FACTORS AFFECTING THE UPTAKE OF IRON IN SPIRODELA OLIGORHIZA (KURTZ) HEGELM

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INTRODUCTION

The uptake of iron by higher plants is a complex process involving the interrelationships of many diverse factors. These include the chemical behavior of the element in soil or culture solution, factors which influence the rate and extent of its passage into the roots, its storage and translocation characteristics and finally its role in the metabolism of the plant. The growth of the plant reflects its reaction to the entire complex of its environmental conditions. If one or two or three of these are varied from an optimal pattern, the summation of the effects of these variations upon the diverse factors listed above will be revealed in the subsequent growth of the plant.

The important role of iron in the economy of higher plants has interested plant investigators for over a century. The problem has been approached in many ways and with numerous different techniques. Much information has been gained. In spite of this, many questions remain unanswered. The literature contains many apparently contradictory statements regarding optimal iron levels, the benefits of chelating agents, the role of competing nutrient ions, pH effects and the relationships between nitrogen sources and mineral requirements. This study was initiated to apply the technique of aseptic culture of green plants to these problems. This method permitted the exercise of close control over the environment of the test plants. The

ability of the worker to alter one factor at a time within narrow limits of concentration seemed to provide a way in which new information might be gained. Such information would, of course, have to be interpreted in the light of the complex of interacting factors mentioned above.

This study involved examining the effect upon plant growth (as measured by weight increase) and iron uptake of the following:

1. Limited changes in the concentration of molybdenum and manganese in the culture solution.
2. The use of different iron levels (0.8 and 0.08 p.p.m.) of ferric chloride, ferric citrate and sodium iron ethylene diamine tetraacetate.
3. The use of ammonia (as $(NH_4)_2SO_4$) instead of nitrate as a source of nitrogen.

LITERATURE REVIEW

Iron

Today there is general agreement that the role of iron in the economy of plants is related to the plant enzyme complex. The cytochrome system of the mitochondria utilizes iron in its electron transport system (30, p. 378) as do the enzymes catalase, peroxidase and the succinnic dehydrogenase complex. This element appears to be necessary for the proper functioning of aconitase, and Nicholas (55, p. 596) has indicated that the activity of hydrogenase is greatly curtailed in states of iron deficiency. Most of the iron in leaves is located in the chloroplasts where it participates in the manufacture of chlorophyll. Cytochrome f, and possibly other enzymes containing iron appear to be needed by the chloroplast in photosynthesis. Examination of chloroplast iron indicates that it may be present in three distinct fractions: a water-soluble form, an acid hydrolyzable form and a heme form. The work of Weinstein and Robbins (98, p. 31), who observed a decrease in protein nitrogen content in iron deficient plants suggests a role, probably indirect, in protein synthesis.

The problem of iron nutrition in plants was not a new one. In 1844, Gris (31, p. 1118), observed that plants lacking iron in their nutrient solutions grew poorly and produced blanched leaves. Since that time hundreds of experiments have been performed in attempts to understand why plants respond as they do to the iron in their environment. In a series of experiments on isolated pea roots, Brown and

Possingham (15, p. 145) noted that iron deficiency resulted in an abrupt arrest of cell division in the apical meristem on the seventh day of deficiency. At this time cyanide-sensitive oxygen uptake ceased to increase, although cyanide-insensitive oxygen uptake continued to increase through the eleventh day. Since cyanide-sensitive oxygen uptake is associated with the cytochrome system, this would suggest the existence of some form of oxygen utilization not connected with iron-containing or iron-activated enzymes. During active growth, amino acids, synthesized in the more mature region of the root, were translocated to the apical meristem, where protein formed. Upon cessation of cell division, these amino acids were observed to condense into proteins in the more mature regions adjacent to the apex. Jacobson (38, pp. 233, 243) analysed chlorotic leaves and found that they contained less total iron than green leaves. He also pointed out that his experiments suggested that iron was present in more than one form. These included an "active" form, which was involved in chlorophyll formation, and an "inactive" or "residual" form, which was the fraction necessary for the other metabolic functions of the leaf. He proposed that the leaves normally satisfy their basic requirement for non-photosynthetic iron first, then supplied it to the chlorophyll-forming fraction. Wallace (92, p. 2398) also noted that plants had to be chlorotic for a week before she could detect a decrease in the activity of the iron-containing enzymes. Bennett (7, p. 100) has also discussed the active and inactive or residual fraction of iron in leaves. He measured these components and estimated that in

chlorotic leaves 32% was of the active form, while in green leaves this form amounted to 72%. He indicated that the active iron was built up quickly, but weakly held, and that the residual portion developed more slowly but was in a more stable form. Sideris (72, p. 319) has suggested that residual iron was found in the proteinaceous matter of the cells, the cell walls and substances with peroxidase activity. This view implied that leaves could appear chlorotic while still containing a considerable amount of iron in the residual form. This iron, being tightly held, was not considered to be available for chlorophyll formation. Thus, Twyman (89, p. 222) has indicated that the concentration of iron in the tissues bears little or no relation to the incidence of chlorosis. The observation that iron appeared to be more effective in plants early in the growing season than later tended to support this hypothesis. In addition, Bennett measured the active iron fraction through the growing season, and observed that it decreased continuously and significantly as the season progressed. This tendency of an ion to accumulate in a stable complex was also recognized by Overstreet (63, p. 492), who suggested it as a basis for a new general view of ion absorption and accumulation. In his discussion he indicated that ions formed complexes with protoplasmic constituents based principally upon valence characteristics. Ions possessing the same valence compete with one another for sites in the protoplasmic and absorption complexes.

Factors, such as pH and phosphate or potassium concentration of the culture solution, add to the complexity of the absorption and

translocation processes. Acidity has been observed to produce a variety of effects. Where iron is not protected by a chelating agent, a high pH results in the formation of an iron precipitate (97, p. 428). At low pH the excessive availability of certain other ions, notably manganese, has been observed to depress iron uptake (93, p. 159). Thorne et al. (84, p. 258) have stated that the effect of high pH did not concern iron absorption, but served to inactivate it within the plant. Acidity effects were considered so important by Allison et al. (1, p. 502) that they stated that Lemna could not be grown at a pH below 6.0.

Phosphate effects have been variously treated by different workers. One group (84, p. 258) has stated that there is no evidence that high phosphate concentrations cause a precipitation of iron in soils or plants, and thus produce deficiency symptoms in so-called lime-induced chlorosis. Miller and others (49, p. 624), however, have indicated that high phosphate concentrations inactivate iron, and Biddulph (8, p. 265) has stated that ferric phosphate may precipitate either on the surface of the root epidermis or in the xylem of roots, stems or leaves. In examining the action of potassium in relieving chlorosis under high phosphate conditions, Bolle-Jones (11, p. 170) has suggested potassium increased the amount of available iron by depressing the phosphate concentration and the inorganic/organic phosphate ratio.

The influence of oxygen has been considered in iron uptake. Granick (30, p. 378) stated that it seems clear that it is necessary

for the root to utilize oxygen to absorb ions to any appreciable extent. Keeler (40, p. 582), however, measured 4 times as much Fe^{59} taken up by non-aerated cultures as by rapid-shake cultures. This did not mean that oxidative respiration had ceased in the non-aerated cultures, but surely the rate of oxidation was lower than in those being shaken.

Iron deficiency chlorosis has been observed in plants which apparently possessed an adequate supply of the element. As mentioned earlier, iron, upon being taken up into the plant, appears to form stable complexes with the protoplasm. Unless additional supplies of the element are available, as new leaves and stems are produced, these organs may suffer from a local iron deficiency. Although translocation of iron from old organs to new has been observed, the process is not rapid enough to provide adequate amounts to young growing tissues (8, p.274). Factors involved in the mobility of iron within the plant have been investigated by Rediske and Biddulph (66, p. 593). Their results indicated that the iron concentration within the plant was the primary factor in its mobility. They observed that the total amount of iron translocated decreased with increases in iron concentration. The concentration of phosphate and the pH of the nutrient solution were secondary factors. The rate of iron movement within the plant increased with decreasing phosphate concentration and decreasing pH. The supposition that iron has remained in the plant roots because of its complexed form or because it might have precipitated there became questionable in

the light of the work of Corin et al. (19, p. 388). His group supplied iron to petioles and leaves and noted that 60% of it moved from the leaves to the roots.

Part of this study is devoted to examining the effects of altering the ratio of the concentrations of iron and manganese. The results to be examined reflect not only their competitive or additive effects in absorption and translocation, but also the effects of these combinations on growth. The paragraphs above discussed the role of iron in the economy of the plant; a similar discussion of manganese follows.

Manganese

Like iron, manganese has appeared in recent investigations to be involved in a variety of plant processes. Pirson (65, p. 92) has observed that under conditions of manganese deficiency in algae, the Hill reaction is completely suppressed, and photosynthesis is strongly inhibited. He has assumed from these observations that manganese is essential in some particular step between the photolysis of water and the evolution of oxygen. Others have found that a deficiency of this element affects the division of chloroplasts (16, p. 54). Arnon (5, p. 26) has noted that the addition of manganese restored photosynthesis to the rate of normal cells, and suggested a direct catalytic participation by the element in photosynthesis. Eyster et al. (23, p. 167) have pointed out that manganese is required for both heterotrophic and autotrophic growth and that its functions in these two cases were distinctly separate.

The observations of Twyman (88, p. 22) have led him to suggest a role for manganese in sugar metabolism. In addition to its role in the functions discussed, manganese is involved in a variety of other plant reactions. Schutte and Schendel (71, p. 959) have observed a decrease in the level of isoleucine and alanine in plants under conditions of manganese deficiency, and several investigators (65, p. 94) have suggested that this element might be involved in the reduction of nitrates. Although it appears to have a wide usage in plants, Reisner and Thompson (67, p. 1473) have pointed out that the manganese requirement for photosynthesis is clearly several times higher than for other functions.

The absorption of manganese into the plant and its subsequent translocation have been studied by many. Rothstein (69, p. 8) has suggested that absorption is a two-step process. First, manganese appeared to bind in an exchangeable form on to the surface of the cell. Then the ion appears to complex with a carrier for entry into the cell. In this work it was observed that the entry of manganese must be accompanied, or preceded by, a corresponding uptake of phosphate. The dependence upon phosphate was apparently related to the synthesis of a phosphorylated carrier system. During the first phase, when the ion was externally bound, it could be replaced by other divalent ions such as calcium, magnesium or iron, which, when present in high concentrations, have been observed to depress the uptake of manganese (47, p. 374). Once in the plant, manganese appears to move readily to the leaves. Redistribution of the element, however, has been shown to be a slow process. Single (73, p. 487)

has noted that it is not easily moved in the phloem, and that its movement from leaves to growing points appears to be significant, but very small. The solubility characteristics of manganese extracted from leaves has suggested that any excess of the ion in the leaf may be in the form of metallo-silicates, phosphates, or perhaps, molybdates (74, p. 501). It has been noted that the formation of such salts at high manganese concentrations, would favor a low, steady rate of release of the element in the leaf, and reduce the possibility of toxic effects.

Iron-manganese interactions

When an investigator alters the concentration of either iron or manganese in the nutrient solution, the ultimate effect is actually a composite of the effects of the change on such diverse functions as photosynthesis, respiration, nitrate reduction, protein synthesis, carbohydrate utilization, etc. The actual observation will be in terms of weight change or iron uptake, but it still may represent the summation of many individual reactions.

Many such observations on the interactions of manganese and iron have been made. De (20, p. 100) has noted that, under certain conditions, the application of iron to sugar cane may result in weight increase and carbohydrate synthesis. Morris and Pierre (52, p. 386) have indicated that in certain cases this may result from an iron-manganese antagonism. They suggested that the iron may, in the presence of large amounts of manganese, exert its effect by competitively

reducing manganese uptake, thus protecting the plant from possible manganese toxicity. Warrington (95, p. 1), Twyman (88, p. 22) and Wallace (92, p. 2398) also have noted this function in relieving manganese toxicity.

The results of an increase in the concentration of manganese supplied to a plant without a corresponding iron increase have been observed frequently. Smith and Specht (75, p. 381) noted that manganese was toxic to roots under these conditions. A decrease in the amount of iron absorbed has been reported (7, p. 94; 18, p. 254; 45, p. 566; 33, p. 75), and much of the iron absorbed has been found to be deposited in the roots (72, p. 319). In some cases this has been attributed to a competition for absorption at the root surface (7, p. 91; 8, p. 265). This condition interferes with translocation into the rest of the plant, and has led Sideris (72, p. 319) to suggest that the root or root surface may be the principal site of iron-manganese interaction. He also grew plants in culture solutions with and without added manganese. Upon measuring the relative amounts of iron in comparable parts of experimental and control plants, he found greater iron uptake and translocation in the plants grown without manganese. Possibly related to these observations are those of Leach and Taper (45, p. 570). They found that while the ratio of iron to manganese varied with different ratios in the nutrient solutions, the sum in p.p.m. of manganese plus iron was nearly constant. An interestingly different observation was that of Epstein and Stout (21, p. 63), who noted that excess manganese

in the medium increased iron uptake by plant roots from colloidal suspensions.

Others, however, have indicated that some of these effects may be more general. Anderson and Evans (4, p. 27) found that the activity of isocitric dehydrogenase and the malic enzyme extracted from leaves suffering from manganese toxicity was 2 to 3 times normal. The addition of high concentrations of iron to the extract reduced this activity to near-normal. This inhibition by iron was considered to be non-competitive. They concluded that the iron combined with other than the manganese sites on the enzymes. Weinstein and Robbins (98, p. 31) have postulated that, in certain cases manganese may vie for a position in the heme nucleus of iron-containing enzymes. This position was supported by the work of Wallace (92, p. 2398) who noted a decrease in leaf catalase and root peroxidase activity in soybeans at high manganese levels. The similarity of this report with that of low catalase and cytochrome oxidase levels (98, p. 31) in sunflower under low iron conditions has suggested that such a competition may indeed exist. The observation that high manganese levels interfered less with iron metabolism under conditions of high iron concentration has also lent credence to the possibility of such a competition.

Recognizing the competitive aspects in absorption and utilization of iron and manganese, many have come to believe that the important consideration in the relation between these two elements is the ratio of their concentrations rather than the absolute amount of either. Thus Leach and Taper (45, p. 566) have reported that

in dwarf beans, iron/manganese ratios below 1.5 in the culture solutions cause iron deficiency symptoms, and those above 3.0 result in manganese deficiency. That the iron/manganese ratio is peculiar to the species was indicated in the same report by the notation that the ideal ratio in tomatoes fell between 0.5 and 5.0. Oulette (62, p. 284) has reported that soybeans grew without abnormality in iron/manganese ratios between 5.0 and 100 provided the manganese concentration in the solution was between 0.1 and 2.5 p.p.m. and the iron above 2.5 p.p.m. Continuing their work on dwarf bean, Taper and Leach (83, p. 777) have indicated that these may be further modified by changes in the calcium concentration of the nutrient solution. In cultures containing 42 p.p.m. calcium they found good growth with iron/manganese ratios between 0.5 and 5.0, but when the calcium concentration was raised to 143 p.p.m., only ratios very close to 2.0 gave satisfactory growth. Carlson and Olsen (18, p. 254) have referred to iron/manganese ratios in their work on sorghum. They found that although the iron/manganese ratios present in the plant materials was proportional to those used in the nutrient solutions, the iron content of the expressed plant sap was not affected by either. They also reported that the ratios appear to exert their maximum effect under conditions of medium iron concentration, and appear to be less significant under conditions of either high or low iron supply.

Another explanation for apparent iron deficiency has involved the possibility of an oxidation-reduction relationship between manganese and iron. Somers and Shive (77, p. 601) have observed

that high concentrations of soluble manganese are invariably associated with low concentrations of soluble iron and vice versa. To them this suggested that active manganese, in excess, oxidized ferrous iron to the ferric state resulting in its inactivation and precipitation in the form of ferric organic complexes. Kenton and Mann (42, p. 185) have suggested that the function of manganese as an essential element in plant nutrition may depend, to some extent, upon this sort of activity. Hopkins (36, p. 610) has stated that his experimental results indicated that the level of manganese in the culture medium and in the cell tends to control the activity of iron through an oxidation-reduction system. Sideris (72, p. 319), in contrast, has indicated that no precipitation of iron could be detected in the nutrient solutions containing manganese, which could be expected to result from such an oxidation-reduction system, and others (65, p. 95) have stated that no evidence exists for the recognition of such a system between these two elements.

The suggestion has also been made that iron toxicity symptoms are identical to manganese deficiency symptoms, and the converse (77, p. 600). Hewitt (33, p. 75), however, has pointed out that they are not the same, since these elements have many independent functions. Nevertheless, it has appeared that, "iron and manganese are definitely interrelated in their metabolic functions, the biological effectiveness of the one being determined by the proportionate presence of the other." (77, p. 601)

Molybdenum

Another element which appears to interact with both iron and manganese in the plant and in the culture solution is molybdenum. In studies on the uptake of molybdenum, Peterson (64, p. 2144) has noted some correlation between the amount of molybdenum removed from different soils and their available molybdenum content. The ability of organisms to store large quantities of molybdenum has been demonstrated, and Keeler (40, p. 583) considered it probable that most of the molybdenum taken up was incorporated into a molybdoprotein. Attempts to separate the molybdenum from the protein have indicated that the complex is quite stable (41, p. 307). Amin and Joham (2, p. 297) have investigated the forms of molybdenum in the cotton embryo and have found a water-soluble fraction, an ammonium hydroxide soluble fraction and an insoluble fraction. Their observations of the rate of movement of this element supported the concept of a stable storage form. They found that after 400 hours the bulk of the seedling molybdenum remained in the cotyledon. In cotton there appears to be a critical level of molybdenum in the plant below which more of the element is absorbed with difficulty (3, p. 106).

Three basic functions for molybdenum in plant metabolism are indicated at this time. Evans (22, p. 208) has pointed out that it is essential for nitrogen fixation, being a constituent of the enzyme hydrogenase. Nicholas and Nason (57, p. 137) have identified it as the metal in nitrate reductase in Neurospora and in soybean leaves.

It has been shown that flavin and molybdenum function as electron carriers in the enzymatic transfer of electrons from DPNH to nitrate. Nason (54, p. 291) has reported that under conditions of molybdenum deficiency tomato plants convert less inorganic phosphate to the organic form than do the controls. Spencer (79, p. 433) had also noted this condition. He found that from 4-10 times as much inorganic phosphate was present in molybdenum deficient plants as was present in normal ones, and suggested that this was due to a heightened activity of acid phosphatases. Nicholas and Commisiong (56, p. 706) demonstrated in vitro that molybdenum inhibits the activity of acid phosphatases competitively, presumably by forming a molybdophosphate addition compound with the substrate. Since this reaction occurs at physiological concentrations, it has been suggested that this may be a normal function for the element.

Manganese-molybdenum interactions.

Investigation of the direct interactions between manganese and molybdenum have not been extensive. Geroff et al. (26, p. 612) have noted that varying the concentration of manganese or molybdenum had no effect upon inducing or accentuating a deficiency in the order, but Mulder (53, p. 414) has reported that high concentrations of manganese in acid soils inhibited the uptake of molybdenum by cauliflower. Candela and Hewitt (17, p. 158) have reported significant reductions in yield in tomatoes under conditions of excess/low manganese/molybdenum ratios. Yields were not appreciably reduced,

however, when an excess/normal ratio was tested. They also reported that increasing the manganese concentration of the nutrient solution from 0.01 to 11 p.p.m. resulted in an increase in the uptake of molybdenum. They suggested that unfavorable results of excess manganese on molybdenum deficient plants are not due to changes in molybdenum content, but to interrelationships between these two elements in plant nutrition. The work of Margolis (48, p. 2220) illustrated this sort of relationship. He observed that recovery from molybdenum deficiency coincided with a massive immediate synthesis of glutamine, and secondarily of other soluble amino acids. He suggested that the concentration of these soluble amino compounds is controlled by manganese through its ability to govern the level of the organic acid precursors of amino acids and to control the conversion of glutamine to protein. The actual contribution of molybdenum to this amino acid synthesis appears to lie in its function in nitrate reduction and phosphatase inhibition.

Molybdenum-iron interactions

As in the case of the molybdenum-manganese interrelationships, workers have not widely explored molybdenum-iron interactions. Warrington (47, p. 428) has investigated the ability of molybdenum to relieve or prevent iron deficiencies. Using three iron sources she observed that molybdenum helped to prevent chlorosis in peas, sorghum and flax cultures. Regardless of the source of iron (organic, inorganic or chelated) the presence of molybdenum delayed the tendency

of pH to drift towards alkalinity. She suggested that the capacity of this element to offset chlorosis was due to the formation of a complex with phosphorous which rendered iron more available by delaying the formation of ferric phosphate. She also found that at high molybdenum levels, the addition of iron depressed the uptake of molybdenum. This relationship was confirmed by Kirsch (43, p. 268) who also found that in tomatoes, yield was decreased when molybdenum was added to nutrient solutions low in iron, but that the same addition increased the yield at high iron levels.

Iron-manganese-molybdenum interactions

Investigators have found experiments involving the use of varying levels of iron, manganese and molybdenum very complex and difficult to interpret. Gerloff (26, p. 612) found that both manganese and molybdenum are capable of affecting the availability of iron. In a more general way, Hoaglund (35, p. 242) observed that "different ions were absorbed at different rates, but definite evidence was obtained of the significant influence of one ion on the absorption of another." The simultaneous consideration of the effect of a third ion has produced varying and sometimes apparently contradictory results. For example, Kirsch (43, p. 269) noted that the yield response to manganese depended not solely upon the level of iron, but on the levels of both iron and molybdenum. He noted that at low iron levels manganese additions decreased molybdenum uptake, while at high iron levels, such additions increased it. Similarly,

he noted that manganese absorption was depressed at low iron levels by the addition of molybdenum, while at high iron levels it was increased. Apparently contradictory results have been obtained by workers investigating the addition of molybdenum to plants suffering from manganese-induced chlorosis. Some (26, p. 612; 33, p. 75; 94, p. 624) have found that such additions accentuated the iron deficiency, while in other cases (43, p. 273; 50, p. 185; 96, p. 709), it appears to alleviate the condition. These apparent discrepancies involved different plants, and, more important, different levels of molybdenum being added to the culture solutions. In general the addition of molybdenum in low concentrations accentuated the chlorosis, while larger increments relieved the deficiency symptom.

Iron level

The question of the proper nutrient level of iron and the best form in which iron may be provided for optimum growth in green plants has received a great deal of attention. Bitcover and Sieling (10, p. 301) examined the iron requirements for Spirodela polyrhiza and concluded that while other factors affected the utilization of the element, a concentration of approximately 2 p.p.m. in the nutrient solution seemed ideal. Steinberg (80, p. 45), working with Lemna minor used 0.4 p.p.m. and indicated that the mineral requirements of Lemna were identical with those of other higher green plants. This iron level was the lowest found to be suggested as optimum, although others (92, p. 2398; 39, p. 413), using other plants, have

suggested levels much greater than this.

Iron source

The reactions of test plants to conditions of iron deficiency have been discussed earlier in this paper. The suggestion that inorganic iron is not efficiently absorbed by plants has been made by Oertli and Jacobson (60, p. 683), who also state that the internal requirement for iron in plants, as judged by the iron-chlorophyll relationships is mostly independent of the species, form of iron, and ratio to other nutrients. Biddulph and Woodbridge (9, p. 442) on the other hand have called attention to the possibility of precipitate formation of ferric phosphate on the container, the roots, and in the xylem. As far back as 1916, Gile and Carrero (27, p. 527) suggested the use of organic iron forms to avoid such precipitation effects. They pointed out that ferric citrate and tartrate would provide adequate iron for plant growth, but that the inorganic ferric chloride was an inferior source of iron. A possible explanation for the effectiveness of ferric citrate was offered by Olsen (61, p. 7398) who indicated that this compound would not release its iron to phosphate precipitation, but that iron would enter the plant and be translocated as the citrate. Bitcover and Sieling (10, p. 302) worked with iron and citric acid combinations and concluded that the optimum ratio of citric acid to iron was 4 to 1.

The use of certain organic compounds which form stable complexes (chelates) with metals such as iron has been examined by several

workers. Leonard and Stewart (46, p. 109) have experimented with ethylene diamine tetraacetic acid (EDTA) and have reported that the addition of this compound to certain types of soils will overcome iron deficiency chlorosis in plants growing on them. Klein and Manos (44, p. 422) have obtained optimum growth in carrot and sunflower at neutral pH, where iron precipitation is normally a problem, by adding EDTA to the nutrient solution. They stated that the mode of action is unknown, but suggested that certain additives, such as coconut milk, may exert their beneficial effect through a chelating action. Jacobson (39, p. 412) has examined various ratios of iron to EDTA, and reported that several species responded well to a ratio of 1 to 1 at an iron level of 5.0 p.p.m.

Some difficulties have attended the use of chelating agents and Hill-Cottingham (34, p. 348) found that they were somewhat unstable in the light and could deposit iron hydroxide on the walls of the container. He also noted that if such reduction occurred in leaves, ferrous iron might be released which could more easily complex with proteins. Heck and Bailey (32, p. 581) reported difficulty in the use of chelating agents and stated that the use of such agents in nutrient solutions in direct contact with plant roots was not a feasible method of trace element control. They maintained that concentrations of chelating agents in which plants would grow did not chelate metal ions effectively, while concentrations adequate for chelation produced severe plant injury.

In view of the conflicting reports on the use of these agents, efforts have been made to determine their fate and mode of action.

Wallace et al. (90, p. 15) found that the EDTA was actually absorbed by the plant, but that most of it was in the fine roots and the bark of the older roots. The suggestion that these agents were important in absorption but not in translocation has received support from several others. Tiffin and others (86, p. 123) have noted that if iron and EDTA were administered separately (split-root technique), the chelating agent had little effect on iron uptake. They suggested that iron chelating agents make the iron soluble and available to the root for absorption. In the same report, however, they pointed out that these agents did have some effect on the translocation of foliar applied radioiron. Bould (12, p. 55), by contrast, has indicated that he feels the iron EDTA complex is translocated as such to the leaves where the iron exchanges without becoming immobilized. Warrington (97, p. 428) used iron complexed with EDTA in her nutrient solutions at pHs of 4.6-5.2 and reported that pH drift was delayed and that iron tended to accumulate in the roots. In studying the metabolic effect of chelating agents, Wallace (91, p. 374) found that in cell-free preparations of plant materials, heavy metal inhibition of two carboxylation reactions was overcome by the addition of certain of these agents. Huffaker et al. (37, p. 449) also suggested that chelating agents might have growth promoting effects by inactivating excess iron which might be inhibiting. He indicated that iron inhibited carbon dioxide fixation when ribose-5-phosphate and glucose-6-phosphate were used as substrates, and that the inhibition was reversed by the addition of chelating agents.

Plant exudates have been examined to determine the amount of chelating agents being translocated within the plant. Tiffin et al. (87, p. 366) collected samples of exudate from the cut stem surface of plants grown with chelating agents in the nutrient solution. They found that the concentration of the chelating agent in the exudate was only 0.3% of its concentration in the nutrient solution. They also found that the average total iron in the exudate was eight times the average concentration of the total iron in the solution. With the uptake of the iron by the plant, the chelating capacity of the nutrient solution increased seven-fold. They concluded that the plants were absorbing the iron and leaving the chelating agent in the solution. In another set of experiments, Tiffin and Brown (85, p. xxiv) working with soybean roots used radioactive iron and tagged the chelating agent with C^{14}. After 20 hours they found the chelate almost entirely in the nutrient solution, while 70% of the iron had been absorbed by the plants. Schmid and Gerloff (70, p. xxiii) have found what appears to be a naturally occurring chelate in xylem exudate. Its filtration and chromatographic characteristics indicated that it was a large molecule and not an iron-phosphate mixture. The exudate iron was removed by anion exchange resins, suggesting that the iron might be attached to an anionic structure.

Brown and others (13, p. 885) have suggested that there appears to be a competition for iron between various chelating compounds in the nutrient solution and the plant roots. They postulated that roots reduce iron from the ferric to the ferrous form for absorption and

compared this process with that in animals in which iron is thought to be stored in one valence state and moved to another. They have observed that high concentrations of chelating agents in the nutrient solution decreased the amount of iron absorbed by the roots. In another set of experiments, Brown et al. (13, p. 885; 14, p. xxiii) have demonstrated that roots compete with chelating agents for iron and have indicated that there is an apparent relationship between the reductive capacity of soybean and hawkeye roots in their ability to absorb iron and their susceptibility to iron-deficiency chlorosis.

In addition to the use of organic iron and chelating agents, another iron form, called the frit, has been investigated. Wildon (103, p. 629) has described frit as "partly-fuzed unvitrified material of which glass is made." Trace elements may be introduced into the frit in specific amounts. The assumption is made that trace element cations were suspended within the amorphous matrix of the frit and could be withdrawn from the surface by plant roots through contact absorption. It has been observed that the production of alfalfa and tobacco in soils treated with frit exceeded production in soils treated with equivalent amounts of chelate.

Ammonia versus nitrate

Suggestions have been made by several (33, p. 75; 75, p. 379) that the nitrogen source used in nutrient solutions affected the mineral requirements of green plants. Many experiments have been performed in which ammonium salts replaced nitrates as sources of

nitrogen. Not all of these have been successful (68, p. 236). Allison et al. (1, p. 502) reported that they were unable to produce growth in Lemna minor or Spirodela polyrhiza. Gorham (29, p. 366) found that when Lemna was grown in the dark, concentrations of ammonium salts as low as 1.5×10^{-3} molar appeared to be toxic. He noted, however, that green algae, diatoms, moss protonema, excised embryos and tissue cultures can use nitrate, ammonium salts and various organic nitrogen sources. Nightingale (58, p. 191) noted that green plants would grow on ammonium salts only under certain conditions. The pH of the culture solution had to be controlled. Plants needed to be able to produce a sizeable carbohydrate reserve and the cultures had to be aerated. The suggestion was made that plants growing with nitrate as a nitrogen source may derive additional oxygen during the process of nitrate reduction, and thus not need aeration for equivalent growth. Hewitt (33, p. 75) has pointed out that the plant's iron requirement is reduced when it is grown on ammonium salts.

Changes in the response of plants to this nitrogen source have been noted. Nightingale (58, p. 191) indicated that a rapid drift toward acidity is a common observation when ammonium nitrogen sources are used. Although the lower pH resulted in greater solubility of iron and manganese, cation uptake in general was greatly reduced. Manganese mobility was depressed, but the translocation of iron was not affected (75, p. 379). Under the most favorable conditions of stable pH and generous carbohydrate reserve, more rapid growth was

observed with ammonium salts than with nitrates. In iron deficient plants, Fujiwera and Tsutsumi (24, p. 405) have measured an increase in the concentration of sugars, proteins and minerals (except iron) when the nutrient solution contained half nitrate and half ammonia.

MATERIALS AND METHODS

All plants for this study were grown with continuous illumination and constant temperature in a growth chamber in which the temperature was maintained at 18° C ± 1°. Illumination was provided by two 100 watt T-12 "warm white" colored fluorescent tubes positioned twenty inches above the culture platform. These tubes provided approximately 125 foot candles of light at culture level as measured with a Weston barrier cell with a Viscor filter and a Cocor Diffusing Disc. Effects due to differences in illumination were minimized by selecting samples for harvest from different locations on the culture shelf.

The culture solutions used were based upon those of White (102, p. 103) except that the trace elements were those of Nitsch (59, p. 269) with variations suitable to the study. The culture solution of White was preferred to that of Gautheret (25, p. 119) because of its low phosphate content. Since iron was to be varied in concentration, the use of White's solution avoided much of the possibility of its being precipitated with phosphate. The iron used in these experiments was provided by dissolving pure iron wire in hydrochloric acid and adding either citric acid or ethylene diamine tetraacetic acid in equivalent amounts to form the appropriate iron complex. The same iron solution was used throughout the study for both solutions and standards. The basic culture solution contained three increments as indicated in table I. below. Table II lists the concentrations of iron, manganese and molybdenum used in the various

treatments involved in the experiments. All cultures contained 50 milliliters of nutrient solution in 125 milliliter Erlenmeyer flasks, and were sterilized at 15 pounds pressure for 15 minutes.

Table I. Stock nutrient solutions

Salt solution	Per liter	Trace elements	Per liter
$MgSO_4 \cdot 7\ H_2O$	0.52 gm	$MnSO_4 \cdot 4\ H_2O$	3.0 mgm
$Ca(NO_3)_2 \cdot 4\ H_2O$	0.26 gm	$ZnSO_4 \cdot 7\ H_2O$	0.5 mgm
Anhydrous Na_2SO_4	0.2 gm	H_3BO_4	0.5 mgm
KNO_3	0.08 gm	$CuSO_4 \cdot H_2O$	0.025 mgm
KCl	0.045 gm	NH_4MoO_4	0.025 mgm
$NaH_2PO_4 \cdot H_2O$	0.145 gm	$CoCl_2 \cdot H_2O$	0.025 mgm
		H_2SO_4 (36 N)	0.005 ml
Vitamin supplement			
Glycine	7.5 mgm	Sucrose	20.0 gm
Niacin	1.25 mgm		
Thiamin	0.25 mgm		
Pyridoxine	0.25 mgm		
Calcium pantothenate	0.25 mgm		

The plant used for these investigations was Spirodela oligorhiza (Kurtz) Hegelm. These plants were subcultured from a clone maintained in this department under sterile conditions. The identity has been verified by Dr. H. K. Phinney and the late Dr. A. N. Steward.

Table II. Concentrations of cations tested

Iron (p.p.m.)	*Molybdenum (p.p.m.)	**Manganese (p.p.m.)
0.8	0.013	1.0
0.6	0.0098	0.75
0.4	0.0065	0.50
0.2	0.0033	0.25
0.08		

* Used with Mo-free trace solution
** Used with Mn-free trace solution

Stock cultures of this plant were maintained under conditions of near deficiency by monthly transfers in a culture solution containing iron at a concentration of 0.08 part per million. This procedure was used in order to provide inoculum containing a minimum amount of iron for the various treatments. Plants possessing four visible fronds were aseptically lifted from stock culture and placed into the appropriate sterile nutrient solutions. These cultures were then placed in the growth chamber, permitted to grow for from 1 to 39 days (depending upon the experiment), and harvested. Four replicates were grown for each treatment; three to be tested and one to be used in case of contamination.

Following the period of growth dictated by the plan of the particular experiment, cultures to be harvested were separated from the nutrient solutions in a Buchner funnel. The plants were then transferred to tared 25 milliliter Erlenmeyer flasks, dried in an

oven at 100° C overnight, removed, cooled and weighed. The plants were wet ashed in the same 25 milliliter flasks in which they were dried.

Ashing was accomplished in a solution consisting of concentrated nitric and concentrated sulfuric acids in a ratio of 4:1. Four milliliters of this solution were placed in each flask of dried plants. The flasks, including an acid blank were placed on a cold hot plate. The plate was then heated until boiling and digestion occurred. This procedure permitted any foaming to take place without becoming vigorous enough to result in a loss of part of the sample.

When ashing was complete and the samples clear, they were permitted to cool and were neutralized with six normal sodium hydroxide. Neutrality was indicated by a small circle of litmus paper of constant size added to the sample. The samples were quantitatively transferred to 25 milliliter graduated cylinders in which iron was determined using the Nitroso-R-salt method of Welcher (99, p. 335). This method involves adding to each sample one milliliter of a 10% aqueous solution of hydroxylamine, one milliliter of a 0.5% solution of Nitroso-R-salt (disodium salt of 1-nitroso-2 napthol-3, 6-disulfonic acid) in a 30% acetone/water solution, and two milliliters of a 54.43% aqueous solution of sodium acetate. The samples were mixed after each addition of solution and finally allowed to stand for 30 minutes while the color developed. The sample solutions together with the acid blank and at least two iron standards were then read at 660 millimicrons on a Beckman model DU spectophotometer. Optical

densities were recorded and corrected against the acid blank. A standard iron curve was constructed from the standard solutions and the iron content of the tissue determined and recorded in milligrams. From these data the concentration of iron in milligrams of iron per gram (dry weight) of tissue was computed. Thus from each sample three items of data were derived: the dry weight of the sample, the total iron absorbed by the sample from the nutrient solution and the milligrams of iron per gram dry weight of the tissue.

In most cases fluctuations in pH were negligible and did not affect the uptake of iron or the growth of the tissue. In one experiment, however, marked changes in pH occurred. A Beckman pH-meter was used to determine the pH of these cultures and the results were recorded in the appropriate data tables.

In general the experiments involved in this study may be divided into three broad categories. These are:

1. Experiments on the effects of limited variations in the iron, molybdenum and manganese content of the nutrient solution.
2. The study of the rate of growth of Spirodela oligorhiza at different iron levels and with different iron compounds.
3. The effect of ammonia versus nitrate as a nitrogen source on growth and iron uptake.

In the first set of experiments cultures were prepared using all possible combinations of iron, molybdenum and manganese in which the three elements were present (a) in complete amounts according to

the general formula of the nutrient solution (iron at 0.8, molybdenum at 0.013 and manganese at 1.0 p.p.m.), (b) in 75% of the complete amounts, (c) in 50% of the complete amounts and (d) in 25% of the complete amounts. This provided 64 possible treatments.

The second set of experiments involved growing plants under different levels of iron supplied in different forms. The levels used were 0.8 p.p.m. and 0.08 p.p.m. The forms included iron with the chelating agent, ethylene diamine tetraacetic acid (EDTA), iron with citric acid (Cit.) and inorganic iron as the chloride (Cl). In these experiments samples were taken initially, and after 1, 3, 6, 10, 14, 17, 21, 25, 29, 33, 36, and 39 days of growth. The use of two levels, three sources and 12 time periods provided 72 treatments in this area of experimentation.

To investigate the role of the nitrogen source in iron uptake and growth, an ammonium salt was substituted for nitrate in a limited set of nutrient solutions. As in the experiments described immediately above, two levels of iron and three sources were used. In this instance plants were harvested after 10, 15, 20 and 25 days of growth. This combination of levels, sources and time intervals resulted in 24 treatments.

RESULTS

The test plants were grown in the culture solutions described in the previous section for periods of from one to thirty-nine days. At the times appropriate to the particular experiment, they were harvested, dried, weighed and assayed for iron. These weights and measures of iron taken into the plant comprise the results of these experiments.

Each culture was inoculated with four fronds of Spirodela oligorhiza having a dry weight of approximately 2.0 milligrams. This inoculum carried into the culture about 0.0002 milligrams of iron. A definitely measurable amount of growth was obtained in all series of cultures except one. The group of cultures in which no appreciable growth occurred included those in which the plants were grown at a level of 0.8 p.p.m. iron in a solution using ammonia instead of nitrate as a nitrogen source. This may have been due to the inhibitory effect of excess iron, since, as Hewitt (33, p. 75) has pointed out, the requirement for iron is reduced when the plant is grown on ammonium salts.

Data on the growth and iron uptake by Spirodela in these experiments has been collected into tables in the appendix as follows:

Table III. This table records the effects of supplying two levels (0.8 and 0.08 p.p.m.) of ferric chloride, ferric citrate or sodium iron ethylene diamine tetraacetate.

Table IV. This table records the effects of four levels of iron (0.2, 0.4, 0.6 and 0.8 p.p.m.), molybdenum (0.0033, 0.065,

0.098 and 0.013 p.p.m.) and manganese (0.25, 0.50, 0.75 and 1.0 p.p.m.) over a period of 30 days.

Table V. This table records the effects of substituting an ammonium salt for nitrate as a nitrogen source.

Table VI. This table presents a comparison of weight and iron uptake between plants grown on ammonia and plants grown on nitrate.

Data from table III has been used in the preparation of the growth curves presented in figure 1, which represent the rates of growth and iron uptake for two levels and three sources of iron.

Iron source and level

At the level of 0.8 p.p.m., growth was greatest when iron was supplied as ferric chloride. At the 0.08 p.p.m. level, ferric citrate gave the greatest growth. Iron uptake from all three sources was greatest from the solutions containing 0.8 p.p.m. iron. Under the conditions of this study, the three sources used provided adequate iron for very good growth. When iron was supplied as the citrate, growth at the level of 0.08 p.p.m. exceeded that at the 0.8 p.p.m. level. When EDTA was the source, growth at the two levels was very nearly equal, while in the plants grown in ferric chloride, growth in the solution containing 0.8 p.p.m. exceeded that in the 0.08 p.p.m. level. In the cultures containing 0.8 p.p.m., iron uptake was highest from the chloride source, intermediate from the EDTA source and lowest from the citrate solutions. In the cultures containing 0.08 p.p.m., uptake was highest from the citrate

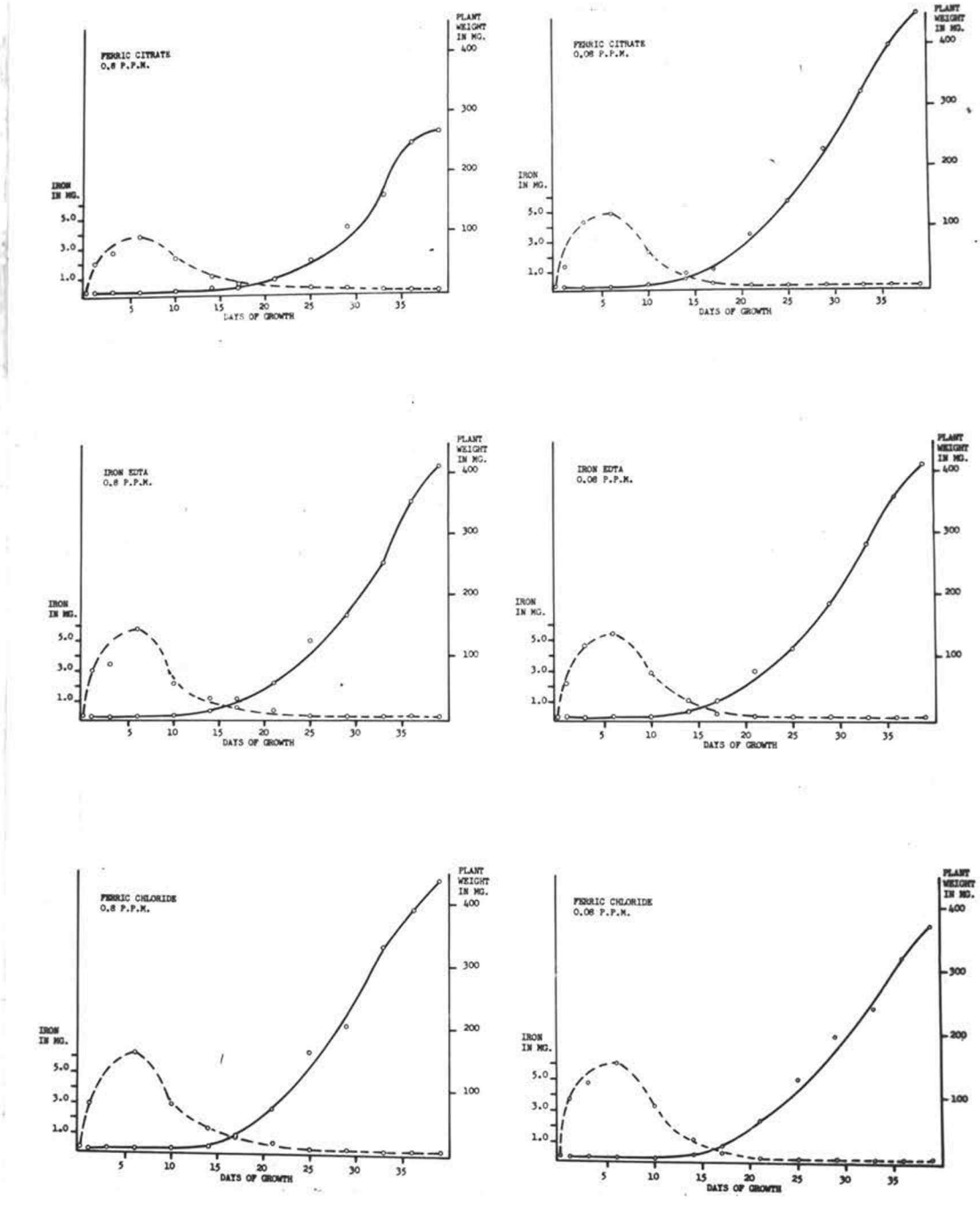

Figure 1. Weight gain (_____) and iron uptake in milligrams of iron per gram dry weight of tissue (- - -) in six treatments.

source, intermediate from the chloride source and lowest from the EDTA solutions at the end of the experiment. The differences in iron uptake between the sources at the 0.08 p.p.m. level were of extremely low magnitude, probably due to the nearly complete absorption from these dilute solutions. The data in table II was so arranged that the iron uptake per gram of tissue is easily followed. In each of the six categories it reached a maximum on the sixth day, and declined thereafter with minor variations later in the period of growth.

Table IV has been segmented in the following manner:

A. The table as a whole is divided into four major sections, each of which contains data on treatments in which the iron concentration remains at a fixed level. Each section occupies one page.

B. Each section is divided into four units, each of which contains data on treatments in which both iron and molybdenum concentrations remain at a fixed level.

C. Each unit presents data on the effect of different concentrations of manganese upon the weight and iron uptake under the fixed iron and molybdenum concentrations.

Iron-manganese-molybdenum interactions

Since maximum weight increase was realized at 0.4 p.p.m. iron, the data presented in this section of table IV is of particular significance. At all molybdenum levels, weight increased as manganese

concentration was raised from 0.25 p.p.m. to 1.0 p.p.m. At three of the four molybdenum concentrations (0.0035, 0.011 and 0.013 p.p.m.), this weight increase was accompanied by a decrease in the amount of iron absorbed per gram of tissue. A similar relationship existed between molybdenum concentration and weight increase on the one hand and iron uptake per gram of tissue on the other. At all manganese concentrations, weight increased as molybdenum level was raised from 0.0035 p.p.m. to 0.0113 p.p.m. At three of the four manganese concentrations (0.25, 0.75 and 1.0 p.p.m.), this weight increase was accompanied by a decrease in the iron absorbed per gram of tissue.

Ammonia versus nitrate

If ammonium salt was used instead of nitrate as a nitrogen source, plants were dark green in appearance when harvested after 10, 15, 20, and 25 days of growth. Table V was constructed from the dry weight and iron uptake data derived from this experiment. Since fluctuations in acidity have been noted in ammonia cultures (58, p. 191), a record of the pH of the cultures upon harvesting was kept, and was included in this table. Although it has been reported that Spirodela will not grow at a pH of less than 6.0 (1, p. 502), slow growth was observed at the 0.08 p.p.m. iron level through the entire 25 day period even though the final pH reached values as low as 3.05. Since the cultures that did not produce appreciable growth had a final pH much higher than this, it appeared

that this factor by itself did not limit growth. Most striking was the observation that those plants grown at the 0.08 p.p.m. level increased their weight 10 to 15-fold, while those grown at the 0.8 p.p.m. level grew little if at all. Table VI has been prepared to show the relative growth and iron uptake per gram of tissue between cultures grown with nitrate and those grown with an ammonium salt as a nitrogen source. Although the rate of growth on nitrate greatly exceeds that on ammonia, the initial iron uptake of the two groups appears comparable. The observed persistent high iron level of the ammonium cultures may have inhibited their emergence from the lag phase of growth.

These results show a need for more work in this area. The growth of plants in properly buffered ammonium nutrient solutions could indicate whether the iron uptake relates more closely to pH or to nitrogen source. It would also indicate whether the iron requirement for adequate growth is lower when ammonia is the source of nitrogen.

DISCUSSION

In the evaluation of results from a study such as this, certain cautions must be observed. One of these is related to the nature of the test organism. Spirodela oligorhiza is a plant that grows primarily by asexual reproduction. Daughter fronds, which initially appear to be marginal, actually develop in a fold at the margin of the parent frond. As they develop, it becomes evident that they are situated on the underside of the parent frond. As the daughter fronds approach full size, the connection with the parent frond is broken, and they assume an independent existence. Depending upon the conditions of growth, these fronds may remain tightly clumped together, or they may separate completely. As in Lemna (6, p. 376), each parent frond appears to live five or six weeks and gives rise to a limited number of daughter fronds.

Workers who have used this type of organism as a test plant have reported that certain unexplainable aspects of its growth pattern have complicated their experiments. Gorham (28, p. 100) has reported difficulty in maintaining uniformity in the growth of his controls. They seemed to vary in growth rate without discernible cause. White (100, p. 832) also noted that the same strain of Lemna minor grew differently at different times under identical environmental conditions. This peculiar growth habit was also observed in this study. In order that the most reliable results might be realized, it was necessary to grow all plants, whose analyses were to be compared, at the same time. A lapse of a

month between identical treatments produced similar, but different results.

With plants of this type, the use of identical inocula was next to impossible. Each culture was started with a small clump of Spirodela containing four mature fronds. No doubt the number of immature daughter fronds varied between treatments. For this reason a certain small error was recognized in all determinations. It is also evident that precise continuing measurements could not be obtained on a single culture since each culture had to be sacrificed each time in order to determine dry weight and iron content.

Since dry weight was one of the measurements used in this investigation, it should be pointed out that it is not necessarily a perfect measure of growth. It is probably a fairly good indication but it might be complicated by storage or accumulation of foods such as starch when growth is inhibited.

The final circumstance which should be recognized in the interpretation of these results is the near-deficient condition under which many of these cultures were grown. When one approaches a condition in which an essential element is nearly absent in a biological system, wide variations in plant reaction may be expected. Sprecht (78, p. 90) refers to this phenomenon as an impairment of a "variation control mechanism" within the plant. He states that because of this "relatively wide variations may occur in either acute deficiency or acute toxicity ranges."

This study was concerned with the effects of different iron

sources, competing ions and different nitrogen sources upon the uptake of iron and gain in weight of a test plant. Stated differently, it involved deficiency, optimal and supra-optimal concentrations of iron. A theory concerning the interaction of factors involved in growth, assimilation and the development of deficiency states has been formulated by White (101, p. 645). He stated that, "the effect of the increase of a factor is to increase the rate of one or many physiological processes, referred to as 'growth' and thereby create increased demand for other factors. Should any of these factors be present at a low level, symptoms of starvation due to their deficiency occur, for the same supply has now to satisfy a larger demand. Whether increased growth actually occurs or not is the resultant of a positive effect due to the increase of the major factor and a negative effect due to the greater relative deficiency of all other factors, and is determined in any particular case by the relative importance of the factors at work. The effect of a decrease of a factor is similar in principle, but works in the opposite direction, i.e. with consequent decreased growth is associated a relative excess of any other factors contributing to growth, that were previously present in sufficiency. Should any of these factors be harmful in excess, supra-optimal symptoms appear." White has thus called attention to the great importance of nutrient balance, which exceeds the need for a particular absolute quantity of any single nutrient under most conditions.

Iron sources and levels

In this study, experiments were conducted to determine the effect of different iron levels and sources upon the rate and extent of weight gain and upon the uptake of iron by test plants. Figures 1 and 2 show graphically the results of these experiments. Under all of these treatments considerable growth was achieved (figure 1). In 39 days of culture the original weight of the inoculum was increased by a factor varying between 133 and 225. After a period of rapid growth, the rate fell off more or less rapidly and may have reflected senility and/or a deficiency of some mineral constituent. The fact that the curve fell off first in the complete iron solution suggested that it was not due to iron deficiency. As suggested by White, above, the relatively high concentration of iron in the complete solutions may have induced deficiency effects in one or more nutrient elements. The results indicated also (figure 1) that iron was freely available to the plants from all three sources. This information appears to conflict with the opinion expressed by Gile and Carrero (27, p. 527) who considered ferric chloride an inferior source of iron. They, however, used nutrient solutions which were neutral or slightly alkaline, while the results reported here were derived from plants grown under acidic (pH 5.2-5.5) conditions. The slow rise and relatively low weight gain for the plants growing in the complete citrate solution actually suggested that iron may have been too available and therefore inhibitory. This is in agreement with Olsen's observation that iron furnished as the citrate was unavailable to precipitation

by phosphate (61, p. 7398).

The results presented in figure 1 also illustrated the pattern of iron uptake in these plants from the various sources and levels. As has also been observed by Granick (30, p. 379), the great bulk of iron withdrawn from the solution was taken up within the first few days. After this initial uptake, the iron was apparently redistributed to the daughter fronds as they were formed. Iron concentration within the plants reached a maximum of approximately 600 p.p.m. during the first week of culture. This extremely high concentration was built up during a prolonged lag phase in the growth pattern. Once growth had begun and redistribution had occurred, this concentration steadily dropped, until, in mature cultures, it varied between 2 and 8 p.p.m. These extreme concentrations, known to inhibit certain metabolic processes (91, p. 374), may have affected the duration of the lag phase of the growth of the test plants. Information on the concentrations of manganese and molybdenum within the plants during the time of these extreme iron levels might well contribute to an understanding of the plant reactions during this lag phase. Figure 2 which compares the emergence of the test plants from the lag phase in different treatments suggests several interesting generalizations. This figure was prepared to indicate how plants grown in the lower iron level begin to recover from the inhibitory effect of high iron concentration earlier and recover more rapidly than do those grown in the higher level. The results also indicated that recovery was earlier, more rapid or both in plants

supplied with organic or chelated iron than in those grown on ferric chloride. The interesting observation that plants grown at both iron levels and all sources initially lost weight also became apparent from these graphs. These data suggested that perhaps these extreme iron concentrations inhibit synthesis, including photosynthesis, more than respiration. According to Bennett's partition of the iron fractions (7, p. 100), the active (involved in photosynthesis) fraction was built up more rapidly than the inactive or residual.

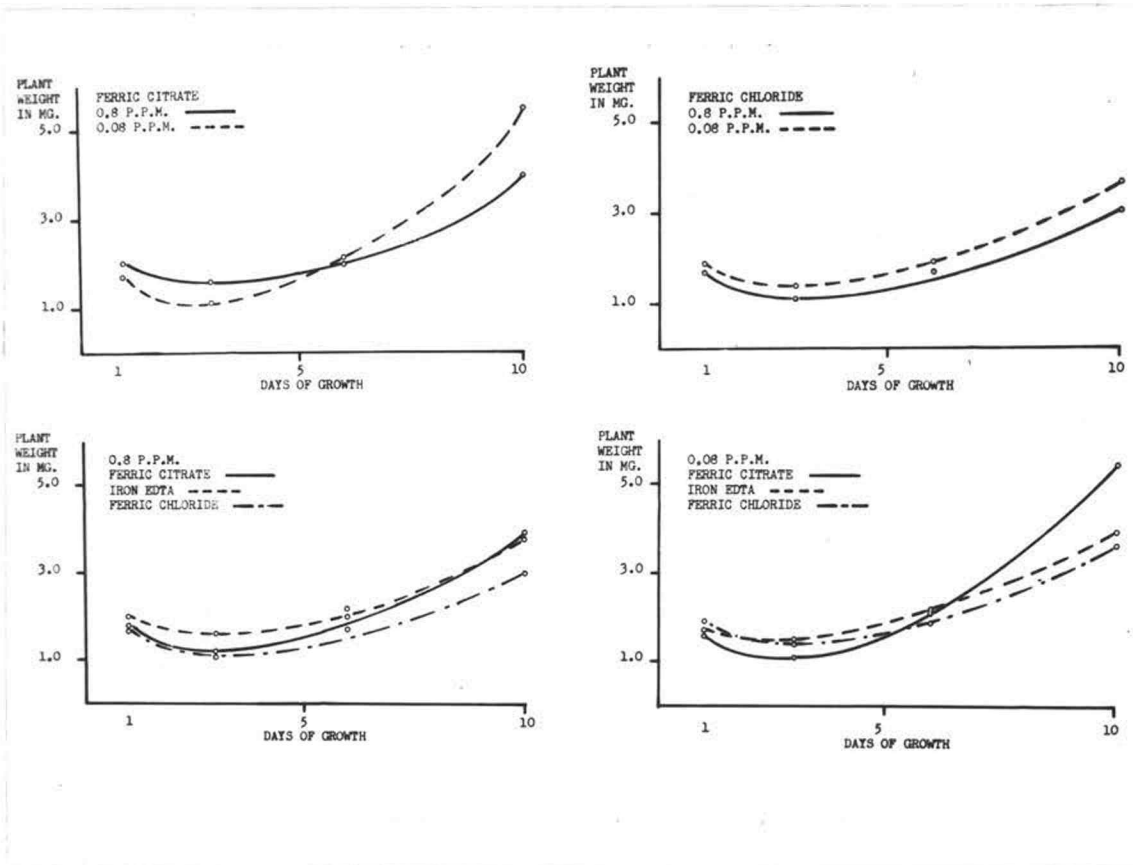

Figure 2. Weight changes in cultures of different iron sources (top), and different levels (bottom).

If functions were inhibited by high iron concentrations, it would appear, from this view, that photosynthesis would be inhibited early.

At the inception of this study a certain iron concentration was chosen as the possible optimum level of iron for the test plant in this particular nutrient solution. This was based partly upon the level specified in the original nutrient formula, partly upon suggestions from the literature and partly upon the results of preliminary experiments. This level was 0.8 p.p.m. iron. The results of these experiments indicate rather clearly that maximum weight increase in Spirodela oligorhiza, grown in the nutrient solution used, occurred at an iron level close to 0.4 p.p.m. iron for the conditions of this experiment. At a higher light intensity, a more concentrated or larger volume of nutrient solution and possibly even a larger inoculum or a longer growing period, the results might have been different.

Very few generalizations could be made regarding the influence of iron levels and sources upon the uptake of that element. Initially, as observed above, the amount of iron per gram of tissue was high, and of the same general order in all treatments. After the cultures had developed for three weeks, the iron levels in the various treatments assumed certain fairly stable relationships to one another. The iron level in the tissue grown at 0.8 p.p.m. was virtually the same for all sources. This was also true for the iron in the plants grown at the level of 0.08 p.p.m. The iron taken up by these plants, however, did not reflect the total amount of iron in their respective

culture solutions. The ratio between the iron supplied at the levels of 0.8 and 0.08 p.p.m. was, of course, 10 to 1. The ratio of the iron withdrawn by the plants from these solutions, from all sources, was, however, approximately 10 to 4. This seemed to reflect either the observation of Steward (81, p. 391) that ions accumulate freely from dilute solutions, or a saturation effect in the higher level solutions which inhibited further withdrawal.

The capacity of the test cultures as a whole to withdraw iron from the nutrient solution revealed some interesting patterns. Figure 3 has been prepared to illustrate these. Plants grown at the complete iron level showed similar patterns of withdrawal from all three sources with a slight early depression in uptake in the citrate cultures. Near the end of the growth period, all the plants grown at the complete level showed a slight tendency to lose iron to the nutrient solution. The plants grown at the lower iron level showed a different pattern in their iron absorption. Regardless of the nature of the iron source, these cultures began to lose iron back into the solution at about the tenth day and began to withdraw it again late in the growth period. The late withdrawal of additional iron was understandable due to the increasing plant population, but the losses of iron by all cultures regardless of iron level remains unexplained. Attempts to find reference to this phenomenon in the literature were unsuccessful.

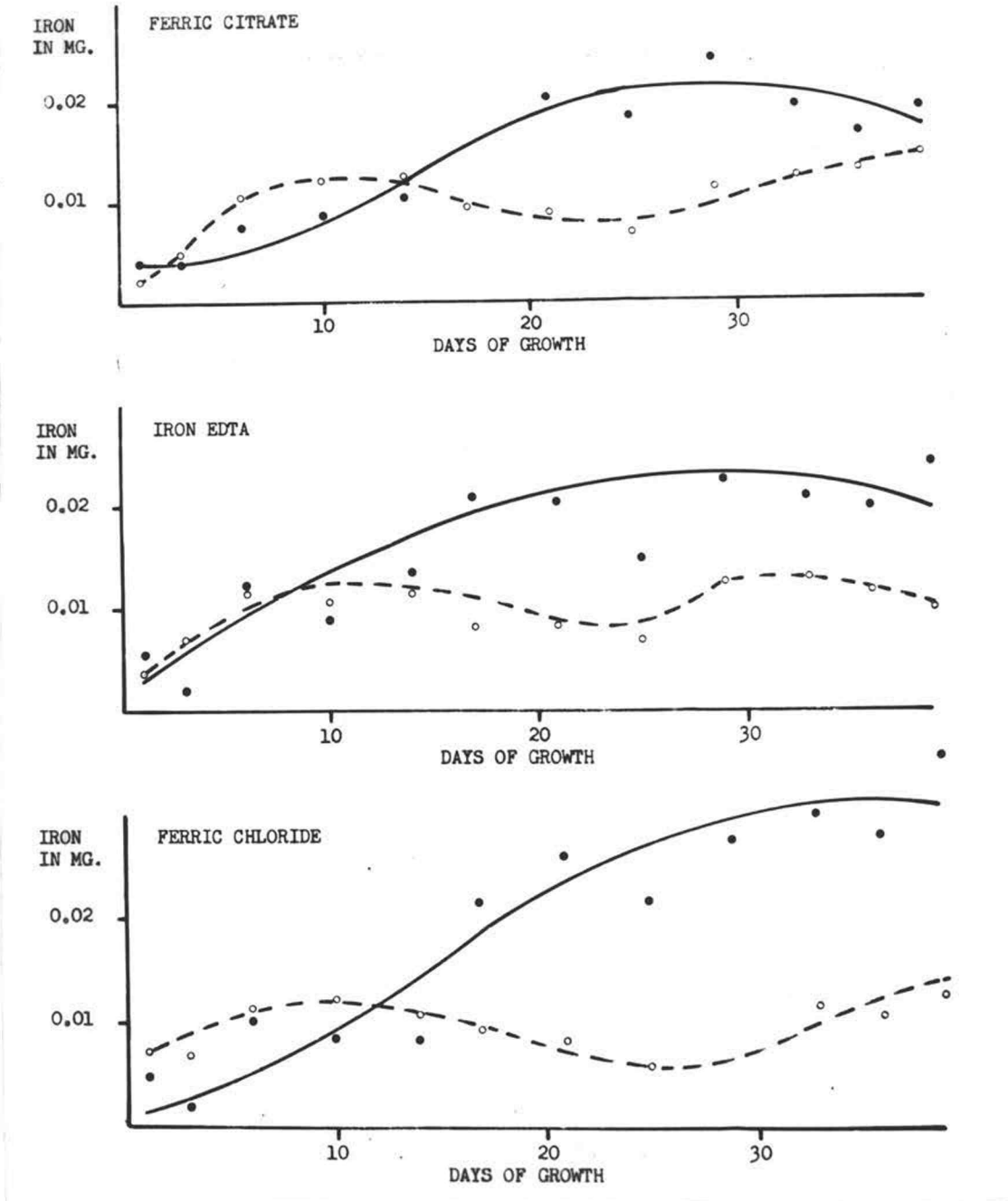

Figure 3. Patterns of total iron absorption from three sources by plants grown at 0.8 (———), and 0.08 p.p.m. iron (- - -).

Iron-manganese-molybdenum interactions

The experiments which were concerned with the interactions between iron, manganese and molybdenum produced complex and sometimes conflicting results. It appeared that many reactions were occurring within and without the plant which involved all three of these ions to varying degrees. Almost any generalization of these results is drawn in the face of at least one conflicting and contradictory sample.

In spite of the above, certain broad overall trends and tendencies have emerged from the results. The first of these concerned the effect of changes in the concentration of manganese upon the weight of the test plants. Since maximum weight increase was achieved with 0.4 p.p.m. iron, this level of iron together with 0.013 p.p.m. molybdenum was adopted as representing a balance between these two nutrients. Under these circumstances, an increase in the manganese concentration from 0.25 p.p.m. to 1.0 p.p.m. resulted in an increase in weight in all cultures. This was also true at 0.2 p.p.m. iron at three of the four molybdenum levels. Increasing the iron above its optimum level produced varying responses unless molybdenum was kept at the 1.0 p.p.m. level. When molybdenum concentration was maintained at the 0.0065 p.p.m. level, and iron varied at 0.2, 0.4, and 0.6 p.p.m., an increase in manganese increased the weight of the cultures, while at 0.8 p.p.m. iron, it caused a decrease in weight. This is difficult to explain in view of the well-recognized antagonism between iron and manganese, but may reflect the weight versus growth

question discussed earlier. In spite of these variations, generally speaking, in a balanced nutrient solution, when manganese is varied between 0.25 p.p.m. and 1.0 p.p.m., the weight of the plants can be expected to vary in the same direction as the manganese concentration. In general this interpretation is in agreement with the report of Kirsch (42, p. 269), who concluded that, at higher iron levels, the addition of manganese stimulated yield. Gerloff (26, p. 612) has also reported an increase in plant tissue with increasing nutrient manganese.

The effect of variations in the concentration of manganese upon the uptake of iron was also examined. In the balanced solution (0.4 p.p.m. iron and 0.013 p.p.m. molybdenum) an increase in manganese from 0.25 to 1.0 p.p.m. uniformly resulted in a decrease in the uptake of iron per gram of tissue. In solutions containing less molybdenum, the same general results were obtained in approximately three out of four cases. Gerloff has also recognized in his work with tomatoes that increases in nutrient manganese tended to decrease the iron content of the tissue. It is recognized that this might be only an apparent effect. If iron uptake was constant within such a series, the additional tissue formation produced by additions of manganese would automatically decrease the iron content per plant or per gram of tissue.

As has been pointed out previously, many workers have considered iron/manganese ratios more important than the absolute quantities of either nutrient present. Data from this study was examined from this

point of view and curves (figure 4) were drawn to illustrate the experimental results in terms of these ratios. Two such curves were prepared. One of these showed the effect of different iron/manganese ratios on the weight of the test plants. This curve is the average of four very similar curves reflecting this condition at the four different molybdenum levels used. The second curve in this figure was based upon the total iron withdrawn from the culture solutions by plants grown in these iron/manganese ratios. This curve was also the average of those for the four molybdenum levels used.

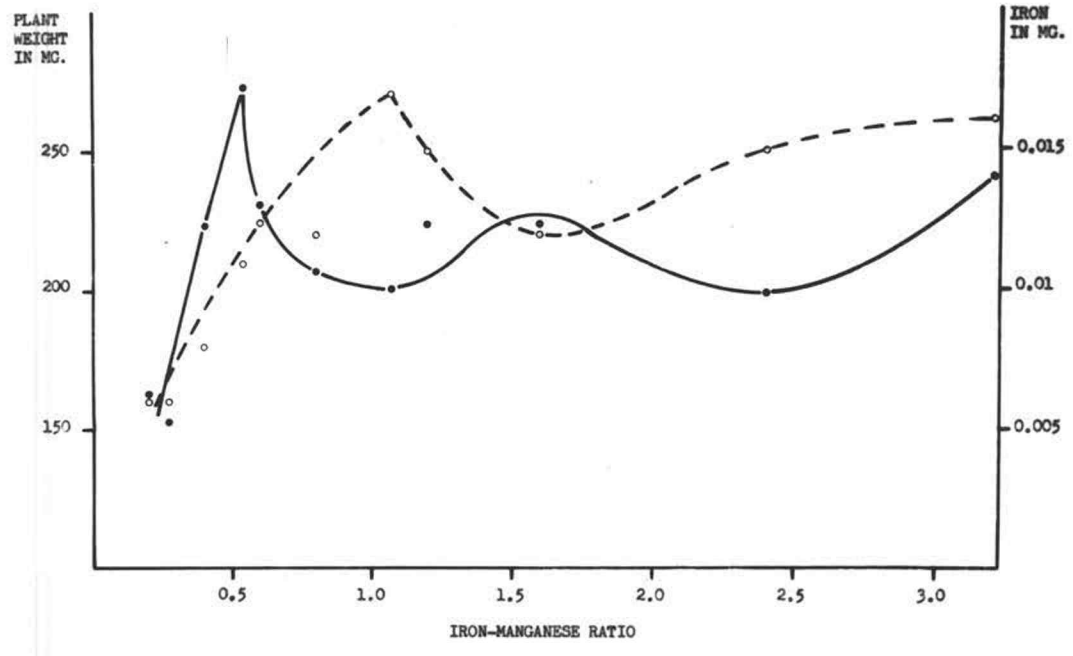

Figure 4. Effects of different iron/manganese ratios on the weight of plants cultured in them (———), and on the total iron withdrawn from the nutrient solution (- - -).

An examination of these curves revealed certain important considerations. The peaks of maximum iron uptake and maximum growth do not coincide. Indeed, the peak of iron uptake occurred at the same iron/manganese level as one of the low points on the weight curve. Similarly, the weight peak occurred at a point where the iron uptake by the plants was rapidly decreasing. These observations suggested an inhibition of growth under conditions of high iron uptake resulting from an iron/manganese ratio of approximately 1.1. Anderson and Evans (4, p. 27) have suggested that excess iron may inhibit certain enzymic reactions, and Rediske and Biddulph (66, p. 593) have indicated that iron mobility may be impaired by high concentrations of the element. It was also observed that in the middle range, between ratios of 0.5 and 2.5, weight of the plants increased as the iron absorbed decreased, and conversely.

The effects of changes in molybdenum concentration on the weight of the test plants was also examined. At the level adopted as balanced in this paper (0.4 p.p.m. iron and 1.0 p.p.m. manganese), increasing the molybdenum content from 0.0033 to 0.013 p.p.m. resulted in a small increase in growth. This was true at all manganese levels and at three of the four iron levels tested.

The effects of changes in molybdenum concentration upon iron uptake were quite stable at optimum and near optimum manganese levels, but became more variable as the concentration of this element was decreased. At 1.0 and 0.75 p.p.m. manganese concentrations, increase in molybdenum resulted in a decrease in iron uptake regardless of the

iron level involved. The balanced nutrient level fell within this range, and thus it seemed that in general this relationship was valid. This position is supported by the observations of Gerloff (26, p. 612), who noted that increases in molybdenum induced a reduction in iron uptake.

The nature of this study provided information regarding the effect of a variety of different iron-manganese-molybdenum combinations on total weight and iron uptake. Dry weight, total iron uptake and iron absorbed per gram of dry weight was determined for all samples. These data were analyzed in several ways. Certain patterns of plant response to nutrient combinations were observed which bear only indirectly upon this problem. Two such patterns are presented here.

Other analyses

Certain highly consistent results were obtained when one nutrient element was maintained at a constant level and the other two were varied together. When molybdenum was held at any of the four concentrations used, and iron and manganese were increased together from 0.2 p.p.m. iron and 0.25 p.p.m. manganese to 0.8 p.p.m. iron and 1.0 p.p.m. manganese, iron taken up by the plants increased strikingly in all cases. When the level of manganese was held constant and iron and molybdenum were varied together, a similar response was observed at all manganese levels. When iron was held constant and manganese and molybdenum were varied together, a somewhat more

complex relationship became apparent. At low iron levels (0.2 and 0.4 p.p.m.) an increase in the other two nutrients increased total iron uptake, while at higher levels (0.6 and 0.8 p.p.m.) the increase of the two nutrients resulted in a decrease in the total iron taken up.

Another example of the same type of response involved holding the concentration of one nutrient at a constant level while the other two were varied in an inverse relationship to one another. It was found that with molybdenum held constant while iron concentration was increased as manganese was decreased, a consistent pattern developed. At all levels of molybdenum, the simultaneous increase in iron and decrease in manganese resulted in increased weight, increased total iron absorbed and increased iron per gram of tissue. When manganese was held constant and iron increased as molybdenum decreased, a weight increase was observed in three of the four manganese levels, and total iron and iron per gram of tissue increased at all manganese levels. Finally, the effect of simultaneously increasing iron, molybdenum and manganese together was examined. In this instance the total iron increased, with the most striking increase being observed between the 0.2 p.p.m. level and the 0.4 p.p.m. iron levels, a moderate increase between the 0.4 p.p.m. level and the 0.6 p.p.m. ones and a small increase between the 0.6 p.p.m. level and the 0.8 p.p.m. cultures. Weight and increase in iron per gram of tissue did not follow the same consistent pattern.

Ammonia versus nitrate

Nitrogen was supplied as ammonia instead of nitrate to a series of cultures in order to investigate the reported effect of ammonia enhancing iron uptake (58, p. 191) or the lowered iron requirement as reported by Hewitt (33, p. 75). The failure to obtain growth with ammonia as a nitrogen source has usually been attributed to a marked decrease in pH. No attempt was made to buffer the nutrient solutions to control excess acidity. During the course of the experiment, the pH of the nutrient solutions fell from 5.0 to values ranging between 3.05 and 3.79. In general plants grown at 0.8 p.p.m. iron showed virtually no growth with ammonia nitrogen regardless of iron source. Plants growing at 0.08 p.p.m. iron showed growth, increasing their weight approximately 10-fold in 25 days. Failure to grow was not correlated with low pH in any of the treatments. On the contrary, the low-iron cultures (in which growth occurred) averaged 0.2 pH units below the high-iron ones (in which no growth occurred). These results are in conflict with previously cited studies.

An inverse relationship was noted between weight increase and nutrient iron absorbed as well as culture iron level. Plants grown in high levels absorbed more than those grown in the lower level. Neither, however, absorbed as much iron as did comparable cultures utilizing nitrate as a nitrogen source. The iron level within the plants grown in ammonia, however, greatly exceeded that of those

grown in nitrate at the end of the experiment. Thus it appeared that some factor other than pH or absolute iron level was involved in limiting the growth of these plants. The tendency of the chelating agent ethylene diamine tetraacetic acid to slow the drift of pH as observed by Warrington (97, p. 428) was also noted in these experiments.

CONCLUSIONS

Spirodela oligorhiza (Kurtz) Hegelm was grown in sterile nutrient cultures with different levels and sources of iron, different levels of competing ions and different nitrogen sources.

Good growth was achieved at levels between 0.08 and 0.8 p.p.m. iron as the chloride, citrate, and EDTA complex.

Iron was rapidly absorbed by the plants, which attained concentrations as high as 600 p.p.m. shortly after inoculum was placed in fresh cultures.

Plants grown at low iron levels recovered from growth inhibition due to excess iron more rapidly than did those grown at higher iron levels.

The optimum iron concentration for Spirodela oligorhiza grown under the conditions of this experiment was found to be approximately 0.4 p.p.m.

Uptake of iron from solutions was not directly proportional to the nutrient levels.

Plants grown at both levels and all sources of iron showed a tendency to lose iron to the nutrient solution.

Increasing manganese concentration resulted in an increase in plant weight and a decrease in iron absorbed.

Iron/manganese ratios which provided the greatest weight increase did not coincide with those resulting in maximum iron uptake.

Increase in molybdenum concentration resulted in a small increase in weight and a decrease in iron uptake.

The substitution of ammonium salts for nitrates as nitrogen sources resulted in the development of lower pH and less growth and total iron uptake than were obtained when nitrates were used, but some factor other than pH or iron level probably limited the growth of these plants.

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APPENDIX

Data Tables

Table III. Effects of using different levels (expressed in p.p.m.) and sources of iron on total weight and iron uptake.

Days of growth	Iron level	Iron source	Dry weight in milligrams	Total iron in milligrams	Mg. Fe/g. dry weight of tissue
1	0.8	Cit.	2.0	0.0040	2.0027
3	0.8	Cit.	1.6	0.0034	2.6868
6	0.8	Cit.	2.0	0.0075	3.7908
10	0.8	Cit.	3.9	0.0087	2.2277
14	0.8	Cit.	8.9	0.0102	1.1617
17	0.8	Cit.	6.9	0.0038	0.5680
21	0.8	Cit.	22.9	0.0202	0.8688
25	0.8	Cit.	51.0	0.0182	0.3588
29	0.8	Cit.	106.3	0.0238	0.2370
33	0.8	Cit.	159.3	0.0191	0.1283
36	0.8	Cit.	248.3	0.0164	0.0752
39	0.8	Cit.	266.5	0.0188	0.0753
1	0.08	Cit.	1.7	0.0022	1.4078
3	0.08	Cit.	1.1	0.0049	4.3398
6	0.08	Cit.	1.9	0.0113	6.1245
10	0.08	Cit.	5.4	0.0121	2.2679
14	0.08	Cit.	13.5	0.0121	0.9130
17	0.08	Cit.	29.4	0.0094	0.3261
21	0.08	Cit.	86.0	0.0087	0.1028
25	0.08	Cit.	140.6	0.0066	0.0474
29	0.08	Cit.	228.1	0.0110	0.0501
33	0.08	Cit.	321.7	0.0120	0.0380
36	0.08	Cit.	399.1	0.0127	0.0311
39	0.08	Cit.	450.4	0.0141	0.0320
1	0.8	EDTA	1.8	0.0055	3.1196
3	0.8	EDTA	1.2	0.0020	3.5188
6	0.8	EDTA	2.2	0.0124	5.8128
10	0.8	EDTA	3.8	0.0088	2.2337
14	0.8	EDTA	10.8	0.0135	1.2194
17	0.8	EDTA	30.3	0.0208	0.6999
21	0.8	EDTA	55.7	0.0201	0.4436
25	0.8	EDTA	131.3	0.0146	0.1132
29	0.8	EDTA	166.2	0.0224	0.1418
33	0.8	EDTA	251.7	0.0207	0.0842
36	0.8	EDTA	353.7	0.0196	0.0512
39	0.8	EDTA	349.8	0.0239	0.0682

Table III. (Continued) Effects of using different levels (expressed in p.p.m.) and sources of iron on total weight and iron uptake.

Days of growth	Iron level	Iron source	Dry weight in milligrams	Total iron in milligrams	Mg. Fe/g. dry weight of tissue
1	0.08	EDTA	1.6	0.0036	2.2666
3	0.08	EDTA	1.5	0.0069	4.7397
6	0.08	EDTA	2.2	0.0118	5.4291
10	0.08	EDTA	3.9	0.0106	2.9345
14	0.08	EDTA	10.2	0.0115	1.1708
17	0.08	EDTA	28.3	0.0082	0.3037
21	0.08	EDTA	75.9	0.0083	0.1097
25	0.08	EDTA	112.0	0.0069	0.0624
29	0.08	EDTA	184.9	0.0125	0.0687
33	0.08	EDTA	280.2	0.0129	0.0456
36	0.08	EDTA	357.5	0.0116	0.0312
39	0.08	EDTA	411.5	0.0096	0.0230
1	0.8	C1	1.7	0.0049	2.9597
3	0.8	C1	1.1	0.0029	2.6292
6	0.8	C1	1.7	0.0104	6.2672
10	0.8	C1	3.0	0.0087	2.9550
14	0.8	C1	6.0	0.0086	1.4556
17	0.8	C1	25.1	0.0217	0.8569
21	0.8	C1	69.1	0.0260	0.4352
25	0.8	C1	162.1	0.0218	0.1339
29	0.8	C1	204.4	0.0275	0.1408
33	0.8	C1	330.9	0.0300	0.0916
36	0.8	C1	393.4	0.0279	0.0702
39	0.8	C1	437.8	0.0358	0.0819
1	0.08	C1	1.9	0.0071	3.7611
3	0.08	C1	1.4	0.0066	4.8029
6	0.08	C1	1.9	0.0113	6.1245
10	0.08	C1	3.6	0.0122	3.3939
14	0.08	C1	8.3	0.0108	1.3053
17	0.08	C1	22.7	0.0094	0.4213
21	0.08	C1	64.1	0.0083	0.1293
25	0.08	C1	128.4	0.0059	0.0456
29	0.08	C1	196.9	0.0150	0.0773
33	0.08	C1	240.7	0.0118	0.0493
36	0.08	C1	320.1	0.0106	0.0331
39	0.08	C1	371.4	0.0126	0.0331

Table IV. Effects of different levels of iron, molybdenum and manganese (in p.p.m.) on total weight and iron uptake.

Nutrient levels			Dry weight	Total iron	Mg. Fe/g. dry
Fe	Mo	Mn	in milligrams	in milligrams	weight of tissue
0.8	0.013	1.0	219.7	0.0150	0.0681
0.8	0.013	0.75	247.3	0.0193	0.0780
0.8	0.013	0.50	179.8	0.0134	0.0762
0.8	0.013	0.25	212.4	0.0197	0.0928
0.8	0.0098	1.0	188.3	0.0195	0.1040
0.8	0.0098	0.75	263.3	0.0140	0.0529
0.8	0.0098	0.50	254.8	0.0132	0.0652
0.8	0.0098	0.25	257.1	0.0127	0.0492
0.8	0.0065	1.0	224.5	0.0182	0.0848
0.8	0.0065	0.75	158.4	0.0168	0.1063
0.8	0.0065	0.50	181.6	0.0177	0.0995
0.8	0.0065	0.25	246.4	0.0128	0.0519
0.8	0.0033	1.0	319.6	0.0175	0.0546
0.8	0.0033	0.75	137.0	0.0175	0.1275
0.8	0.0033	0.50	209.3	0.0138	0.0635
0.8	0.0033	0.25	245.3	0.0163	0.0663

Table IV. (Continued) Effects of different levels of iron, molybdenum and manganese (in p.p.m.) on total weight and iron uptake.

Nutrient levels			Dry weight	Total iron	Mg. Fe/g. dry
Fe	Mo	Mn	in milligrams	in milligrams	weight of tissue
0.6	0.013	1.0	255.8	0.0119	0.0468
0.6	0.013	0.75	220.1	0.0111	0.0603
0.6	0.013	0.50	229.9	0.0110	0.0486
0.6	0.013	0.25	206.4	0.0115	0.0557
0.6	0.0098	1.0	190.2	0.0091	0.0481
0.6	0.0098	0.75	212.7	0.1400	0.0669
0.6	0.0098	0.50	247.2	0.0156	0.0630
0.6	0.0098	0.25	199.2	0.0147	0.0741
0.6	0.0065	1.0	244.1	0.0130	0.0537
0.6	0.0065	0.75	223.7	0.0139	0.0619
0.6	0.0065	0.50	222.8	0.0168	0.0753
0.6	0.0065	0.25	191.7	0.0157	0.0830
0.6	0.0033	1.0	181.0	0.0159	0.0885
0.6	0.0033	0.75	230.9	0.0152	0.0656
0.6	0.0033	0.50	196.3	0.0151	0.0777
0.6	0.0033	0.25	198.7	0.0157	0.0792

Table IV. (Continued) Effects of different levels of iron, molybdenum and manganese (in p.p.m.) on total weight and iron uptake.

Nutrient levels			Dry weight	Total iron	Mg. Fe/g. dry
Fe	Mo	Mn	in milligrams	in milligrams	weight of tissue
0.4	0.013	1.0	342.8	0.0113	0.0331
0.4	0.013	0.75	296.0	0.0089	0.0299
0.4	0.013	0.50	221.7	0.0141	0.0652
0.4	0.013	0.25	275.6	0.0095	0.0345
0.4	0.0098	1.0	301.2	0.0098	0.0235
0.4	0.0098	0.75	318.6	0.0116	0.0363
0.4	0.0098	0.50	192.0	0.0083	0.0430
0.4	0.0098	0.25	262.2	0.0102	0.0387
0.4	0.0065	1.0	284.5	0.0114	0.0466
0.4	0.0065	0.75	254.0	0.0113	0.0441
0.4	0.0065	0.50	271.2	0.0096	0.0354
0.4	0.0065	0.25	234.2	0.0104	0.0441
0.4	0.0033	1.0	322.1	0.0118	0.0371
0.4	0.0033	0.75	221.7	0.0131	0.0605
0.4	0.0033	0.50	196.3	0.0117	0.0594
0.4	0.0033	0.25	197.2	0.0095	0.0482

Table IV. (Continued) Effects of different levels of iron, molybdenum and manganese (in p.p.m.) on total weight and iron uptake.

Nutrient levels			Dry weight	Total iron	Mg. Fe/g. dry
Fe	Mo	Mn	in milligrams	in milligrams	weight of tissue
0.2	0.013	1.0	168.4	0.0073	0.0427
0.2	0.013	0.75	213.9	0.0073	0.0339
0.2	0.013	0.50	154.3	0.0059	0.0392
0.2	0.013	0.25	162.1	0.0067	0.0453
0.2	0.0098	1.0	183.1	0.0068	0.0373
0.2	0.0098	0.75	169.8	0.0045	0.0263
0.2	0.0098	0.50	122.9	0.0054	0.0436
0.2	0.0098	0.25	116.0	0.0055	0.0494
0.2	0.0065	1.0	157.2	0.0045	0.0289
0.2	0.0065	0.75	131.0	0.0051	0.0385
0.2	0.0065	0.50	74.1	0.0061	0.0847
0.2	0.0065	0.25	107.9	0.0052	0.0528
0.2	0.0033	1.0	144.4	0.0064	0.0432
0.2	0.0033	0.75	98.9	0.0065	0.0660
0.2	0.0033	0.50	188.4	0.0033	0.0179
0.2	0.0033	0.25	207.7	0.0030	0.0145

Table V. Effects of the use of an ammonium salt as a nitrogen source on total weight and iron uptake. Iron level is expressed in p.p.m.

Days of growth	Iron level	Iron source	Final pH	Dry weight in milligrams	Total iron in milligrams	Mg. Fe/g. dry weight of tissue
10	0.8	Cit.	3.53	2.5	0.0065	2.8409
15	0.8	Cit.	3.43	3.2	0.0064	2.1611
20	0.8	Cit.	3.47	4.9	0.0079	1.6013
25	0.8	Cit.	3.40	4.8	0.0089	2.0071
10	0.08	Cit.	3.70	4.1	0.0078	1.9464
15	0.08	Cit.	3.47	7.8	0.0076	0.9091
20	0.08	Cit.	3.22	20.2	0.0079	0.3950
25	0.08	Cit.	3.05	28.0	0.0900	0.3229
10	0.8	EDTA	3.62	3.2	0.0072	2.3596
15	0.8	EDTA	3.48	3.4	0.0054	1.7616
20	0.8	EDTA	3.43	4.1	0.0062	1.5018
25	0.8	EDTA	3.95	3.8	0.0078	2.0661
10	0.08	EDTA	3.75	3.6	0.0071	1.8684
15	0.08	EDTA	3.49	4.4	0.0079	1.3672
20	0.08	EDTA	3.23	16.8	0.0070	0.4179
25	0.08	EDTA	3.72	28.1	0.0095	0.3360

Table V. (Continued) Effects of the use of an ammonium salt as a nitrogen source on total weight and iron uptake. Iron level is expressed in p.p.m.

Days of growth	Iron level	Iron source	Final pH	Dry weight in milligrams	Total iron in milligrams	Mg. Fe/g. dry weight of tissue
10	0.8	Cl	3.50	2.7	0.0069	2.5330
15	0.8	Cl	3.41	1.6	0.0047	3.1207
20	0.8	Cl	3.41	2.7	0.0040	1.5368
25	0.8	Cl	3.81	2.8	0.0105	2.8648
10	0.08	Cl	3.79	3.5	0.0051	1.3783
15	0.08	Cl	3.47	8.0	0.0081	1.0359
20	0.08	Cl	3.22	16.2	0.0082	0.5089
25	0.08	Cl	3.50	25.0	0.0106	0.4215

Table VI. Comparison of dry weights and iron uptake by plants grown in nitrate versus ammonium at 0.08 p.p.m. iron.

Days of growth	Iron source	Dry weight in milligrams		Mg. Fe/g. dry weight of tissue	
		Nitrate	Ammonium	Nitrate	Ammonium
10	Cit.	5.4	4.1	2.2679	1.9464
14	Cit.	13.5		0.9130	
15	Cit.		7.8		0.9091
20	Cit.		20.2		0.3950
21	Cit.	86.0		0.1028	
25	Cit.	140.6	28.0	0.0474	0.3229
10	EDTA	3.9	3.6	2.9345	1.8684
14	EDTA	10.2		1.1708	
15	EDTA		4.4		1.3672
20	EDTA		16.8		0.4179
21	EDTA	75.9		0.1097	
25	EDTA	112.0	28.1	0.0624	0.3360
10	Cl	3.6	3.5	3.3939	1.3783
14	Cl	8.3		1.3053	
15	Cl		8.0		1.0359
20	Cl		16.2		0.5089
21	Cl	64.1		0.1293	
25	Cl	128.4	25.0	0.0456	0.4215