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SIGNIFICANT RÔLES OF TRACE ELEMENTS IN THE NUTRITION OF PLANTS^{1, 2}

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During the past quarter of a century much has been written concerning the place of the so-called trace elements in the economy of plant life. Most of this literature deals with the effects produced upon plants when these elements are absent from the growth substrate and is purely descriptive in nature, portraying the symptoms of deficiency. There is also, in the physiological literature of the past decade and a half, much descriptive material portraying the pathological symptoms which appear in plants when these elements occur in the plant environment in concentrations in excess of those required for growth and development. Thus far these qualitative observations make up practically all of the great mass of the investigational literature upon this subject. So much of this foundation in natural fact has already been accomplished, that intensive activity along this line of categorical description can no longer be considered an adequate field of investigation in this particular phase of plant nutrition. It should be remembered, however, that the extension of our knowledge in this phase of nutrition cannot progress without at least some continued activity along these descriptive lines; but our chief interest is no longer in the qualitative pathological symptoms themselves, which develop as the result of trace element malnutrition, but in their relation to those more basic phenomena which can be evaluated only through careful quantitative experimental investigation. From almost the beginning of the present century studies have been directed toward ascertaining whether or not certain elements, which are found in trace quantities in both the plant and in its environment, might be included in the category of essential factors for growth and development. There is much contradictory evidence, as might be expected, relative to this question, which

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is an important one. The energetic search for such elements which began early in the century is still in progress. It has been in the past a profitable field of investigation and it is predictable that it will provide an equally profitable field of study in the future. The inability of experimenters to grow plants successfully through a complete cycle in artificial culture, in the early years of the present century, initiated a search for missing essential factors. The usual experimental technique which had long been employed in solution culture and in sand culture was too crude, however, and the materials used were too unrefined to disclose the need of specific elements required in minute quantities in nutrient substrates for plants.

Search for the missing factors, therefore, led to great improvements in experimental techniques. Great strides have been made in the refinement of methods and in the purification of nutritive materials for accurate study in the endeavor to demonstrate the need of these elements in the nutrient substrate and to prove their essentiality. Through this refinement of technique, improvement in the methods of operation, and purification of nutritive material, it is now possible to add to the previous list of essential elements for plants boron, manganese, zinc, and copper. Undoubtedly other elements will be added to this list with still further purification of chemical materials and further refinements of physical equipment and methods of procedure. The degree of refinement at present attainable, however, imposes a rigid limitation upon experimental accuracy and this is particularly true with respect to the analytical data from quantitative studies in the field of trace element nutrition. The suggestion has recently been made that an element may not be regarded as non-essential if it is present in a nutrient substrate in concentrations greater than one part in a billion (10). Such a degree of purity may not be attainable at the present time. It is this point, as well as that relating to the absolute importance of the trace elements in nutrient substrates, which has recently been so strongly emphasized in connection with the development and refinement of methods for growing isolated plant tissues *in vitro* (12, 13). It appears that the degree of purity and the state of refinement are determining factors in any attempt to establish proof of the essentiality of a given element which is present in the plant environment in very minute concentration. It is not so simple a matter to differentiate between that which merely stimulates growth and that which is essential for growth. The fact that the presence of a minute trace of a given element in a nutrient substrate may stimulate growth of the plant does not constitute proof of essentiality, nor does failure to stimulate constitute proof that an element is not essential. In order to provide proof of the essentiality of a given element it is necessary to show that a plant cannot complete its cycle in the absence of a measurable concentration of the element in question.

In order to obtain quantitative information concerning the place which the trace elements occupy in the nutrition of plants and the rôles which they play in metabolic processes, elaborate precautions must be taken to eliminate all possible sources of contamination derived from the experimental set-up, and careful attention must be given to such matters as the purity of salts, distilled water, and cleanliness of the apparatus involved. These precautions are indispensable if the objectives of quantitative research in this field are to be attained. With the discoveries made during recent years, and with the refinement of the experimental method, it has now become a simple procedure to demonstrate the effect of deficiencies of boron, manganese, and zinc with many species of plants under the conditions of control which characterize the usual routine of solution culture experimentation.

While superficial descriptions relating to the effects of trace element deficiencies upon the various species continue to accumulate, as is to be expected, it is most encouraging and even inspiring, that a pronounced change toward the more quantitative type of investigation has been in progress during the past several years and some very important quantitative work dealing with trace element nutrition has appeared in the literature (2, 5, 7, 8). Difficult as it has been to demonstrate beyond a doubt that boron, manganese, and other trace elements are essential for plants, it may, however, be infinitely more difficult to prove why they are essential. But the present status of trace element nutrition in plants makes it unnecessary to dwell upon either the economic or the theoretical importance of the elements required by plants in only very minute amounts. The economic importance of these elements is obvious in view of the host of plant diseases, many of which are very destructive, which have been traced either directly or indirectly to deficiency or excess of one or more of these elements in the soils of many agricultural districts. It is to be emphasized also that not one of the many problems relating to the important rôles which these elements play in the metabolic processes of plants, has yet completely emerged from the theoretical category to take its place in the realm of fact established through experimental study. The importance of theoretical consideration of such problems is, therefore, apparent.

It has early been pointed out by several investigators that the pathological symptoms produced in plants by boron deficiency are remarkably similar to those produced by calcium deficiency under like experimental conditions. The similarity has been recognized in virtually all of the species thus far studied. This has suggested the possibility that boron is in some mysterious manner involved in those processes in the plant in which calcium is a direct reactant. This suggestion is supported by the well-known fact that both boron and calcium are especially effective in the intensive metabolic processes which are confined largely to the meristematic regions, and recent investiga-

tions have shown also an intensive utilization of boron by the reproductive organs of the plant (6).

Attention will now be directed to the consideration of quantitative experimental evidence relating to an important rôle which boron plays in the accumulation, assimilation, and metabolism of calcium in several species widely separated in their morphological, physiological, and taxonomic characteristics. But, before presenting this evidence it will be necessary to describe very briefly, the experimental procedure by which these data were obtained. Relatively large numbers of plants of each species dealt with were grown in a standard culture medium until they had attained the age and phase of growth and development desired before the experimental treatments were begun. At the beginning of the treatment period the cultures were divided into three groups or series: The cultures of the first series covered a range of boron concentrations in the standard nutrient substrate which, by previous tests, was known to produce visible symptoms of deficiency in the species in question. The cultures of the second series covered a range of approximately optimum boron concentrations; and the cultures of the third series covered a range of boron concentrations sufficiently high to produce symptoms of toxicity in the plants of the species in question. With the exception of the boron treatments the nutrient substrate used in the cultures of the three series was identical. For the sake of convenience the three series may be designated the deficiency series, the optimum series, and the toxicity series, respectively. In all cases the experimental period, during which the cultures were under treatment, was continued until pronounced visible symptoms of boron deficiency appeared in the plants of the deficiency series, and of boron toxicity in the plants of the toxicity series. This required different intervals of time for the different species. The cultures were conducted under a system of continuous flow of the culture solution at a rate sufficiently high to prevent material alteration in the proportions of nutrient ions through the activity of the plants.

At the end of the experimental interval the various tissues of the plants were analyzed for calcium and for boron and an attempt was made to differentiate between total and soluble or active quantities of the elements in the tissues tested, in the hope that such a procedure might throw some light upon the connection between these two elements in the metabolic activity of the plants. While the experimental evidence reveals absolutely no information concerning the actual physiological function of either boron or calcium in the plant, it does indicate at least one important rôle which the trace element boron plays in the functional processes of the major element calcium.

From a consideration of the experimental data (7) it became immediately obvious that the boron in the tissues of the particular species in question (corn) was virtually in a completely soluble state and could therefore be

assumed to be in a mobile, active condition. It was obvious also that its accumulation in the tissues was determined by its concentration in the nutrient substrate. When now the experimental data relating to boron were examined in connection with corresponding data representing the status of calcium in the plant, the outstanding feature which was at once apparent was the almost perfect correlation between the boron of the tissues and the soluble or active calcium of the tissues. On the other hand, there was no apparent relation between the boron content and total calcium in the tissues of this monocot, and there was no obvious relation between soluble and total calcium in tissues of these plants. It must be concluded, therefore, that the proportional part of the total calcium in the plant which was maintained in the soluble, active state in which it could be translocated from points of supply to centers of metabolic activity, was determined not by the total calcium content of the plant but by the supply of available boron in the corresponding tissues which in turn was determined by the boron concentration of the nutrient substrate. This peculiar property of boron by which calcium is rendered mobile and active is not confined to organic substrates. The principle involved is effectively utilized in certain industrial procedures to eliminate calcium in the soluble form from complex inorganic (3), as well as organic (1, 4) systems in which it occurs as an impurity during the process of large-scale preparation and manufacture of certain important commercial products.

The corresponding analytical data of a representative dicot, *Vicia faba*, were next considered. When these were treated in a manner similar to those of the monocot, corn, it was found that a relatively small fraction of the total boron in this dicot was soluble and in this respect the dicot was strikingly different from the monocot. But the soluble boron fraction of the dicot, like that of the monocot, was directly related to the total boron content of the tissues and also to the concentrations of this element in the nutrient substrate.

In the dicot, *Vicia faba*, the soluble calcium, as in the monocot, was directly related to and determined by the soluble boron fraction, which in turn was related to the total boron content and determined by the concentration of this element in the nutrient substrate. There was no direct relation between soluble and total calcium either in the monocot or in the dicot, but in the toxic range of boron concentrations of the substrate the accumulation of calcium was impeded, and this was directly associated with a corresponding retardation of the actual rates of calcium absorption and of the rates of growth.

One of the characteristic differences between the monocot and the dicot relative to this particular phase of inorganic nutrition was the fact that under identical experimental conditions with respect to the chemical and

physical properties of the nutrient substrate, the total calcium content and the total boron content were always much higher in the dicot than they were in the monocot. At present there is no adequate explanation for this fairly general phenomenon. In all of the species so far investigated the calcium and boron values for the dicot are usually much higher than those for the monocot. But this did not hold for the soluble fractions of these elements in the tissues of the plants. The soluble fraction of boron relative to the total was always much lower in the dicot than in the monocot and this invariably resulted in a low fraction of soluble calcium, relative to the total.

TABLE I

AVERAGE SOLUBLE BORON AND AVERAGE SOLUBLE CALCIUM, PERCENTAGE OF TOTAL, PRESENT IN THE TISSUES OF MONOCOTS AND DICOTS

RANGE OF SUPPLY	MONOCOTS		DICOTS	
	SOLUBLE BORON	SOLUBLE CALCIUM	SOLUBLE BORON	SOLUBLE CALCIUM
	%	%	%	%
Deficiency boron range	62.7	28.3	6.5	16.5
Optimum boron range	78.4	34.7	28.7	22.9
Toxicity boron range	86.1	55.7	32.2	25.4

This is clearly brought out in table I showing percentages dealing with relative solubilities of the two elements in representative species of the two groups of plants, monocots and dicots, so important in general agriculture. A consideration of these data provides an explanation for the well-known fact that the boron requirement of the monocot for normal growth and development is very much lower than is that of the dicot but equally essential for both, and it accounts also for the frequent appearance in the literature of statements to the effect that boron is not essential for growth and development of the agricultural monocots such as oats, wheat, and some grasses. The optimum boron requirements of the dicots thus far investigated were from five to ten times as high as those of the monocot, but this is only a rough estimate, since it is extremely difficult to evaluate accurately, in terms of growth criteria, the effective concentrations of boron in the extremely low ranges required by the monocots.

In the course of these investigations it was shown frequently that if at any stage in the cycle of these experimental plants the boron was excluded from the nutrient substrate so that a deficiency of active boron occurred within the plant, such deficiency rapidly destroyed the potential metabolic possibilities of the calcium even when calcium was present in adequate concentrations both in the tissues and in the substrate. Under such conditions

the plant quickly manifested deficiency symptoms. Were they boron deficiency symptoms or were they calcium deficiency symptoms? Who knows, since a deficiency of available boron in the tissues was directly associated with an inadequate supply of active calcium, and since the symptoms produced by an apparent deficiency of one element were identical with those produced by a deficiency of the other? If, on the other hand, boron was maintained in adequate concentration and calcium was excluded from the nutrient substrate at any stage in the growth cycle, calcium deficiency symptoms did not manifest themselves until the calcium previously acquired by the plant became inadequate in quantity to maintain the normal growth status of the plant. This required a relatively long period of time, since in the presence of an adequate supply of active boron the calcium already in the plant functioned quite effectively.

There is considerable experimental evidence to indicate that boron is a vital factor in the processes involved in organic syntheses. During the course of microchemical investigations of the terminal meristematic tissues of both monocots and dicots, it was discovered incidentally through the use of methods of staining, that striking differences occurred in both the pectin content and the fat content of the cells of the tissues from plants grown in the deficient, the optimum, and the toxic boron concentration ranges. An attempt was then made to determine qualitatively and roughly by quantitative methods whether the differences observed might in any way be related to the effective boron concentrations in the nutrient substrate and in the tissues. It is interesting and perhaps important that plants grown within the range of deficient boron concentration yielded strong positive tests for pectins and negative tests for fats. Plants grown within the range of toxic boron concentrations always yielded negative tests for pectins and strong positive tests for fats. Plants grown in the range of optimum boron concentrations always showed the presence of both pectins and fats. It must be strongly emphasized that these observations and tests are merely suggestive and will require confirmation by repeated checks and tests making use of much more exact methods of quantitative analyses than those employed in these preliminary investigations which strongly suggest that boron plays an important indirect rôle in carbohydrate synthesis and fat metabolism. In such a rôle its effects can not be direct but its influence is made effective through other factors in some such manner as has already been discussed, and these factors may be inorganic in nature, such as calcium involved in the mechanisms of organic synthesis. This becomes understandable in view of the fact that in green plants with high concentrations of active calcium, carbohydrate synthesis may give way to the formation of fats and fatty substances (9). Further experimental evidence also suggests that boron may play similar indirect rôles in certain metabolic processes in-

volution of the cations and perhaps the anions of major elements other than calcium (11), but this evidence is too fragmentary and inadequate for broad generalization.

There is another important phase of trace element nutrition which involves the activity of the two elements iron and manganese. That these two elements are intimately interdependent in their effects upon the plant, and that the nature of the activities of one of these is determined by the proportionate presence of the other, can no longer be doubted. Such interrelationships between these two elements, relative to their active influence in the metabolic system of the plant, have been suggested by many investigators. These suggestions have usually been associated with the fact that symptoms of chlorosis, and other characteristic effects resulting from deficiency or excess of one of these elements, are either identical with or reciprocally related to each other.

The theoretical explanation of the rôle which manganese plays in the metabolic processes, in which iron also assumes an important rôle, revolves around two facts: first, that the active, functional iron in the tissues is in the reduced state, that is, in the ferrous condition; and, second, that the oxidizing potential of manganese is higher than that of iron. If iron in the ferric state is absorbed by the plant, much of it is immediately reduced to the ferrous form under the powerful reducing systems in the cell, unless it is restrained by some counterreactant. If such a reactant is not present, or is present only in deficient quantity, a very low concentration of iron in the active state may become a powerful toxic agent resulting in a type of chlorosis which is readily recognizable as an iron toxicity symptom, or it might with equal correctness be designated as a manganese deficiency symptom. If, however, a strong oxidizing agent with an oxidizing potential considerably higher than that of iron is present in adequate concentration, the reduction of iron is restrained, or if iron is already present in the reduced state, it may be oxidized to the ferric state, in which condition it may be precipitated, probably in the form of ferric organic complexes. Manganese is the element which possesses the chemical characteristics necessary for such a theoretical system, and there is little doubt that it reacts with iron in the plant in accordance with the chemical principles suggested and in some such manner as is here described, but the sequence of events and the details of the exact processes involved are utterly complex and not at all understood.

Assuming that such a dynamic relationship exists in the plant between iron and manganese, it then becomes evident that so long as a supply of iron and manganese is available in the nutrient substrate a dynamic equilibrium system involving both oxidized and reduced ions of both iron and manganese must be maintained. Theoretically, however, complete oxidation or complete reduction cannot be attained in such a system so long as a supply of

these elements is available in the nutrient substrate and absorbed by the plant; but the oxidation of ferrous ions to ferric ions and accordingly the precipitation of iron in the form of ferric organic complexes is determined by the relative quantity of manganic ions present in the system. Thus, when the relative proportion of manganic ions in the plant is high, the active ferrous ions are maintained at a proportionately low level through this process of oxidation reduction. But when the relative proportion of manganic ions is low, the active iron, which is capable of functioning in the metabolic processes, is maintained at a proportionately high level. When these active ferrous ions become excessive in the tissues through a shift in the dynamic equilibrium system in their direction because of a proportionately low supply of manganese in the nutrient substrate, the plant will manifest this excessive iron activity by the development of toxicity symptoms. On the other hand, when the equilibrium is shifted in the opposite direction, as it is with relatively high manganese in the substrate, so that a deficiency of active iron results, the plant will manifest this by the development of symptoms associated with iron deficiency. These symptoms might with equal correctness be designated as manganese toxicity symptoms.

These processes have been followed analytically both with plant materials and in inorganic systems and the several steps described have been experimentally verified. Their effects upon the plant can readily be demonstrated and this has been done in the following manner: Three series of cultures were grown in a standard culture solution. In each series of cultures the iron supply was maintained at a constant level. This was very low in one series, approximately optimal in the second, and high in the third. In each series the manganese concentration range extended from very low to very high concentrations so that in one direction the only variable factor was that of iron concentration and in the other direction the only variable factor was that of manganese concentration.

Inspection of the analytical data from these series of cultures brings out the fact that a shift from low to high iron in the substrate causes a corresponding shift in the concentration of manganese required to produce good growth of the plants, free from symptoms of toxicity or deficiency. In other words, when iron is low in the substrate good growth is obtained only when manganese is correspondingly low, and when iron is high in the substrate good growth is obtained only when manganese is correspondingly high. The data further show that good growth can be obtained with a relatively wide range of iron concentrations in the nutrient substrate but only when accompanied by a corresponding range in the concentrations of manganese. This demonstrates the importance of maintaining in the nutrient substrate the proper ratio of iron to manganese, which has a value of approximately two (2) for the species investigated although good growth may be obtained

within a limited range of values for this ratio. It demonstrates also, that, within certain limits not attained in these investigations, the total supply of these two elements is not at all important, provided only that the proper ratio of active concentrations of iron to manganese is maintained. The analytical data show that within the tissues of the plant a similar relationship exists, not between total quantities of iron and total quantities of manganese, but between active iron and active manganese, although here a somewhat greater fluctuation than that which is permissible in the nutrient substrate, may occur in the value of this ratio and still be consistent with good growth and development of plants which are free from pathological symptoms. The effective internal range of these ratio values extends from approximately 1.5 to 2.5 for the species investigated, but it is not expected that the same range of values would be effective with all species with reference to either the nutrient substrate or the active plant tissues.

As a final consideration and as the result of these studies, it may be pointed out that the rôles which have here been ascribed to the trace elements boron, iron, and manganese, important as they may be, are mere incidents in the whole complex maze of physiological functions which they may indirectly assume in the vital activities of any species. While it is at present impossible to assign any one particular process as the special function of a given trace element, it is probably safe to assume that each of these elements is a critical factor in every important physiological process involved in the nutrition of a plant.

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