SELENIUM AND NUTRITION OF ASTRAGALUS

II. IONIC SORPTION INTERACTIONS AMONG SELENIUM, PHOSPHATE, AND THE MACRO-AND MICRONUTRIENT CATIONS

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SUMMARY

Growth of Astragalus species was shown to be adversely affected by concentrations of phosphate in solution which are often used in inorganic nutrient media. Several ionic interactions in sorption by plants were demonstrated including depression of phosphate and the micronutrient metals by seleniteselenium, depression of selenium by phosphate, and direct or indirect enhancement of the macronutrient cations and the micronutrient metals by phosphate. Plant growth affects sorption and, conversely, repressed or excessive sorption may limit yield if the element is essential and deficient, or too high in effective concentration in the plant. Relevant interactions are considered.

INTRODUCTION

Another report concerned the effects of selenite or selenate supply on growth and selenium content of Astragalus species 3. In order to have attained favorable plant growth it was necessary to consider possible direct effects of ions on growth *(e.g.* heavy-metal toxicity or nutrient deficiency *per se*) or indirect effects (*e.g.* an ion excess of one element) inducing a nutritional deficiency of another through modified ratios of the elements, perhaps related to so-called cation-sum constancy 1 or to organic anion contents 4. The indirect effects concern a number of possible interactions among the ions supplied in solution cultures. Fried and Broeshart have summarized and discussed a number of such interactions ⁵. One such effect could be causal, or more than one could be eoincidentally influencingthenet **result. Because of these complex interactions and their confounded reciprocity with concomitant plant growth, including differential top-to-root development, results of element applications purported-**Iv affecting growth must be interpreted with caution. The probable sequence of events during growth and development must be consider**ed. Development, or a variation thereof, is a result of a series of antecedent conditions and processes.**

The other article presented evidence of repressed phosphate sorption induced by selenite and concomitant enhancement of growth of *Astragalus bisulcatus 3.* **Allusions to other interactions of ions were made. It is the purpose here to present further evidence for some of the interactions which were involved in the study of growth of the Astragali. Further, a comprehensive consideration is made of possible effects of an increasing supply concentration of an independently variable ion on sorption of a dependent ion with relation to concomitant plant growth using solution culture media.**

MATERIALS AND METHODS

Astragalus species were used as in a previous report $³$. As such, the proce-</sup> dures were generally the same as previously reported for the Astragali. Seeds were germinated in vermiculite to which a dilute macronutrient solution had been added. Four or five seedlings each were transplanted into aerated solution cultures in 4-liter Pyrex beakers. The containers were wrapped with cloth skirting to exclude light from the solution media. The basal nutrient solution was (μM) : nitrogen (NO₃) 7000; potassium 3000; calcium 2000; magnesium 500; sulfur 1000; nitrogen (NH_4^+) 1000; chlorine 25; iron (as 1:1 EDTA) 25.0; boron *12.5;* manganese 1.0; zinc 1.0; copper 0.1; and molybdenum 0.1. The solution pH was frequently adjusted to about 5.5. Salt solution was applied at transplanting and periodically as necessary depending on the length of time before plant harvest. Supply concentrations of phosphorus and selenium varied as necessary to demonstrate the effects concerned. Phosphorus was supplied as KH_2PO_4 , and selenium as Na_2SeO_3 .

Plants were usually harvested at about 6 to 7 weeks from transplanting, unless otherwise indicated. Plant materials were separated into roots, stems (including leaf petioles), and leaf blades. The parts were dried, weighed, and ground. Appropriate plant aliquots were wet ashed 8. Selenium concentrations were measured by the fluorometric method of Watkinson 14, totalphosphate phosphorus by a molybdenum blue color method 8, and the micronutrient metals and macronutrient cations by atomic absorption spectrophotometry 9.

RESULTS AND DISCUSSION

In order to interpret experimental observations related either to direct effects between element supplies and plant growth, or to the reciprocal effects of growth and ion sorptions as modified by ionic interactions, it was necessary to consider the possible interrelations. These are outlined in Table 1 (compare discussion in Ref.⁷, p. 109). The present results concern plant growth and composition, and certain ionic interactions in sorption of mineral elements.

Different whole-plant compositions between a solute treatment and its control may be related to ionic interaetions in sorption (other conditions being constant) or interrelated with concomitantly modified plant yields. Thus, at least in part, the concentration changes of ions may be caused simply by differential growth diluting or concentrating effects (designated by \pm in Table 1). Considerations here are applicable for small differences in yield, assuming the rate of sorption to be independent of the rate of growth under any particular set of experimental conditions. Possible simple dilutions, at least in part, are evident where the control-to-treatment yield ratio increase exceeds the comparable ratio of the total plant sorptions of a dependent ion. A concentrating effect could apply to inverse situations. Thus, for increased yield eomparisons, at least a part of a dependent ion concentration difference may be attributable to simple growth dilution. Here, where actual treatment coneentrations are greater than theoretical values, calculated from the ratio of the total content of the ion in the control to the yield under treatment, the concentration difference of the dependent ion could be due entirely to growth dilution; where actual plant treatment concentrations are less than calculated theoretical eontrol values, the concentration difference could be due only in part to growth dilution, but then differences could be due also in part to an interference interaction on sorption. Converse results would apply to a simple concentrating effect on the dependent ion and a synergistic interaction, where treatment yields are less than controls. In short, such computations and interpretations are valid only where \pm designations occur in Table 1; designations therein with three asterisks*** conclusively determine the type and validity of ionic interaction. (See Table 2 for interpretation of causal relations between yield and plant concentration changes with treatment.) It should be

TABLE 1

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Possible effects of an increasing supply concentration of an independently variable ion on sorption of a dependent ion, with relation to concomitant plant growth, using solutionculture media *

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* Assume tbat the aetual rates of growth and total sorption, with time, remain reasonably constant or deviate inappreciably from their determined averages for the period of growth.

** Variable top-to-root ratios can modify the results. Yields per culture, and concentrations in plants as a whole and total amounts per eulture of a dependent ion are expressed on the dry-weight basis.

 $=$ indicates no change; $-$, a decrease; $+$, an increase; and \pm , either decrease or inerease depending on the relative ratios of the total yields (treatment/control) to the total sorptions (treatment/control).

*** Effect particularly evident, and conclusive.

noted that apparent dilutions or concentrations could be enhanced or diminished respectively by moisture percentage differences if fresh weights of plants are compared; dry weights were used here to avoid this consideration.

Seasonal effects on the relative differences in yield of top to root between a control and a treatment can markedly affect the concentration and the total distribution of a dependent ion, and thus the interpretations 2; relations for the plant as a whole are particularly considered here, relative to involvement of interactions in ion sorptions.

E//ect o/ selenite on phosphate sorption

A. bisulcatus plants were cultured with maintenance of a reasonably constant and favorable phosphate concentration within the plant by monitoring twice a *day,* having started with an external supply of 3 μ g-at P per liter. The selenite supply concentration was varied over a concentration range of zero to 50 μ g-atoms per liter of culture. Plant yields and phosphate contents were measured.

Possible causal relations where ionic total sorptions are variable with a partieular change in plant yield under treatment *

** C(theoretical) = **Yield under treatment**

i **Assume that the rate of sorption is independent of the rate of growth for relatively moderate to small differenees in yield.**

Concentrations of the micronutrient metals were determined in the plants since from a yield decrease at the 50 μ g-at Se per liter supply **level a nutritional deficiency was suspected, especially for manganese; these elements were not monitored in supply as was the phosphate. The experimental data are presented in Table 3. Yield was** significantly restricted at the culture level of 50 μ g-at Se per liter. **This was evidently not due to a toxicity** *per se* **of Se, but presumably caused by an induced deficiency of manganese in the plant; the plant coneentration was near the critical for Iavourable growth, as determined in a preliminary study with** *A. bisulcatus* **to approximate 0.4 mg-at Mn per kg dry weight for the whole plant. The phosphorus concentrations were low, but adequate for favorable growth. The total phosphate per plant culture significantly decreased. The phosphate concentration decrease in spite of frequent solution applications to substantially maintain the plant concentration, especially concomitant with a significant yield decrease, demonstrates that sorption of phosphate was suppressed as a consequence of selenium supply. An interference interaction is implied.**

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In another experiment with *A. bisulcatus,* plants were cultured at a single concentration of phosphate supply $(500 \mu g-at/l)$ with increasing supply concentrations of selenite 3. With increased selenite supply concentration, yield was enhanced and the selenium concentration in the plant increased. The concentration of phosphorus in the plant concomitantly decreased. Presumably phosphate was toxic at the nil level of selenite supply and yield was increased with progressively decreasing phosphate excess, while selenium concentrations inocuously increased in the plant. This was borne out by observation of decreasing symptoms of what was considered to be phosphate injury 3. An interference interaction of selenite on sorption of phosphate was evident.

Effect of selenite on macronutrient cation sorption

A limited number of observations thus far with whole plants of Astragalus species have not revealed such interaction. Other, subsequently considered, interionic effects suggest that where experimental conditions are proper, an interference interaetion might occur. Further, ancillary studies with abscised roots of barley *(Hordeum vulgare)* have shown that selenite applied at 10 µg-at per liter repressed sorption of potassium from a solution of KC1.

Effect of selenite on micronutrient metal sorption

In addition to the interfering effect of selenite on phosphate, reported above, there was an evident concurrent repression of sorption of each of the micronutrient metals (Table 3). The concentrations of these metals generally decreased in the plants with increased selenite supply, emphasized for manganese where an induced deficiency was suspected, concomitant with a depressed plant yield. At the highest selenite supply level, the otherwise progressive decrease of concentration of the metals was counteracted by a concurrent restriction on yield presumably induced by a manganese deficiency. The resultant effect of high selenite on the micronutrient metals is explicable, then, as a direct effect of the independent ion $(SeO₃)$ on plant yield and composition (Table 1). The overall effect between Se-O and Se-50 remains as an interference interaction. The data of Table 3, therefore, demonstrate that selenite can interact repressively on the sorption of the micronutrient metal ions. It may be noted that here, where the internal phosphate concentration was low but adequate for growth, results for iron closely paralleled those of the other micronutrient metals; this result may be eontrasted later herein, where interaction of iron and phosphate in nutrition is particularly considered (Table 6).

The previous experiment demonstrated concurrent interference interactions of selenite on phosphate and on micronutrient metal sorptions. Another trial confirmed the latter effects. In this experiment, the phosphate concentration in the plants was again maintained substantially constant and at a level favorable for plant growth. With the *A. bisulcatus* plants here, however, the manganese coneentrations in the plants were always at a favorable nutritional concentration (compare with the previous experiment where plant Mn was presumably deficient at the highest level of selenite supply). The results are presented in Figure 1. Plant growth did not vary

Fig. 1. Effect of selenite supply on the growth and manganese content of *A. bisulcalus* plants.

Data represent averages of three replicate cultures of live plants each. Cultures were supplied with macro- and micronutrients, exclusive of manganese, at the basal level. Manganese was supplied at 0.75 μ g-at/liter, which was adequate here for growth as determined by frequent plant analysis. The total Mn content of plants at Se supply of of 12μ g-at/liter was significantly lower (0.1% level) than the nil controls.

Fig. 2. Effect of phosphate supply on the growth and selenium content of *A. canadensis plants.*

Data represent averages of two plants from three replicate cultures of six plants each, harvested after two weeks' growth. Cultures were supplied with maeronutrients at 0.1 basal and micronutrients at 0.01 basal. Selenite was supplied at 0.0125 μ g-at Se/liter, since a non-indicator species was used.

significantly at the various levels of selenite supply. Both the manganese concentration and the total content decreased (the latter highly significantly) in the plant with increasing selenium supply. It is evident that selenite induced an interference of manganese sorption.

Effect of phosphate on selenite sorption

In an experiment with *A. canadensis,* a non-indicator Se species, phosphate supply was varied at two different levels of selenium application. Yield and plant composition for selenium were determined. Results at one of the two Se application levels are shown in Figure 2. Plant growth was unaffected by the various supply concentrations of phosphate. Both the selenium concentration and the total plant content decreased with increase of phosphate appliea-

TABLE 4

Effect of phosphate on the sorption of selenium

Macro- and micronutrient supplies were basal. Selenium was applied at 50 µg-atoms/liter. Plant yields and their contents represent averages of three replicate cultures of six plants each. 'e' is used to designate 'culture.'

Treatment		Plant compositions		
Ρ,	Yield,	P	Se	
μ g-at/l	g/c	$mg-at/kg$	$mg-at/kg$	μ g-at/c
5	0.94	154	28.9	27.1
50	$1.45*$	356	17.4	25.2
500	1.05	519	9.0	9.45

The yield was significantly higher at P 50 than at either P 5 or P 500, at the 5% level.

tion. These data clearly demonstrate an interference interaction of phosphate on selenite. Like results were attained when the selenite supply concentration was ten times that reported in Figure 2.

Similar results were obtained with *A. bisulcatus,* an accumulator species. Data are presented in Table 4. Plant yield was repressed at the 5 μ g-at P/liter supply level due to a deficient manganese concentration in the plant (see Table 6), the repression here probably being due to an interference interaction of Se on Mn sorption (see earlier results and discussion of Table 3). Decreased yield at the 500 μ g-at P/l level was related to an excessive phosphate concentration in the plant particularly the top, where visual symptoms were evident; see Table 6). As would be expected, phosphorus concentrations in the plants increased with increased supply concentration. Selenium concentrations and the totals per plant culture decreased with phosphate increase. For the increased yield comparison (P-5 to P-50), only part of the selenium concentration change could be attributed to dilution. This is evident since, if the total Se in the plant, at the 5 μ g-at P/l ('control') supply level, of 27.1 μ g-at Se per culture were distributed in the plant dry weight of 1.45 grams per culture at the 50 μ g-at P/I ('treatment') supply level, the calculated theoretical concentration of selenium would be 18.7 mg-at Se per kg dry weight. The actual measured concentration was 17.4 mg-at Se per kg, which is less than the theoretical value; therefore, an inter-

TABLE 5 Effect of phosphate on the sorption of the macronutrient cations

Conditions were the same as indicated in Table 4. $(c = culture)$.

ference interaction occurred for P on Se sorption. (See Tables 1 and 2 for interpretation of the data and computations.) Ad ilution explanation, even in part, cannot be invoked for the yield and selenium concentration changes of P-5 or P-50 to P-500, since in this comparison yield was either constant or decreasing with concurrent decreases of both selenium concentration and the total sorbed per culture. Here, then, the results can be attributed to an interference interaction. Further, under the conditions of these experiments at least, phosphate and selenite appear to reciprocally inhibit each other in ionic sorption.

Effect of phosphate on macronutrient cation sorption

In the same experiment just reported (Table 4), where effects of phosphate on sorption of other ions from the culture media were sought, possible effects on the macronutrient cations were determined. Results of interactions of phosphate on sorption of potassium, calcium, and magnesium are presented in Table 5. With increased plant yield between P-5 and P-50, concentrations and total contents per culture increased for K, Ca, and Mg. Synergistic ionic interactions of phosphate on the macronutrient cations are evident. With yield decrease between P-50 and P-500, concentrations of K, Ca, and Mg increased, which results are not contrary to consideration of a synergistic interaction of phosphate on the macronutrient cation. Considering the computation suggested for \pm designations in Table 1 (see Table 2) where treatment yields are less than controls, it is clear that calculated theoretical concentra-

tions exceeded the actual measured values for K, Ca, and Mg. The indeterminate causal relation (Table 2) is explicable then as a direct effect of the independent H_2PO_4 ion on plant growth and cation content; the overall effect between $P-5$ and $P-500$ remains synergistic for the approximately equal yields (Table 1).

Effect of phosphate on micronutrient metal sorption

A preliminary growth trial was performed with *A. bisulcatus* to establish favorable levels of solution culture supply of phosphate and the micronutrients with increasing concentrations of selenium appli-

Fig. 3. Effect of phosphate supply on the growth and manganese content of *A. bisulcatus* plants.

Data represent averages of three replicate cultures of five plants each. Cultures were supplied with macronutrients at the basal level and micronutrients at 0.4 basal. Selenite was supplied at 50 μ g-at/liter of solution culture. Yield increased significantly at 500 μ g-at P/1; manganese was deficient (by plant analysis) at 5 and 50 μ g-at P/1.

TABLE 6

Effect of phosphate on the sorption of micronutrient metal ions

Conditions were the same as indicated in Table 4. Plant fractions are designated T , tops; R, roots; and P, plant as a whole.

cation. The results showed that the plants with higher selenium supply became deficient in manganese at a 0.4 basal micronutrient supply where phosphate was applied at either 5 or 50μ g-atoms per liter; visual deficiency symptoms were evident at 5 μ g-at P per liter. At a 500 μ g-at P/liter supply level, growth was greater and the plants revealed no manganese deficiency. Experimental plant yields and chemical plant analyses confirmed the manganese deficiency at the lower phosphate supplies where selenium was applied at 50 μ g-at per liter (Fig. 3). The data further showed that the incrased growth was not due to an increased plant phosphorus coneentration, it being approximately constant and within a favorable nutritional range. Since both the manganese concentration and its total content in the plants increased as growth increased with the phosphate-500 application, clearly phosphate supply enhanced the sorption of manganese with a concomitant increase in plant growth. Similar results were obtained in this experiment, where the selenium was supplied at a lower concentration (viz 20 μ g-at Se/l); however,

the manganese concentration and yield were greater at 20 than at 50 μ g-at Se per liter. This comparison between the two selenium supply levels, and like trends with Zn and Cu, implies that the selenium interference interaction and the phosphate synergistic interaction ean be effective simultaneously, and thus counteracting on sorption of these micronutrient metals.

Similar synergistic effects of phosphate on the sorption of all of the micronutrient metals are evident in another part of the experiment discussed immediately above. Here, the micronutrient level was basal including the manganese which was applied at one μ gatom per liter; this coneentration should have been fully adequate for growth at a nil level of selenium supply (compare Fig. 3 and discussion). The results are presented in Table 6. The data are separated into top and root values (and their sums) in order to contrast the distribution effects of phosphorus on iron from those of the other micronutrient metals. The yield at 50 μ g-atom P per liter was significantly higher than that at either the 5- or 500-P level. At the 5-P level, i. anganese was deficient in the plants; at the 500-P level, phosphoru, was toxic. The 50-P level was apparently favourable from these t "o standpoints. With improved yields (comparë $5-$ to $50-P$), particularly evident with the tops, the concentrations and totals per culture for Mn, Zn, and Cu increased in both plant fractions and in the plant as a whole, with increasing phosphate supply and sorption. Definite synergistic interactions of these metals and phosphate are evident. The results for iron, for the plants as a whole, are definitely synergistic for the total sorption differenees, but unconfirmed by concentrations. (Dilution computation is invalid here, since the treatment total exceeds the control; computations hold true only where $+$ total ion differences occur in Table 1.) The plant part data suggest a synergistic effect for iron with the roots; concentrations and totals per culture increased with increasing phosphate. The tops, however, show an inverse trend in eoncentration. This is probably related to the phenomenon of induced chlorosis in plants under conditions of high phosphate supply where iron is restricted in its mobility from roots to shoots. Thus, where results of synergistie or interference interactions on sorption are sought, whole plants rather than parts should be considered.

Microsutrient metal interactions

Relations between or among the micronutrient metals on sorption and plant yield were not specifically studied in this work with the nutrition of the Astragali. Such effects have been reported between iron and manganese, for example, with *Soya max*.¹². There, Somers and Shive demonstrated a reciprocal interference interaction between these micronutrient metals. Excess non-toxic concentration in the plant of either one produced a deficiency of the other, concomitant with depressed yield. Ratios of the ions as well as their particular concentrations seem to be implicated. Certainly, in some measure, this type of interaction was effective in the present research. Such would produce yet another interrelated influence on the overall results, reported here.

Resultant ef/ect o/ ionic interactioss in nutrition

The net effects of selenium application on growth and plant composition were in part reported elsewhere 3 . It was shown there in Figures 2 and 3 that with increasing selenium supply, and with phosphate and manganese concentrations in the plant adequately maintained, the yield decreased while the selenium concentration in the plant increased. Although the selenium concentration in the plant could have been toxic, hut since no foliar symptoms developed, the growth decrease was probably related to a progressively deficient supply of some micronutrient element (uncharacterized) other than manganese. This suggestion is supported by the observation that growth was not suppressed at the 50 and was less suppressed at the 100μ g-atom/liter selenite supply levels where additional P and micronutrients (exclusive of Fe) were initially supplied, concomitant with decreased selenium and increased micronutrient sorption. The complexity of the resultant effects is obvious.

Phosphate toxicity in plant growth and development could arise through chemical mass action of inorganic phosphate repressing otherwise favorable energy production or transfer reactions. Numerous competitive and non-competitive ionic interferences in sorption, in addition to those described herein, have been reported 5. They have concerned unilateral or reciprocal effects with single or more carrier sites and mechanisms, and related to different values or ranges of ion supply concentrations. The biomechanics, particularly related to synergistic interactions, are not clearly evident here. The

result with iron and phosphate obviously involved a secondary interference (probably including precipitation in roots) causing decreases of iron concentration in the plant tops. Whole plants must be used where interionic sorption effects are considered. Thus, for sorption by the whole plant here, it is possible that with the relatively high phosphate supply concentration, iron was 'fixed' as Fe-P, sequentially decreasing a competitive Fe to Mn ratio (for example) and leading to a particularly effective enhancement of manganese sorption. Such a succession would then reveal a synergistic increase of sorption of the dependent ion (Mn) with increased supply concentration of the independent ion (H_2PO_4) . In like manner, the resultant effect among selenite, phosphate and micronutrient supplies on the growth of, and ionic concentrations in, *A. crotolariae* may be considered 3. Here, the enhanced intial supply of micronutrient could have itself allowed greater growth with dilution of the selenium concentration in the plant and/or the added phosphate could have offset an increasing interference of selenite on micronutrient sorption thus allowing better growth with a decreased selenium concentration in the plant. Thus, although enhanced sorptions may be direct between two ions, they may be rather indirect among more, through auxiliary direct interferences allowing other dependent ions to be sorbed more effectively. In any event, the observed growth and plant composition are the resultant of all the interrelated internal conditions and processes which have previously occurred under the environmental conditions and within the limits established by the hereditary potentialities of the organism.

Greenwood 6 has reported a synergistic enhancement of phosphate sorption by copper, which may be the inverse of effects reported here. A synergistic interaction of phosphate on molybdate sorption was shown by Stout et al. with subterranean clover ¹³; sulfate, on the other hand, appeared to interact interferingly on molybdate. The unique precipitation and induced immobilization deficiency of iron by higher phosphate concentrations in plants has been demonstrated and discussed by Rediske and Biddulph 10; compare with P toxicity 6. With lower phosphate supply, the present synergistic interaction is exemplified. Antagonistic actions between copper and iron have been suggested 4. Sharma *et al.* have reported a mutually competitive interaction of Zn and P in plants 11 The interpretation would appear to be subject in part to another

effect, however; application of phosphate increased growth, thereby allowing an enhanced zinc deficiency to develop through a dilution effect. The P on Zn interaction reported here is of the opposite sort from that shown by Sharma *et al.*¹¹. Dissimilarity of experimental conditions no doubt determines the different results. The latter experiments were with soils, which variably modify the effective, indeterminate P and Zn supply concentrations and could confound the interpretation of results.

Recently, Ziebur and Shrift have reported results of studies with appliedse lenium using undifferentiated callus from Astragalus species 15. Data on the chernical composition of the dependent ions in the callus were not presented. In the absence of such, we submit that the yield decreases may have been related either to Se toxicity *per se,* or to an induced deficiency, through interference interaction on sorption, of some dependent essential nutrient ion.

In recapitulation, here, selenite tended to depress the sorption of phosphate and the micronutrient metals; phosphate tended to restrict selenite sorption; and phosphate tended to enhance the sorption of the macronutrient cations and the micronutrient metals in interactions which often resulted in yield differences. Where the influences were relatively well balanced, plant growth was favorable and yields were relatively constant 8

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