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THE INFLUENCE OF PHOSPHORUS, ZINC AND MANGANESE ON ABSORPTION AND TRANSLOCATION OF IRON IN WATERCRESS

by I. P. CUMBUS, D. J. HORNSEY and L. W. ROBINSON

School of Biological Sciences, University of Bath, Bath, Avon, U.K.

SUMMARY

Absorption and translocation of iron by intact watercress plants (*Rorippa nasturtium-aquaticum* (L) Hayek) was studied in short period uptake experiments utilising ^{59}Fe labelled ferric chloride. Total translocation of iron was inhibited by increasing levels of phosphorus, zinc and manganese in the nutrient medium; the elevated phosphorus and zinc concentrations enhanced iron absorption into roots, but increased retention of absorbed iron in translocating portions of the plant. High levels of manganese in the medium reduced the initial absorption of iron into the root system.

INTRODUCTION

Severe cases of chlorosis have been reported in commercial watercress crops over a period of many years[2 14]. The condition has generally been diagnosed as an iron deficiency characteristic of lime-induced-chlorosis, described and documented in many other crop species[6]. Although watercress is usually successfully cultivated in alkaline irrigating waters, rising from chalk and limestone, instances of iron deficiency in crops have been recorded recently[8] and this investigation examined some of the possible factors concerned.

Previous studies with other crop species have implicated phosphorus as a causative factor in iron chlorosis[13]. Reduction in iron availability through precipitation in the nutrient medium is suggested as one possible cause of chlorosis[4 15]. Experiments on the foliar application of iron, with varying levels of phosphorus in the nutrient medium, have demonstrated an internal immobilization of iron in

translocating portions of plants[5 9]. The specific accumulation of iron in conductive tissue of bean plants (*Phaseolus vulgaris*) was shown to be correlated with the presence of phosphorus in the same tissues[9].

It has long been established that manganese can induce iron chlorosis in plants. A special reciprocal relationship was stressed between these two elements[18], based on a mechanism controlled by oxidation-reduction potentials. The reduced ferrous ion is accepted as the more readily available ionic form of iron to be absorbed by plant root systems[19]. Other micronutrients, including zinc, have an antagonistic relationship with iron in the processes of absorption and translocation, although no such valency change associated with oxidation-reduction is possible[12].

Direct iron-zinc interaction has been reported in rice seedlings (*Oryza sativa*)[11] and a possible influence of zinc on the reducing capacity of root sap and nutrient solutions has been suggested[1].

The elements phosphorus, zinc and manganese were selected for this study, in relation to iron nutrition, on the basis of their documented effects shown in other crop species and several more specific associations with watercress. Phosphatic fertilizers are the main nutrient supplement used in watercress cultivation to improve yields, and zinc levels are artificially raised in the irrigating water to prevent 'Crook Root' (*Spongospora subterranea*) fungal infections[21]. Under submerged substrate conditions the availability of iron and manganese for plant nutrition would be influenced by the degree of oxygenation of the medium[22]. The variable conditions of aeration in irrigating waters may affect the oxidation-reduction state, thus altering the ratio of ferrous and manganous ions which are available for root absorption.

METHODS

Uniform 6 week old watercress plants, grown under commercial conditions, were selected. Plants were lifted from the beds, washed in distilled water, and transferred to beakers of experimental culture medium (Table 1). Solutions were continuously aerated and renewed every 24 hours. Continuous illumination was provided by 4 × 20 watt white fluorescent tubes. A standard 3 day nutritional pre-treatment was given before isotope application, during which plants are subjected to their particular nutrient regimes.

Phosphorus, zinc and manganese were varied in the culture medium sur-

TABLE 1

Composition of experimental culture medium

Formula	Element	Final conc. (ppm)
$Ca(NO_3)_2 . 4H_2O$	N	10.0
	Ca	14.3
KH_2PO_4	K	1.3
	P	1.0
$MgSO_4 . 7H_2O$	Mg	2.25
	S	2.95
H_3BO_3	B	0.01
$CuSO_4 . 5H_2O$	Cu	0.001
KCl	K	0.055
	Cl	0.050
$MnSO_4 . 4H_2O$	Mn	0.020
$(NH_4)_6Mo_7O_{24} . 4H_2O$	Mo	0.001
$ZnSO_4 . 7H_2O$	Zn	0.005
$FeCl_3$	Fe	1.0
	pH	5.5

rounding the main basal roots, while the ^{59}Fe labelled ferric chloride was supplied to an isolated portion of the adventitious roots produced in the leaf axils. Similar nodal positions and root masses were selected on each experimental plant. This arrangement prevented ^{59}Fe becoming inactivated by any of the nutrient variables in the medium during the experiment.

Treatments consisted of four levels each of phosphorus, 0, 1, 10 and 50 ppm P; zinc, 0, 1, 5 and 20 ppm Zn; and manganese, 0, 1, 5 and 20 ppm Mn; with 5 replicates for each treatment. Although concentrations of the elements phosphorus, zinc and manganese were adjusted for particular treatments as indicated above, the remaining nutrients were present in the medium according to Table 1.

The isotope solution contained ^{59}Fe labelled ferric chloride with an activity of 10 μCi/ml. Aliquots of 0.3 ml were introduced with a syringe into polythene vials of distilled water surrounding isolated adventitious roots. Each vial contained a final volume of 3 ml with a total activity of approximately 3 μCi and 1 ppm Fe in solution.

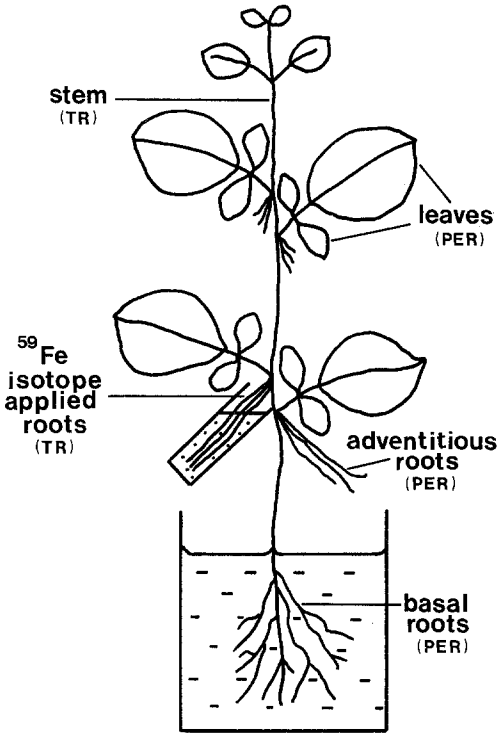

Fig. 1. Experimental arrangement of intact watercress plants for the application of ^{59}Fe and division of plant organs for analysis.

After a 24 h exposure to the labelled iron, plants were washed and their various organs, leaves, stem, adventitious roots, basal roots and isotope applied roots, were excised as separate samples for dry weight determinations. The oven dried tissue (100°C for 24 hours) was subsequently acid digested and the gamma activity determined using a NaI (Tl) well type scintillation counter. Results were expressed on a tissue dry weight basis and all counts were corrected for background activity.

RESULTS

The effect of each variable, phosphorus, zinc and manganese, on the final ^{59}Fe activity distribution between plant organs is shown in Table 2. Increasing phosphorus concentrations from 1 to 50 ppm P reduced the ^{59}Fe content of leaves, stem and adventitious roots, but with the 0 ppm P treatment the activity levels of these organs, on a dry weight basis, were found to be higher than in the 1 ppm P treat-

TABLE 2

Effects of phosphorus, manganese and zinc on ^{59}Fe distribution in watercress

Treatments		Distribution of absorbed ^{59}Fe (cpm/mg) dry weight ± SE)				
ppm		Leaves	Stem	Adventitious roots	Basal roots	Isotope applied roots
Phosphorus	0	39 ± 5	44 ± 6	133 ± 10	156 ± 11	2771 ± 46
	1	50 ± 6	57 ± 7	177 ± 12	37 ± 5	2988 ± 48
	10	17 ± 4	38 ± 5	68 ± 7	31 ± 5	4363 ± 58
	50	4 ± 2	33 ± 5	31 ± 5	9 ± 3	4879 ± 61
Manganese	0	66 ± 7	155 ± 11	245 ± 14	33 ± 5	5364 ± 64
	1	16 ± 3	53 ± 6	74 ± 7	25 ± 4	2719 ± 46
	5	12 ± 3	39 ± 5	77 ± 8	26 ± 4	1845 ± 38
	20	5 ± 2	21 ± 4	71 ± 7	19 ± 4	1764 ± 37
Zinc	0	35 ± 5	9 ± 3	86 ± 8	59 ± 7	3564 ± 52
	1	19 ± 4	13 ± 3	83 ± 8	38 ± 5	2761 ± 46
	5	5 ± 2	14 ± 3	39 ± 5	6 ± 2	4759 ± 60
	20	4 ± 2	30 ± 5	8 ± 3	8 ± 2	7213 ± 74

Mean activity levels of five replicate plants

ment. Basal root activity levels decreased with increasing phosphorus concentration, while the roots to which ^{59}Fe was directly applied showed enhanced activity levels with the higher phosphorus concentration treatments.

With no manganese in the culture medium high activity levels were generally found in all plant tissues including the isotope applied roots. Treatments of 1 ppm Mn and higher concentrations resulted in severe reduction in this activity.

Increasing levels of zinc reduced the resultant activity levels in leaves, adventitious and basal roots, whereas ^{59}Fe activity in stems and isotope applied roots was enhanced.

The plant organs can be arbitrarily divided into two fractions[9]: a translocatory portion (TR) and periphery (PER). For the applied ^{59}Fe to reach the peripheral organs *i.e.* leaves, adventitious and basal roots, it must first be absorbed and distributed through translocating portions *i.e.* isotope applied roots and stem respectively.

From the results shown in Table 2 it is apparent that increased concentrations of the three variable factors resulted in an overall reduction in translocation of ^{59}Fe to peripheral organs. The percentage of ^{59}Fe absorbed by the root system that is subsequently

translocated to peripheral organs is shown in Figures 2–4. High phosphorus concentrations in the culture medium resulted in greater retention of iron in the translocatory portion (Fig. 2). Each of the three component organs of the peripheral plant portion showed a decreased percentage in translocated iron with the increasing phosphorus treatments.

Increasing levels of manganese in the culture medium reduced the total translocation of ^{59}Fe (Table 2), but the percentage of absorbed iron which is translocated is not affected to the same degree (Fig. 3). The highest level of ^{59}Fe translocated to the periphery occurred at 0 ppm Mn; the remaining treatments all resulted in a lower and similar degree of iron translocation. A greater percentage of iron was translocated to adventitious roots at 20 ppm Mn, but the lesser

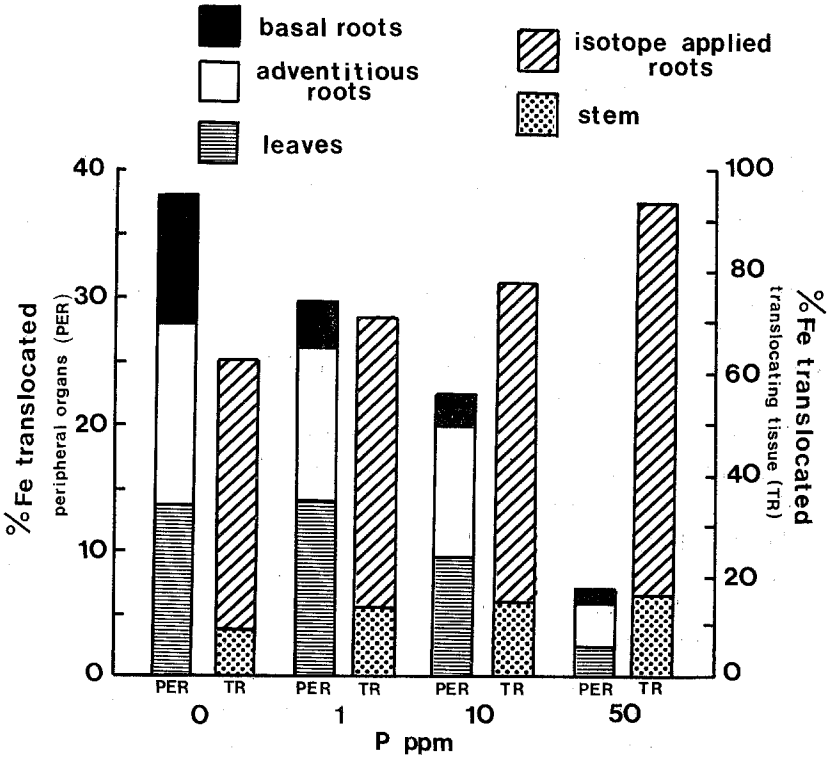

Fig. 2. The effect of phosphorus concentration in the culture medium on the percentage translocation of absorbed ^{59}Fe into plant organs.

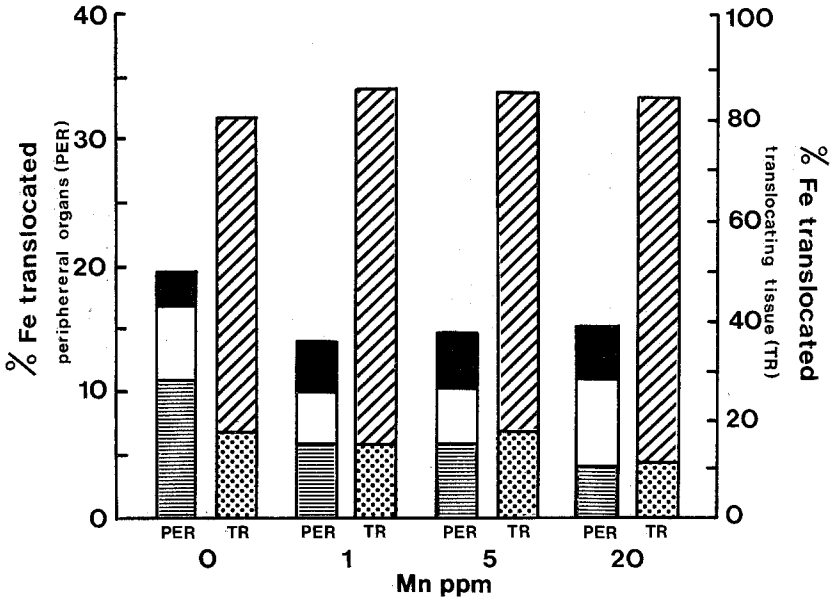

Fig. 3. The effect of manganese concentration in the culture medium on the percentage translocation of absorbed ^{59}Fe into plant organs. (Key to histogram see Fig. 2).

translocation to the leaves in this treatment resulted in only a small overall increase in the 'PER' percentage iron.

Iron translocation was particularly sensitive to zinc treatments (Fig. 4). At a culture medium concentration of 1 ppm Zn the proportion of absorbed ^{59}Fe subsequently translocated was slightly greater than at 0 ppm Zn, but 5 ppm and 20 ppm Zn treatments severely inhibited translocation and increased iron retention, particularly in the isotope applied roots.

DISCUSSION

In the movement of ions from substrate to the shoot, two metabolically active steps have been proposed[10]: active accumulation of ions at the root surface, where they combine with membrane carriers; and a second active process within the root, where ions are deposited into the vascular system. By observing total uptake of iron into the plant and its distribution between organs, the influence of phos-

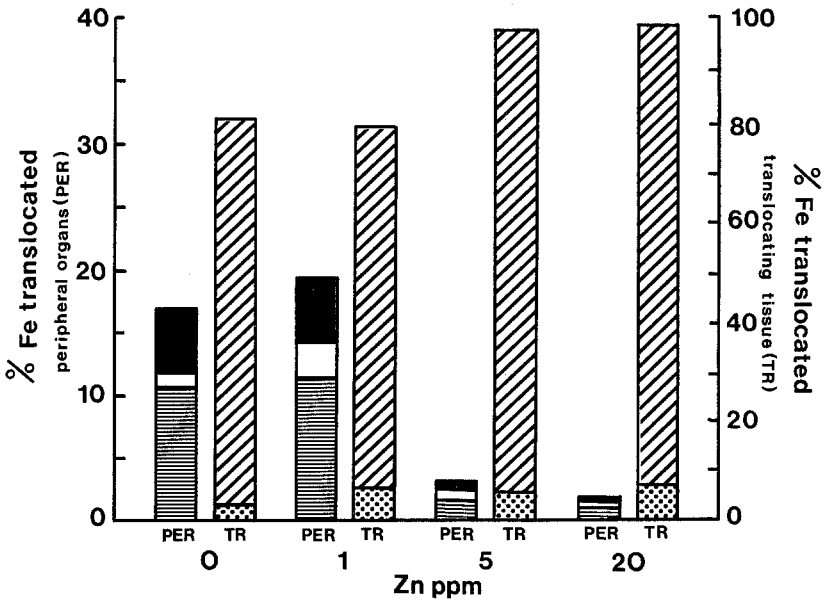

Fig. 4. The effect of zinc concentration in the culture medium on the percentage translocation of absorbed ^{59}Fe into plant organs. (Key to histogram see Fig. 2).

phorus, manganese and zinc on these active processes can be assessed.

Several previous studies of iron transport in plants have suggested that iron deficiencies in leaves can result from precipitation or immobilization of iron in translocatory tissues, caused directly by increased levels of other nutrient factors[3 9]. The results of experiments with watercress will be discussed in the light of these proposed mechanisms.

Total translocation of iron in watercress was severely inhibited by each of the three nutrient variables at the higher concentrations. The reverse effect was reported in *Zea mays* and *Phaseolus vulgaris* with increasing phosphorus in the culture medium[9]. It was concluded that tissue iron status would be low initially, due to inactivation of iron in the nutrient medium by high phosphate concentrations. Consequently, applied labelled iron was readily translocated to the deficient tissues. Mobility of iron is enhanced when tissue levels of this nutrient are at their lowest[16]. The experimental pre-treatment of watercress plants with high phosphorus solutions for 72 hours may have been of insufficient duration to reduce iron levels in tissues, as

iron translocation was found to be inversely correlated with increasing phosphorus levels.

Increased activity counts of ^{59}Fe in isotope applied adventitious roots of watercress, correlates with increasing phosphorus levels in the medium, indicating that the inhibitory action of phosphorus on iron transport does not occur at the initial root absorption site. Increased retention of iron in both stem and roots suggests iron inactivation in these portions. A direct precipitation of an iron-phosphate complex in translocating vessels is a possible explanation of these findings, or an effect on naturally occurring chelating agents which have been shown to be present in plants[17 20].

Zinc inhibition of iron transport was clearly shown in this investigation with little of the initially absorbed iron reaching peripheral plant organs in high zinc treatments. Activity levels in ^{59}Fe labelled adventitious roots rose with increasing zinc concentrations, showing that the inhibitory effect of zinc is not at the initial absorption site of roots, but at the loading site into the stem vascular system. Zinc may act as a competitive cation in the active metabolic transport occurring at this site.

High manganese treatments had little effect on internal distribution patterns of ^{59}Fe although the total translocation was reduced. This indicates that intereference with iron transport occurs primarily at the initial root absorption site with no further interactions at other locations. Manganese is reported as being antagonistic to iron in active absorption in other plant root systems[7 18], and results with watercress confirm such a mechanism. The separate application of ^{59}Fe to isolated adventitious roots avoided direct interaction with manganese in the nutrient medium. Iron uptake was, however, reduced with high manganese treatments, indicating an internal effect of manganese at the root absorption site.

Iron deficiency has been diagnosed as causing a specific chlorosis in watercress and this condition is often associated with variations in tissue levels of phosphorus, zinc and manganese[8]. These experiments have shown that an internal interaction of these three nutrient variables with iron can result in reduced iron transport to leaf tissue. This internal reaction does not preclude the possibility of external interaction of nutrients in the media reducing iron availability.

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