Ion Competition in Manganese Uptake by Barley Plants [1]

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The recommended concentration of manganese in Hoagland's solution produces toxic symptoms in barley plants and must be reduced substantially to avoid injury to the leaves. The suggested rate of 0.5 ppm Mn in this solution resulted in characteristic brown spots on the leaves and affected the yields adversely. When the concentration of Mn was reduced to 0.025 ppm, this difficulty was overcome. Equally effective was the addition of 10 ppm silicon as sodium silicate to the solution (*25*).

Another method of preventing manganese injury to barley leaves was that of increasing the total macronutrient strength of the solutions. This lowered the Mn content of the leaves, reduced the symptoms, and gave higher yields (*25*).

This report deals primarily with subsequent experiments in which the component ions of Hoagland's solution were manipulated to study their individual effect on the uptake of Mn by barley.

Materials & Methods

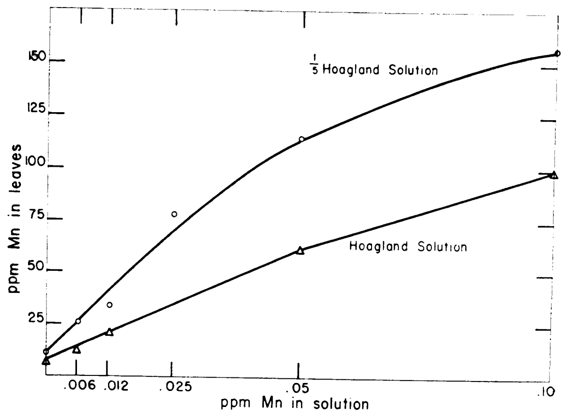
Seeds of Atlas barley (*Hordeum vulgare* L.) were germinated on screens mounted on pyrex dishes containing distilled water. After 1 week, 5 seedlings were placed in corks and transferred to 45-liter tanks in duplicate filled with Hoagland's No. 2 solution, with modified micrcnutrients (table I), and provided with gentle aeration. After growing for 6 weeks in the greenhouse, the plants were harvested, dried, weighed, and analyzed for Mn and Fe. Manganese was determined colorimetrically as permanganate after heating with periodate, and iron was complexed with *o*-phenanthroline.

Results

In the first experiment barley plants were grown in solutions covering a wide range of Mn concentrations comparing full-strength with one-fifth strength Hoagland macro-salts. Micronutrients other than Mn were added uniformly to all cultures. The composition of the solutions used is given in table I.

Analysis of the leaves showed an increase in Mn content with increasing concentration of Mn in solution, as would be expected. The Mn content of the leaves was higher in the plants grown in the one-fifth strength solution compared to the full strength, again as would be expected on the basis of previous evidence. (fig 1).

Of additional interest is the yield curve obtained from the same experiment (fig 2). In the low range of supplied Mn the yields of plants grown in fifth-strength Hoagland's are larger than those grown in full-strength solution, presumably due to greater Mn uptake in the face of lower ion competition. At the higher range of Mn in solution, the Mn content is again higher in the low-salt plants but now this works adversely on the yield because toxicity due to high Mn has entered the picture. The two curves cross at the point where the advantage of a higher Mn content in the deficiency range is overcome by



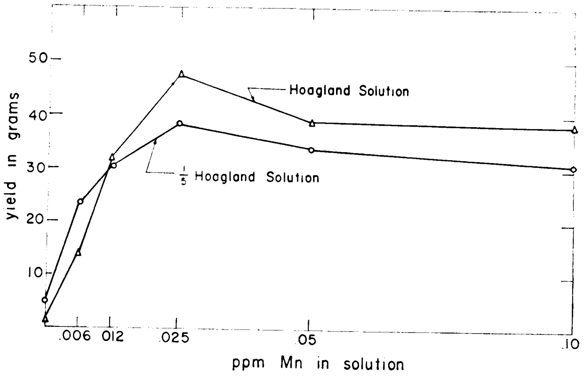


Fig. 1. Manganese content of leaves as a function of the concentration of manganese in solution.

Fig. 2. Yield of barley as a function of the concentration of maganese in solution.

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Table I

Composition of Hoagland's Solution No. 2
With Modified Micro-Nutrients

Salt	Macronutrient salts meq/l Full strength	1/5 Strength
$Ca(NO_3)_2$	8	1.6
KNO_3	6	1.2
$MgSO_4$	4	0.8
$NH_4H_2PO_4$	1	0.2

	Micronutrient Salts mg/l	*Element*	ppm
H_3BO_3	0.143	B	0.025
$MnCl_2.4H_2O$	1.81	Mn	0.5
$ZnSO_4.7H_2O$	0.22	Zn	0.05
$CuSO_4.5H_2O$	0.08	Cu	0.02
$H_2MoO_4.H_2O$	0.02	Mo	0.01
$FeSO_4$	0.1 ppm Fe added alternate days		

the negative effects of toxicity at the higher concentrations of Mn.

The question remained as to whether the depressing action of the higher salt solution on Mn uptake was due to a general concentration effect or a more specific case of competition by certain ions. To clarify this point, barley plants were grown in one-fifth strength Hoagland salts (table I) as background solutions supplemented with single salts added at the rate of 10.8 meq per liter.

Early in the experiment leaf symptoms of calcium deficiency appeared so the seedlings were replanted with an increase in the background concentration of calcium nitrate from 1.6 to 3.2 meq per liter. Following this correction no deleterious effects were noted from the use of the experimental salts. The pH of the solutions was maintained at a value of 5.3 ± 0.2 by adding acid or base corresponding to the single salt under investigation.

This technique made it possible to compare over a long growth period the effect of individual cations, Ca, Mg, K, and NH_4 paired with the anions nitrate, sulfate, and phosphate. An additional cation, Na, although not a constituent of Hoagland's solution, was included in the study because of its biological and agricultural importance. Micronutrients were added in the amounts shown in table I.

The leaves from 160 plants were analyzed for Mn (table II). With the plants grown in the background solution alone considered as controls, the results show that adding Ca, Mg, and NH_4 nitrates or sulfates produced plants with a lower Mn content. Plants grown with Ca or NH_4 phosphate were lower in Mn, those with K or Na phosphate were higher than the control. The nitrates and sulfates of Na and K and the phosphate of Mg were of the same order of magnitude as the control.

Comparing the average values of the cations, Ca, Mg, and NH_4, may be listed in one group which produced plants with less Mn than the check, and K and Na which gave plants with more Mn. For anions the order of decreasing Mn produced in the leaves is: phosphate > nitrate > sulfate.

The roots were also analyzed for Mn content (table II). The same general pattern of Mn uptake, as a function of the supplementary ions added, exists in the roots that was found in the leaves.

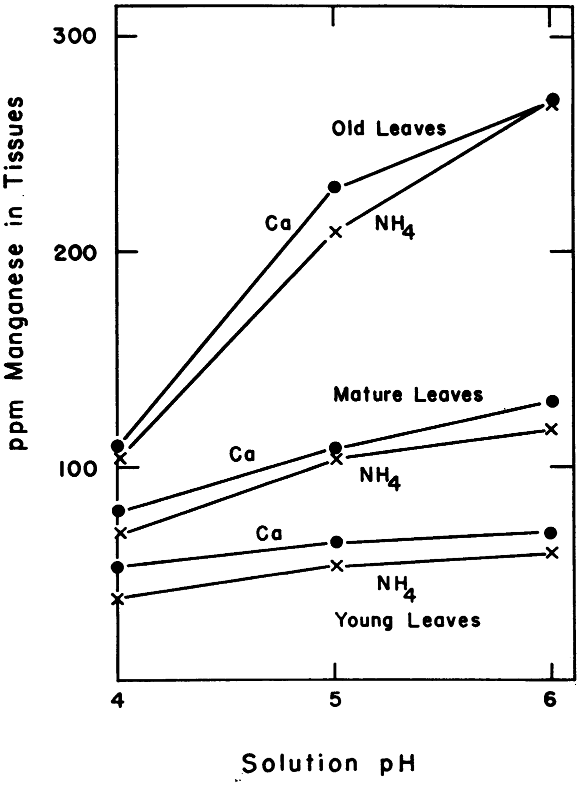
An important factor in ion uptake is the pH of the medium in which plants are grown (11). In the preceding experiments the pH of the nutrient solutions had been controlled in a range of 5.3 ± 0.2. High manganese uptake has been found in acid soils, especially when the pH is below 5. Raising the pH

Table II

Manganese Content in ppm of Barley Plants
Grown in Low-Salt Background Solutions
Supplemented With Single Salts*

Anion of single salt	Cation of single salt: Ca	Mg	Na	K	NH_4	Avg.
			Leaves			
Nitrate	317 ± 19	289 ± 19	424 ± 24	420 ± 19	229 ± 17	340
Sulfate	311 ± 15	259 ± 14	407 ± 27	411 ± 12	217 ± 13	321
Phosphate	356 ± 23	431 ± 16	538 ± 15	501 ± 21	395 ± 23	444
Average	328	326	456	444	280	368
	Unsupplemented low-salt background solution					426
			Roots			
Nitrate	656 ± 32	519 ± 29	963 ± 45	697 ± 38	405 ± 25	648
Sulphate	585 ± 27	480 ± 30	773 ± 27	637 ± 33	469 ± 31	589
Phosphate	715 ± 36	759 ± 41	1042 ± 49	770 ± 42	576 ± 28	812
Average	652	619	959	701	483	689
	Unsupplemented low-salt background solution					730

* In the first week of the experiment some plants showed symptoms of calcium deficiency. This was corrected by raising the 1.6 meq/l of calcium nitrate (table I) to 3.2 meq in the background solution.



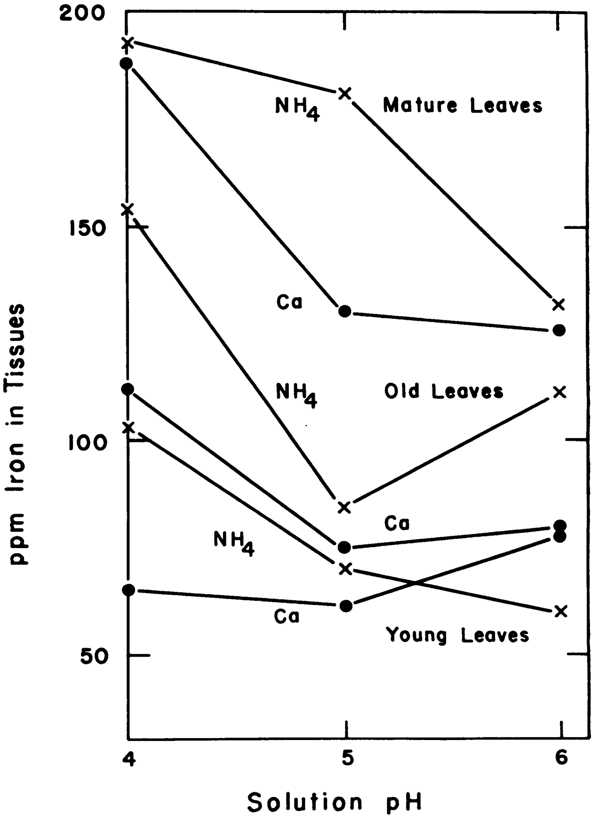


Fig. 3. Manganese content of leaves from plants grown in solutions at different pH values.
Fig. 4. Iron content of leaves from plants grown in solutions at different pH values.

by adding lime to the soil has the effect of reducing the Mn uptake to the point where symptoms of Mn toxicity are eliminated (1, 2, 6, 17).

A study was made of the Mn uptake by barley plants grown in nutrient solutions controlled at pH values of 4, 5, and 6. The variation in reaction was limited to 0.2 pH unit, beyond which it was restored by adding 0.1 N H_2SO_4 or NaOH. The background solution was of the same composition as that used in the previous experiment, and single salt supplements of $Ca(NO_3)_2$ and NH_4NO_3 were compared.

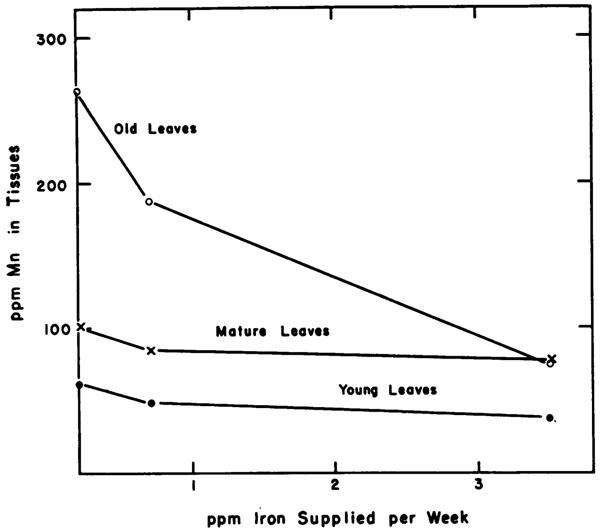
For purposes of analysis the shoots were divided into three parts: young, mature, and old. Of the total leaf tissue, about one-fourth by weight was classified as young, a similar ratio was old, and the remaining half was mature. This separation was made because of sharp differences in Mn content of leaf tissue according to its stage of development.

In figure 3 are shown the results of Mn analysis of plants grown at the three pH values in solutions with a high concentration of Ca or NH_4. The maximum uptake of Mn occurred in the old leaves at a pH of 6 with both salt solutions. It dropped considerably at a pH of 5, and still further at 4. The same trends are apparent for the mature and young leaves but the differences in Mn content as a function of pH

Table III

Mn & Fe Content in ppm of Roots From Plants Grown in Solutions With Controlled pH Values

Solution pH	Supplementary salt: Mn		Fe	
	$Ca(NO_3)_2$	NH_4NO_3	$Ca(NO_3)_2$	NH_4NO_3
4	240 ± 19	230 ± 17	17,900 ± 1140	23,000 ± 1370
5	670 ± 39	440 ± 28	10,900 ± 830	9,000 ± 510
6	485 ± 27	590 ± 31	6,600 ± 410	5,900 ± 385



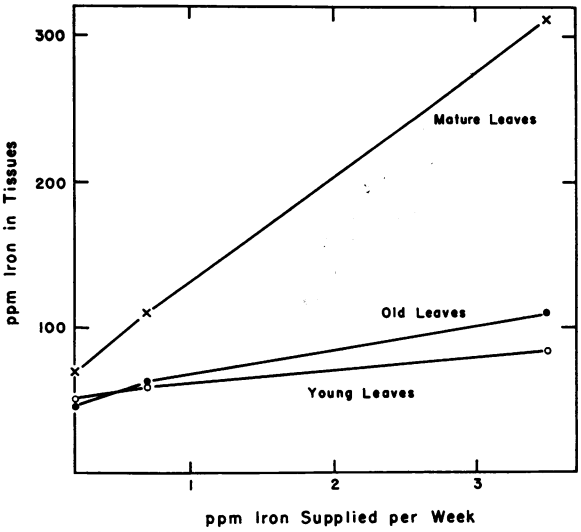


Fig. 5. Manganese content of leaves from plants grown in solutions with three rates of iron supplied.
Fig. 6. Iron content of leaves from plants grown in solutions with three rates of iron supplied.

are not as pronounced as they are in the old leaves. The Fe content of the leaves is given in figure 4 and the results of Mn and Fe analysis of the roots are shown in table III.

The literature contains many reports of iron and manganese interaction (4, 9, 18, 20, 22). Taking this into account, an experiment was conducted to study the influence of iron supply on Mn uptake. Three rates of iron, as ferrous sulfate, were added to one-fifth strength Hoagland solutions and barley plants were grown as previously. Mn was added to each tank at the rate of 0.5 ppm and the solutions were kept at a pH of 5.5 The results of Mn analysis of the leaves are illustrated in figure 5. From these curves it is evident that as the Fe supply is increased the Mn content of the leaves is reduced, especially in the case of the old tissues. In figure 6 are shown the curves of iron content in the three fractions of the leaves as a function of Fe supplied.

The amounts of Mn and Fe found in the roots are presented in table IV. The iron content is extremely high and the proportion of this nutrient on the outer surface of the cell walls was not determined, but it must be considerable since it is visible to the naked eye as a heavy coating of a red iron precipitate. The Mn content of the roots was less as the amount of Fe in solution was increased, but the effect did not compare with that found in the old leaf tissues.

Table IV

Mn & Fe Content in ppm of Roots From Plants Grown in Solutions at 3 Rates of Iron Supplied

ppm Fe per week	Mn	Fe
0.2	480 ± 35	4,400 ± 265
0.7	420 ± 28	9,800 ± 590
3.5	410 ± 25	27,600 ± 1,900

Discussion

The comparison of individual salts on an ion for ion basis showed that Ca, Mg, and NH_4 salts were more effective than K and Na in repressing the uptake of Mn by barley plants. Among the anions, phosphate resulted in higher Mn in the tissues than was obtained with sulfates and nitrates.

In assessing the depression of Mn uptake brought about by high concentrations of the macronutrient salts of Hoagland's solution, account must be taken of the relative proportion of the ions present. Full strength Hoagland's No. 2 solution includes 8 meq of Ca, 4 of Mg, and 1 of NH_4 per liter and these ions were the most effective in reducing Mn uptake. The other cation, K, is present at a concentration of 6 meq per liter, and it was found in the single salt study to have a minor effect on the Mn uptake as the nitrate or sulfate and a stimulating effect as the phosphate. Sodium salts uniformly produced the highest Mn content in the roots, but with respect to the leaves the effect of Na closely followed that of K.

The over-all depressing action of increased Hoagland solution salts must be due primarily to the cations Ca and Mg. The NH_4 ion, while extremely effective in the single-salt study, is present in such a small amount in Hoagland's solution (1 meq/l) that its contribution in retarding Mn uptake must be of a questionable order of magnitude. The tendency of phosphate to increase Mn uptake suggested the possibility that it might be tying up Fe and thus indirectly affect the absorption of Mn.

This question was examined further and it was established that an increase in the supply of iron resulted in a decreased Mn uptake. The influence of Fe was much more effective than was the case with any of the single-salt supplements.

An increase in the hydrogen ion concentration also exerted a marked effect in depressing Mn uptake. It has been shown generally in absorption work that solutions at a low pH, reduce ion uptake (5, 11). In these experiments there was less Mn taken up from the more acid solutions while the Fe absorption was greater. The latter effect may have been due to a higher solubility of Fe with acidity resulting in a greater availability to the plant. It may be assumed that the uptake of Fe involved two forces working at cross purposes. One is the increased competition from hydrogen ions at the lower pH values causing a decrease in Fe absorption, the other being the greater solubility of Fe pushing in the other direction. Ostensibly, this experiment was designed with pH as the variable. Actually, Fe was also a variable because of its pH dependence. While the amount of iron supplied was carefully controlled, its availability was a function of the acidity.

This amounts to saying that, in the pH experiment, the lower Mn uptake in the more acid solutions was a product of competition from higher concentrations of iron and hydrogen ions. This is not the only possible explanation of these results but it appears to be the most consistent with current theories in this field of work.

In those experiments where both Mn and Fe were determined in the tissues, the Mn content varied directly with the age of the leaves whereas Fe was higher in the mature leaves than in the young or old leaves. This could be explained as a migration of Fe from the old tissues to the mature ones in the course of plant growth and development, but this would not be consistent with the notorious immobility of Fe in plant tissues.

The results support the general idea of Tottingham (*22*), Somers and Shive (*20*), and others (4, 9, 18) as to an Fe-Mn antagonism in plants. The finding of Morris and Pierre (15) that the effect of Fe in reducing Mn toxicity was due to a decrease in Mn absorbed by the plant rather than an increase in Fe content is not supported by our results.

The data reported here and elsewhere indicate that, in experiments with nutrient solutions (3, 16) and clay suspensions (5), Mn uptake is increased as the pH of the medium is raised toward neutrality. In acid soils the opposite effect has been noted (8, 12, 23, *24*) and the same may be said for manganiferous soils (9, 10, 19). In these situations the higher Mn concentrations in the soil solution more than compensate for the inhibiting effect exerted by hydrogen ions in the more acid media.

The effect of Ca in reducing Mn uptake in these experiments is in accord with the findings of Hewitt (8) but in contradiction to the work of Morris and Pierre (15). Earlier work by McCool (13) had shown that the chlorides of Ca, Mg, K, and Na each had an ameliorating effect on the symptoms of toxicity from Mn in pea and wheat seedlings.

The greater uptake of Mn in the presence of phosphate compared to nitrate and sulfate was of particular interest since one is a cation and the other an anion. It has been shown by Morris and Pierre (15) that phosphate increases the severity of Mn toxicity. While Bortner (2) showed the opposite effect, the discrepancy can be explained on the grounds that he used trisodium phosphate which would shift the pH and in that way mask the influence of phosphate per se.

It may be more than a coincidence that phosphate also has been reported to increase the uptake of Mo (*21*). The possibility should be investigated that the same mechanism is at work in the stimulation of Mn and Mo uptake by phosphate. One assumption is that phosphate ties up iron either on the roots or within the plant, thus removing an inhibiting influence on Mn and Mo absorption.

Gerloff et al. (*7*) have suggested the production of an iron-molybdenum precipitate of very low solubility as a factor in antagonisms they encountered. In this case, if phosphate were to tie up Fe, the net effect could be a stimulation of Mo uptake.

Where Fe and Mn are concerned we have the possibility of direct competition between two cations. Again, if phosphate were to remove Fe by precipitation, the end result could be an increase in Mn uptake. This hypothesis to explain the stimulation of Mn and Mo uptake by phosphate would be an attractive one except for some work by Millikan (14) and Warrington (*26*) which failed to show the necessary Fe and Mo antagonism. This whole question is worth exploring further to establish the facts in the matter, first of all, and then determine if the data support the hypothesis outlined above, or if a different explanation of the effect of phosphate is forthcoming.

Summary

Barley plants grown in Hoagland's solution developed toxicity symptoms in the leaves which were related to high concentrations of Mn in the tissues. Full-strength Hoagland macro-salts were more effective than one-fifth strength in reducing the Mn content of the tissues and the symptoms of toxicity.

A study of Mn uptake from single-salt solutions superimposed on a weak, balanced nutrient solution revealed that Ca, Mg, and NH_4 nitrates or sulfates were the most efficient in reducing Mn absorption. The nitrate and sulfate of K and the phosphate of Mg produced plants with a Mn content of about the same order of magnitude as the check. Plants grown in Ca or NH_4 phosphate were lower in Mn, while K phosphate plants were higher than the control. Sodium, not a constituent of Hoagland's solution, was also found to behave in general like K in its effect on the Mn content of the leaves but produced the highest Mn values in the roots.

Iron and hydrogen ions were even more effective than the macrosalts in reducing Mn uptake, while at the same time the iron content increased. A hypothesis was considered to explain the reported stimulation of Mn and Mo uptake by phosphate.

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