonium forms. Although information has been published on the uptake of one or the other of these ions by different zones of roots (Burstrom 1939; Brouwer 1954; Tromp 1962), a comparative study of the two ions using different zones of the roots of a single species does not appear to have been made.

As a first step, short-term measurements have been made of the uptake of $^{15}NO_3^-$ and of $^{15}NH_4^+$ by different zones of the unbranched radicle of the pea (*Pisum sativum* L.).

Whole plants were used in these studies in preference to excised roots. Although reliable measures of uptake can sometimes be obtained in short-term experiments with excised roots, particularly with low-salt roots (Hoagland and Broyer 1936), the

* Scoresby Horticultural Research Station, Department of Agriculture, P.O. Box 174, Ferntree Gully, Vic. 3156.

† Agronomy Department, Waite Agricultural Research Institute, Glen Osmond, S.A. 5064.

variation along the length of the root may be altered by excision (Bowen and Rovira 1967). Moreover, when working with nitrate and ammonium in particular, it is unwise to use excised roots, as uptake of these ions decreases rapidly and may cease altogether within a few hours after severing the phloem connection with the tops (Koster 1963).

II. Method

(*a*) *Growth of Seedlings*

After being soaked in aerated, distilled water for 24 hr, seeds of *P. sativum*, cv. White Brunswick, were germinated on moist blotting paper at a suction head of 3 cm of water for 72 hr. Seedlings with radicles 2–3 cm long were then supported on a plastic screen, the radicles being immersed in an aerated, nitrogen-free culture solution having the composition shown in Table 1. The shoots were illuminated continuously, but light was excluded from the vessels housing the radicles. After 5 days growth in this solution, when the radicles had attained a modal length of 18 cm, a sufficient number had at least 13·5 cm of their length free from laterals and these were selected for further measurements.

Table 1

Composition of nutrient solutions

Atom	Amount in Culture Solution (mg-atom/l)	Amount in Labelled Solutions (mg-atom/l)*		Atom	Amount in Labelled Nitrate Solution (mg-atom/l)
		Nitrate Only	Ammonium Only		
^{14}N	0·0	1·05	1·05	Fe	0·025
^{15}N	0·0	0·42	0·42	Mn	0·0025
K	0·50	1·50	0·50	Cu	0·0005
Na	0·20	0·45	0·20	Zn	0·0005
Ca	0·50	0·50	0·50	B	0·0079
Mg	0·75	0·75	0·75	Mo	0·0001
P	0·23	0·23	0·23		
S	0·88	0·75	1·63		
Cl	1·00	1·00	1·00		

* Initial pH 6·8; pH change during uptake ±0·2.

The H_2SO_4–I_2–KI procedure (Scott 1950) was adopted to examine free-hand longitudinal and transverse sections of radicles for the presence of suberized tissue. The area of the curved outer surface of successive zones of fresh radicles was calculated after measuring the radii with an eyepiece graticule at low-power magnification.

(*b*) *Measurement of Uptake*

In each test a single radicle was installed in a potometer similar in principle to that described originally by Gregory and Woodford (1939) (see Figs. 1 and 2). The apparatus, made entirely of Pyrex glass, consisted of a main vessel, containing the basal part of the radicle together with lateral roots, and three zone vessels. The flat-ground joints of adjacent vessels were separated by a 0·7-mm-thick latex diaphragm, and the vessels were held together with spring clamps. Two side tubes on each zone vessel were connected with a bubble pump to provide for the aeration and stirring of nutrient solution during the uptake measurements. Each radicle was installed with the help of a sharp-ended, stainless steel tube of 1 mm bore. After the radicle had been inserted gently into the tube, the tube was pushed through the set of diaphragms, and then withdrawn from the bottom of the apparatus leaving the radicle in place with successive lengths sealed in the zone vessels. Finally the bottom outlet was stoppered and the vessels were filled with appropriate nutrient solutions (7 ml per zone vessel), entrapped air being exhausted through a

1-mm-diameter flexible polyethylene tube. Uptake was allowed to proceed for 30 min. To minimize transpiration the shoot of each plant was enclosed within a thin polyethylene bag lined at the sides with moist tissue paper. During the growth of the plants and the period of uptake the temperature was maintained at $20 \pm 1°C$.

To enable a check to be made on leakage and translocation of the isotope, in any one test the labelled solution was added only to the top- and bottom-zone vessels or to the middle-zone vessel; the remaining vessels contained the unlabelled culture solution described in Table 1. The composition of the labelled solutions used to supply either $^{15}NO_3^-$ or $^{15}NH_4^+$ is also given in Table 1.

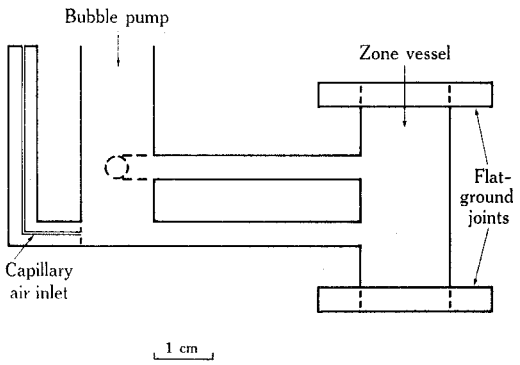

Fig. 1.—Detail of a zone vessel.

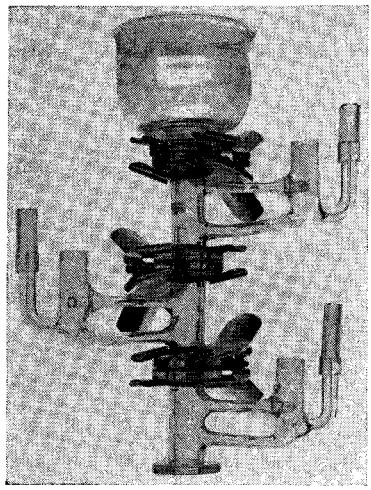

Fig. 2.—Assembly consisting of a main vessel and three zone vessels.

Although this paper is not concerned primarily with the nature of the uptake phenomenon, it is desirable to provide a little interpretative data. Accordingly, uptake was also determined when 1 mM KCN had been added to the culture solution for the 60 min preceding transfer to the labelled solution, and to the labelled solution itself. It became obvious that further information was needed for ammonium. In further tests, following the $^{15}NH_4^+$ uptake period, the radicles were washed for a total of 30 min with six changes of a solution which contained 1·5 m-equiv/l $^{14}NH_4^+$ as the only form of nitrogen, but which was otherwise identical with the labelled solution.

After the treatments described above had been completed, the root zones were severed from one another, washed separately with three changes of distilled water, blotted with filter paper, weighed fresh, dried at 75°C, and finally weighed dry. The following composite samples were prepared from a number of radicles for the determination of total nitrogen and ^{15}N:

(1) basal part of the radicle together with its laterals from the main vessel (18 lengths per sample);

(2) 4·5-cm length of radicle from each zone vessel (18 lengths per sample); and, in separate runs,

(3) apex of 1·0-cm length from the bottom zone vessel (30 apices per sample).

The composite samples were duplicated and triplicated where necessary.

The time course of uptake was not followed for each zone, as to do this with the present technique would have burdened the work excessively. However, data were obtained for seedlings at six times over an 8-hr period, after immersing the distal 13·5 cm of the unbranched part of the radicle in aerated, labelled solution. The radicles, seeds, and shoots were separated at each harvest, composite samples being prepared at each harvest from 10 seedlings.

(c) *Estimation of Total Nitrogen and ^{15}N Content*

Composite samples were digested for 5 hr in Kjeldahl flasks containing 5 ml of sulphuric acid, 0·17 g of salicylic acid, and 1·5 g HgO–K_2SO_4 catalyst (1 g HgO to 20 g K_2SO_4). The digest was diluted to 20 ml, and after the addition of 10 ml of alkali (200 g NaOH plus 12·5 g $Na_2S_2O_3.5H_2O$ in 500 ml of distilled water) the diluted digest was distilled into 5 ml of boric acid containing methyl red–methylene blue indicator (10 g boric acid plus 5 ml of mixed indicator, consisting of 2 volumes of 0·2% methyl red and 1 volume of 0·2% methylene blue—both in 45% ethanol—in 500 ml of distilled water). The boric acid solution containing distilled ammonium was then titrated with 0·01N $KH(IO_3)_2$ solution to a lilac end point.

For the mass spectrometer determination of ^{15}N, each titrated sample was evaporated to 1 mg nitrogen per 2 ml after the addition of one drop of concentrated H_2SO_4. An appropriate amount (100–300 μg nitrogen) of concentrated digest was transferred to Rittenberg tubes. The tubes were attached to an A.E.I. MS2 mass spectrometer; and, after degassing under high vacuum and freezing with liquid nitrogen, the ammonia was oxidized to N_2 gas with excess sodium hypobromite solution. In the preparation of the alkaline hypobromite KI was used to inhibit oxygen formation. ^{15}N enrichment was determined from ion mass peaks 28 and 29 or 28, 29, and 30 as required.

III. Results

The total nitrogen content of the radicle tissues, and the dry weight, fresh weight, and surface area per unit length of radicle are given for each zone in Table 2. No suberized superficial tissues were detected, and the endodermis remained in a primary stage along the 13·5-cm length of radicle examined.

Table 2

WEIGHT, TOTAL NITROGEN CONTENT, AND SURFACE AREA OF SUCCESSIVE ZONES OF THE PEA RADICLE

Values are relative to those for the 0–1·0-cm zone = 100. Absolute values given in parentheses for the 0–1·0-cm zone have the units indicated in the first column

Measurement	Distance from Apex (cm)			
	0–1·0	1·0–4·5	4·5–9·0	9·0–13·5
Surface area per cm* (cm)	100 (0·22)	114	127	146
Dry weight (mg/cm)	100 (0·49)	49	76	116
Fresh weight (mg/cm)	100 (4·13)	116	159	218
Total nitrogen (% dry weight)	100 (7·18)	70	59	51
Total nitrogen (μg/cm)	100 (35·2)	34	45	59

* Neglecting area of root hairs.

The amounts of nitrate and ammonium nitrogen absorbed in 30 min by each zone are given in Table 3. The total amount of nitrogen ($^{14}N+^{15}N$) transferred to unlabelled zones by contamination or translocation did not exceed 0·4 μ-equiv/g for NO_3^- and 2·1 μ-equiv/g for NH_4^+. Neither the seed nor the shoots showed any enrichment with ^{15}N in the 30-min period. The nitrogen content of the labelled solution was not depleted by more than 0·5% during the 30-min uptake period.

The changes in ^{15}N content produced by the cyanide treatment or by washing with ^{14}N are given for the various zones in Table 4.

The time course of uptake for whole seedlings is shown in Figure 3. The amounts absorbed by the seedlings in the first 30 min agreed with the sum of the uptakes that had been measured for successive zones of the radicle.

TABLE 3

UPTAKE OF NITRATE AND OF AMMONIUM INTO SUCCESSIVE ZONES OF THE PEA RADICLE

Values are relative to those for the 0–1·0-cm zone = 100. Absolute values given in parentheses for the 0–1·0-cm zone are expressed as n-equiv/30 min

Uptake Measured	Distance from Apex (cm)				Distance from Apex (cm)			
	0–1·0	1·0–4·5	4·5–9·0	9·0–13·5	0–1·0	1·0–4·5	4·5–9·0	9·0–13·5
	Nitrate Uptake				Ammonium Uptake			
Per centimetre	100 (2·3)	31	41	41	100 (17·4)	25	48	54
Per square centimetre*	100 (10·2)	28	35	29	100 (78·7)	23	38	38
Per mg dry weight	100 (4·6)	63	54	35	100 (35·6)	54	63	46
Per mg fresh weight	100 (0·6)	29	26	19	100 (4·2)	24	30	25
Per mg nitrogen	100 (64·8)	90	91	70	100 (497·1)	78	106	90

* Refers to surface area of root, neglecting root hairs.

IV. DISCUSSION

Difficulties arise with potometers if the length of the zone enclosed is very short (<5 mm) and the uptake time is large (>1 day), as a considerable part of the ion absorbed within the potometer vessel then diffuses longitudinally through the cortex and out into the ambient solution (Russell and Sanderson 1967). It is unlikely that such losses were important in the present potometer experiment (length of zone

TABLE 4

INFLUENCE OF TREATMENTS WITH POTASSIUM CYANIDE AND [^{14}N]AMMONIUM ON UPTAKE

KCN*	$^{14}NH_4^+$†	Nitrate Uptake (μ-equiv/g dry wt.)				Ammonium Uptake (μ-equiv/g dry wt.)			
		0–1·0	1·0–4·5	4·5–9·0	9·0–13·5	0–1·0	1·0–4·5	4·5–9·0	9·0–13·5
–	–	4·6	2·9	2·5	1·6	35·6	19·4	22·5	16·5
–	+	—	—	—	—	22·4	12·7	n.d.	9·9
+	–	1·4	0·6	0·6	0·4	58·3	94·9	n.d.	49·6
+	+	—	—	—	—	22·1	22·0	n.d.	16·8

* 1 mM KCN before and during uptake. † Washed with 1·5 m-equiv/l $^{14}NH_4^+$ after uptake.

⩾1 cm, uptake time = 30 min). However, this kind of difficulty prevents high resolution being obtained. Greater resolution can be achieved with other techniques: excised root segments supplied with a source of energy can be used for potassium-uptake studies (Brown and Cartwright 1953); for elements having convenient radioisotopes, roots can be scanned with a counter after brief exposure to a labelled solution (Bowen and Rovira 1969).

The data in Table 3 show that uptake of nitrate and ammonium occurred over the whole length of radicle examined (0–13·5 cm). This conflicts with older beliefs;

for example, Sinnott (1935) states that "the absorption of material from the soil is (therefore) carried out only by a very small portion of the root system, close to the growing tips of the young roots, and never takes place anywhere in the older portions".

In relating the uptake data to developmental stages in the growth of the radicle, we may picture the 0–1-cm zone as that in which primary cell division and enlargement are performed. There is little cell division or enlargement in the 1·0–4·5-cm zone (Torrey 1963). Lateral primordia are initiated in the 4·5–9·0-cm zone, and laterals are growing within the cortex in the 9·0–13·5-cm zone. The vascular cambium is initiated and secondary growth begins in the 9·0–13·5-cm zone.

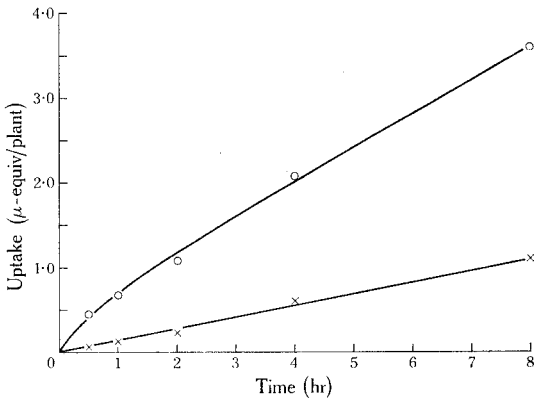

Fig. 3.—Time course of uptake of ammonium (○) and of nitrate (×) by pea seedlings.

It is interesting to note that uptake of nitrate or ammonium per unit length is least in the 1·0–4·5-cm zone, where there is least cell division and enlargement. A similar though less marked minimum in phosphate uptake has been observed by Bowen and Rovira (1967) in the corresponding zone of wheat roots—that is, in the zone intermediate to that of primary growth and that of lateral initiation. The region of cell enlargement immediately proximal to the apical meristem, though not the meristem itself, often absorbs ions rapidly (Burstrom 1939; Bowen and Rovira 1969); and rapid influx in this region probably accounts for the high uptake of nitrate and ammonium shown by the apical centimetre of the radicle.

The divergence in the distribution along the radicle of those properties to which uptake may be referred (see Table 2) implies that the apparent gradient in the uptake of any ion species must depend strongly on the way in which uptake is expressed. It is worth noting that the apical centimetre, in which uptake is most rapid, has a greater nitrogen content per unit length than the other zones. Moreover, uptake of either nitrate or ammonium shows least variation along the length of the radicle when expressed per unit total nitrogen content. Unlike the dry or fresh weights, total nitrogen content provides a good indication of the relative amounts of protoplastic material in each zone. The decrease in uptake of nitrate or ammonium per unit dry weight in the basal part of the radicle can be most simply attributed to the increased proportion of vascular and mechanical tissue.

When formulating models of actual uptake of nitrogen by plant roots, we need to know the radius of each zone and to have a measure of the propensities for uptake of ammonium and nitrate per unit length of each zone. Although studies of

longer duration and of the concentration dependence of uptake are clearly needed, the differences between zones, shown in Table 3, suggest strongly that it is unrealistic to treat the root as if it had a uniform surface. An approach to modelling that permits considerable flexibility in the choice of boundary conditions governing uptake has recently been outlined by Anderssen, Hale, and Radok (1969).

Ammonium is usually absorbed more rapidly than nitrate from neutral or near neutral solutions (Lycklama 1963; Micheal, Schumacher, and Marschner 1965). Depending on the zone considered in the present experiment, ammonium nitrogen was absorbed from 7 to 10 times more rapidly than nitrate nitrogen in the first 30 min of uptake. Figure 3 shows that a large difference between the rates of uptake of the two forms of nitrogen by the intact seedling persisted for at least 8 hr. Part of the ammonium was exchangeable; but adsorption of exchangeable ammonium could not account for the differences in ammonium uptake between zones, as a consistent proportion—nearly 40%—of that absorbed was exchangeable with $^{14}NH_4^+$ in each zone (see Table 4). Treatment of the radicles with 1 mM cyanide reduced the uptake of nitrate in each zone. It should be noted, however, that the uptake of nitrate by the cyanide-treated radicles was not negligible, particularly in the apical zone. In contrast to that of nitrate, the uptake of ammonium was greater in the cyanide-treated radicles than in the controls; this effect was particularly striking in the 1·0–4·5-cm zone. Most of the additional ammonium absorbed was exchangeable with $^{14}NH_4^+$ (see Table 4). Presumably the treatment with cyanide disorganized processes or barriers that limit the adsorption of ammonium in the normally metabolizing root.

V. Acknowledgments

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