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SELENIUM AND NUTRITION OF ASTRAGALUS

I. EFFECTS OF SELENITE OR SELENATE SUPPLY ON GROWTH AND SELENIUM CONTENT

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SUMMARY

Astragalus species were cultured on solutions of repurified nutrients in a greenhouse provided with carbon-filtered air. Earlier results of others which had demonstrated pronounced growth increases with selenium application are now considered to have been largely related to ameliorated toxicity of phosphate through suppressed sorption induced by selenium. Manganese sorption was similarly repressed. Losses of selenium, presumably organo-volatiles, from cultures were confirmed; concomitant inadvertent accretions occurred. Essentiality of selenium for growth has not been conclusively established, but certain observations confirm the possibility. If selenium is required for growth of these species, the critical concentration will probably approximate one $\mu\text{g-atom Se/kg}$ dry weight in leaves or whole plant tops.

INTRODUCTION

Plant species that accumulate high concentrations of selenium when they grow only on highly seleniferous soils – those particularly displaying high Se availability for plant sorption¹⁰ – have been termed as selenium indicators^{8 10}. Essentiality of, or benefit from, selenium for development of the indicator species might be inferred by this definition⁸ except where a restricted population distribution is determined by competitive growth among species. Moderate (secondary) to low selenium accumulators are not limited to highly seleniferous soils^{8 10}. Growth of so-called non-accumulators is reported to be inhibited by even low supply concentrations in solution cultures, although they appear to be able to grow satisfactorily and escape toxicity on seleniferous soils^{10 13}. Uniquely high selenium accumulation by, and distinctly different Se assimilation



patterns of, higher plants and its possible requirement for growth are apparently limited to only relatively few species, particularly in the distantly related families of the Leguminosae, Compositae and Cruciferae^{8 10}. If selenium is essential for growth, an interesting evolutionary development might be implied for these species^{8 10}.

Within the family Leguminosae, yield of *Astragalus crassicaarpus* (now designated *succulentus*⁸), a non-accumulator *Astragalus* species, was limited by a concentration above 100 $\mu\text{g-at}$ per kg dry weight¹³. By contrast, *A. racemosus*, a selenium indicator species, had been shown to tolerate a selenium concentration of at least 9,000 to 40,000 $\mu\text{g-atoms}$ per kg dry weight without obvious ill effect¹³. Yields of both alfalfa (*Medicago sativa*) and subterranean clover (*Trifolium subterraneum*) were directly or indirectly adversely affected when the concentration of selenium in mature leaf laminae was more than 800 to 1,000 $\mu\text{g-atoms}$ per kg dry weight⁴. Comparative effects may depend on the species studied and/or the conditions of experimentation.

A beneficial effect of selenium on growth of either alfalfa or subterranean clover has not been demonstrated⁴. If required for growth of these species, it was suggested that the critical concentration would be less than one $\mu\text{g-atom Se}$ per kg of dry plant material. These results differed from those of other researchers which had suggested that selenium might be essential for healthy growth of at least selenium indicator plants^{8 10}. Data with the latter species showed growth increases with advertent applications of selenium as selenite or selenate to cultures using *A. racemosus*⁸. Similar results, using selenite, were obtained with other selenium indicator plants, including *A. bisulcatus*⁸, which was one of the species used in the present studies. In contrast, however, growth of *A. crassicaarpus* was adversely affected at similar external culture supply and internal plant concentrations of selenium¹³ demonstrating physiological differentiation in the Astragali⁸.

Since an apparently favorable effect of selenium on growth had been reported or implied for *A. racemosus*, it was of interest to confirm the growth promotion with closely related species and relegate any different results to quantitative rather than qualitative relations. (See Shrift¹⁰ for an early report on the present studies.)

MATERIALS AND METHODS

The *Astragalus* species especially studied here included *A. bisulcatus* and *A. crotolariae*. Less intensively, growth of *A. pectinatus*, *A. canadensis*, and *A. succulentus* was observed. The first three of these are considered to be accumulators of selenium; the last two are not⁸. Initially, seeds of these species were from field plants in their native habitat. For more rigorous study of the possibility of selenium essentiality for growth, first generation seeds were secured for *A. bisulcatus* and *A. crotolariae* following experimental culture on solutions with repurified nutrient salts in a greenhouse provided with air passed through carbon.

Procedures were generally the same as reported earlier with alfalfa and subterranean clover⁴. Seeds were germinated in vermiculite to which a dilute macronutrient solution had been added. Seedlings were transplanted into aerated solution cultures, generally 4 or 5 per 4-liter Pyrex beaker, the latter wrapped to exclude light. The composition of the basal nutrient solution (using repurified salts) was as follows (μM): nitrogen (NO_3^-) 7,000; potassium 3,000; calcium 2,000; magnesium 500; sulfur 1,000; nitrogen (NH_4^+) 1,000; chlorine 25; boron 12.5; iron 25.0; manganese 1.0; zinc 1.0; copper 0.1; and molybdenum 0.1. The pH of the culture solutions was adjusted very frequently to pH 5.5., but rapidly drifted by ± 1.5 units during growth (see Trelease and Trelease¹², and Ulrich and Shrift¹⁴). The basal medium was supplied to plants at the beginning of culture and replenished thrice before plant harvest. Selenium was applied initially and generally was maintained at the various levels indicated in the tables and figures.

Phosphorus was initially applied at two levels, depending on the species used, namely $2 \mu M$ for *A. bisulcatus* and $10 \mu M$ for *A. crotolariae*. These supply concentrations are less than those used for many species in physiological study. They were less, since the *Astragalus* species studied here were sensitive to an induced growth restriction at usual levels of phosphorus supply and older morphological parts, especially during early growth, were visibly injured. The phosphate supply (as KH_2PO_4), therefore, was adjusted in accordance with maintenance of concentrations between 50 and 200 mg-atoms total-P per kg dry weight in leaf blades. A similar adjustment of manganese was made; here, a plant content of between 0.7 and 1.5 mg-atoms per kg dry weight in recently mature leaf blades was sought. Manganese supply regulation was necessary since other results had demonstrated that mineral ion contents of plants were influenced by the supply ratios of selenium to other elements.

For selenium sorption and distribution, and plant yield determinations, plants were generally harvested during the vegetative stage of development (7 to 12 weeks after transplanting) and separated into roots, stems (including leaf petioles), and leaf blades. Plant materials were dried and ground. Appropriate plant aliquots were wet ashed for selenium content. The fluorometric method of Watkinson¹⁵ was used to determine selenium. For comparison of supply and recovery of selenium in the system, all known sources of supply

were considered and measured; the total selenium recovered included that in the solution cultures and the sum of that in plant parts.

RESULTS AND DISCUSSION

Plant growth and elemental composition

Selenium sorption and growth of two selenium accumulator species, *A. bisulcatus* and *A. crotolariae*, were studied. With the latter species, effects of selenite and selenate were compared. Yields of plant material and distribution of selenium therein are shown in Table 1.

Total plant yield was significantly increased in one case, with advertent application of selenium. This occurred with *A. bisulcatus* plants from first generation seeds of low selenium content; sequential crops were secured by the low selenium culture technique (Table 1, Expt. II). Possible essential benefit is supported here, by the observation of floral initiation on six of the 25 replicate plants at the 0.25 $\mu\text{g-atoms Se per liter}$ level of selenite application while no reproduction was evident with the controls. This result, indicating a beneficial effect of selenium on growth, confirms those reported earlier by Trelease and Trelease¹³ with *A. racemosus*. However, we believe that the relatively large growth benefit from selenium application reported by them for *A. racemosus* was possibly related, at least in part, to corrected phosphate toxicity. Replacement of its chemical analogue sulfur probably was not concerned since aberrancies in sulfur nutrition were not apparent. Therefore, in addition to possibly demonstrating a selenium requirement for favorable metabolism, the observations of Trelease and Trelease suggest to us that the selenium favorably altered the sorption-accumulation level of other elements and thus plant yield.

Such an interaction between selenite and phosphate was borne out by results of the present authors (Fig. 1). An initial supply concentration at or above 500 $\mu\text{g-atoms}$ of phosphorus per liter of solution adversely affected early growth of phosphate sensitive *Astragalus* species. This effect has been common also to subterranean clover and some other species⁶. Typical visual symptoms of toxicity have been characterized as a bleaching necrosis of older leaf laminae⁹. Observations suggest that different rates of attainment of a nearly similar critically high concentration (about 1 per cent total-

TABLE 1

Growth and distribution of selenium

Treatment	Yield, g†			Se concentration, µg-at/kg		
	Roots	Tops	Plant	Roots	Tops	Plant
<i>A. bisulcatus</i>						
<i>Experiment I</i>						
Se nil**	8.1	9.9	18.0	27	44	36
SeO ₃ 5	6.8	9.3	16.1	3,480	6,200	4,790
SeO ₃ 10	7.1	9.5	16.6	6,650	10,700	8,980
<i>Experiment II</i>						
Se nil	9.9	16.5	26.4	1.72	0.64	1.04
SeO ₃ 0.25	9.8	20.1*	29.9*	252	272	265
<i>A. crotolariac</i>						
<i>Experiment I</i>						
Se nil	9.4	21.7	31.1	22	27	25
SeO ₃ 1	9.1	21.1	30.2	363	452	442
SeO ₃ 10	7.7***	21.6	29.3*	2,400	4,960	4,290
SeO ₃ 50	6.8***	15.1***	21.9***	10,200	26,900	21,700
SeO ₃ 100	4.7***	9.6***	14.3***	20,800	30,300	27,200
SeO ₃ 50 + P&M(-Fe)	7.9**	23.4	31.3	7,500	16,300	14,000
SeO ₃ 100 + P&M(-Fe)	6.2***	15.9***	22.1***	17,200	24,500	22,600
SeO ₄ 1	7.5***	21.9	29.4*	224	483	420
SeO ₄ 10	7.4***	23.1*	30.5	1,390	3,180	2,670
SeO ₄ 50	8.5	20.6	29.1	5,190	14,900	12,100
SeO ₄ 100	4.1***	10.1***	14.2***	12,000	37,400	30,100
<i>Experiment II</i>						
Se nil	7.4	20.7	28.1	1.7	0.97	1.17
SeO ₃ 0.25	6.6	18.3*	24.9*	151	238	215
SeO ₃ 2.5	5.5*	17.1	22.6*	750	1,530	1,300
SeO ₄ 0.25	6.3	18.3	24.6	—	—	—
SeO ₄ 2.5	6.6	20.0	26.6	—	—	—

† Significance between treatment and control (nil) yield: * 5%; ** 1%; *** 0.1%.
All others not significant.

** Selenium supply concentrations µg-atoms per liter.

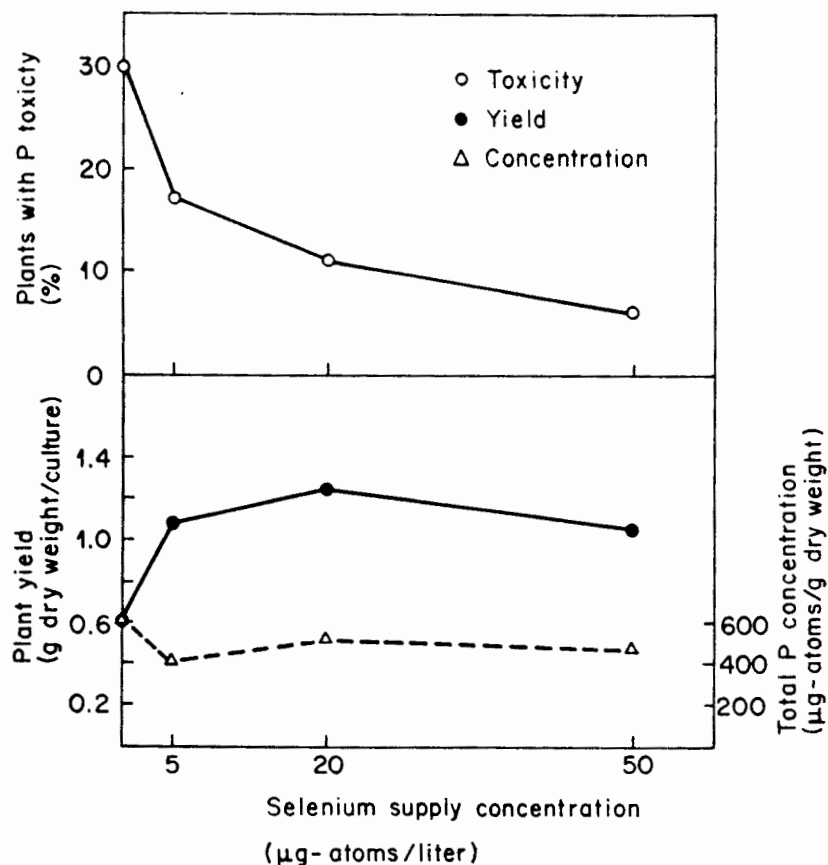


Fig. 1. Effects of selenium concentration in the nutrient culture solution on plant growth, total-P concentration in tops, and percentage of plants affected with P toxicity. No toxic P symptoms have been observed below 600 mg-at total-P per kg dry weight in tops. Plant yields were significant at the 1% level for the 20, and at the 5% level for the 50 μg-at Se per liter supply concentration.

P on a dry weight basis) in the plant tops determined growth⁶. The approximate order of increasing sensitivity to phosphate supply under our cursory examination has been: tomato, subterranean clover, *A. crotolariae* and *A. canadensis*, *A. pectinatus*, *A. succulentus*, and *A. bisulcatus*. In this regard, critical concentrations in plants for growth often appear to be nearly similar; rather, for a particular supply concentration, different rates of acquisition are inferred for

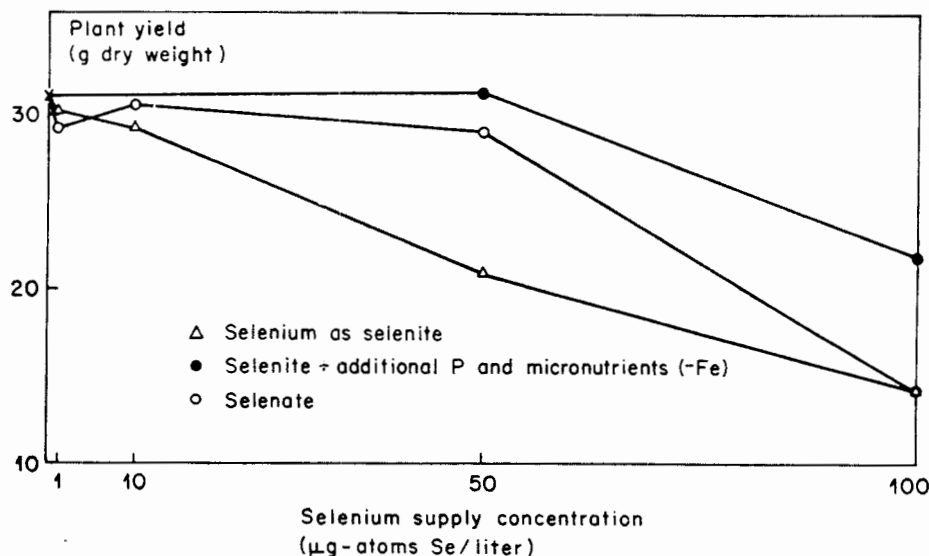


Fig. 2. Growth of *A. crotonariae* plants as influenced by selenium supply concentration. Same experiment as reported in Fig. 3 and Table 1. Selenium supply levels were maintained as practicable.

various species. In short, here selenium supply appears to have depressed phosphate sorption, counteracting the growth limitation due to excessive phosphate concentration in the absence of Se application or inducing an apparent deficiency of phosphorus and growth restriction when applied with otherwise favorable P supplies.

At higher levels of supply of selenium, growth of *A. crotonariae* was depressed (Table 1, and Figs. 2 and 3). This result was probably not related to a physiologically deficient phosphorus or manganese concentration in the plants since they were adequately maintained by frequent foliar analysis and supply adjustment as necessary. We submit that the growth decrease was probably related to a deficient supply of some other micronutrient element (uncharacterized), with (at least) no pronounced specific foliar symptoms. This suggestion is supported by the observation that growth was not suppressed at the 50 and less suppressed at the 100 $\mu\text{g-atom per liter Se}$ (as SeO_3^-) supply levels where additional P and micronutrients (except for Fe) were initially supplied (Fig. 2). Selenate supplied at 50 $\mu\text{g-at per liter}$, as compared with selenite at the same level, produced a less depressing effect on yield, correlated with a lower selenium content in the

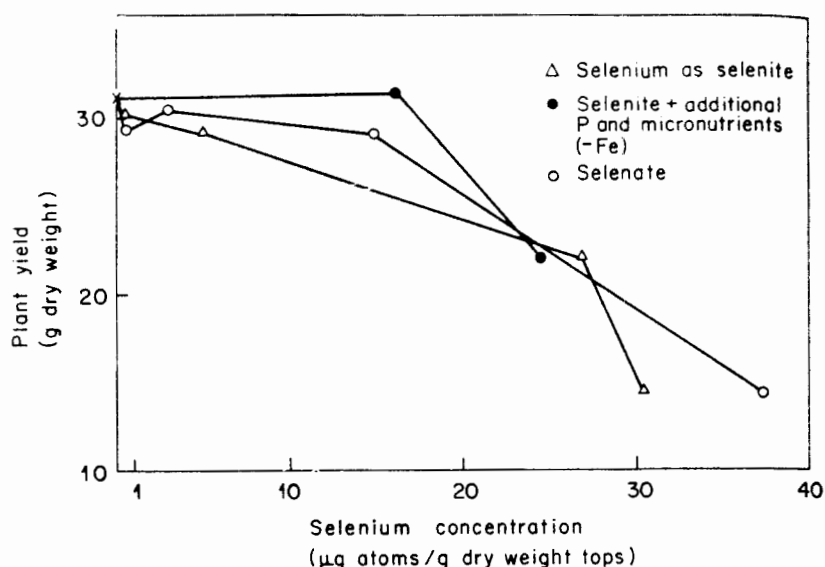


Fig. 3. Growth of *A. crotolariae* plants as affected by their selenium content. Same experiment as reported in Fig. 2 and Table 1. Selenium supply levels were maintained as practicable.

plant (Fig. 3). Further, with selenium concentrations of less than 14 mg-at per kg dry weight, all plant yields were near the maximum.

With regard to effects of additional phosphate and micronutrients (exclusive of Fe) above, on growth, attention may be drawn to the earlier work of Trelase and Trelase^{11 13}. With *A. crassicaepus*, growth was repressed at all levels of selenite added to the solution media. There, the reported copper supply concentration (0.002 ppm) would probably have been low; the other micronutrient metals were probably favorably supplied. It is quite possible, therefore, that copper could have been progressively more deficient and growth concomitantly limited by the higher supply concentrations of selenium. A similar effect has been observed by the present authors, where a manganese deficiency with *A. bisulcatus* was induced when the Mn supply was just adequate in minus selenium cultures.

In addition, or as an alternative, to the above suggested effect of selenium on growth of non-accumulator species of *Astragalus* (most are such), direct influences of applied Se on the development of roots have been reported⁸. Visual symptoms of direct injury were

not observed in the present research. Growth inhibitions are possibly typical for non-indicator species. Impairment of root growth, often concomitant with relatively low (or less high) concentrations in the plant, may be typical for at least some toxicities such as those, for example, induced by heavy metals.

Selenium sorption increased with increasing supply concentration (Table 1). Concentrations in plant parts increased in the order root, stem (including petioles), and leaf blades, which agrees with earlier observations with a number of *Astragalus* species^{5 8}. The opposite has been reported for alfalfa and subterranean clover⁴, other crops and pasture species, and *Neptunia* and *Stanleya* sp.^{5 7 8}. No relationship to top to root weight ratios is apparent in this inconsistency.

Selenium was readily sorbed from selenite or selenate solutions. At similar initial supply concentrations and other culture conditions, selenium concentrations and total sorptions were greater from selenite than from selenate with *A. crotolariae* (Table 1). This is the inverse of earlier observations with *A. racemosus*^{8 12}. This disagreement may be related to species differentiation or possible pH differences of the media in the researches, because the rates of sorption of these forms of selenium appear to differ quantitatively in these respects¹⁴, or cultural differences such as sulfate supply concentration¹². In the present studies, a supply level approximately at or above 50 $\mu\text{g-atoms Se per liter}$, either as selenite or selenate, depressed growth. A selenium concentration in leaf blades or whole tops of the accumulator species of *A. crotolariae* of approximately 20 mg-atoms per kg resulted in repression of growth (Fig. 3). Top and root yields were equally adversely affected above this concentration. An external supply level of 100 $\mu\text{g-atom Se per liter}$ or a plant concentration of about 30 mg-atoms per kg dry weight restricted yield to 50 per cent. Critically toxic (upper tolerance) concentrations of selenium in whole plants would appear to be about 10 times higher in *Astragalus* accumulator species than those in alfalfa and subterranean clover. Average selenium concentrations in *A. bisulcatus* were from equal to about double those in *A. crotolariae* at similar increasing supply levels and like intervals of time. (See Ulrich and Shrift¹⁴, for comparative sorption rates in short time study with abscised roots of *Astragalus* species, and Shrift¹⁰ for suggestions that *A. crotolariae* could possibly be 'intermediate in

some physiological characteristics between accumulators and non-accumulators.) With these *Astragalus* species (Table 1) and the alfalfa and subterranean clover⁴, the plant concentrations of selenium increased about 10-fold with 10-fold increases in supply concentration.

Selenium supply and recovery

In the earlier investigation on growth and nutrition with selenium for alfalfa and subterranean clover⁴, it was shown that those plants at a low level of supply acquired more selenium than that advertently provided. Further, with greater known supply, there were marked losses, attributable to volatilization of organo-selenium compounds^{1 4}. This same effect was found in the present investigation with *Astragalus* species (Table 2). For equal growth of *A. crotonariae* cultured from seeds from plants in their native habitat, a generally increasing absolute and percentage loss of selenium occurred from culture systems provided with increasing advertent supply concentrations. The absolute loss per gram of plant tops also increased with increasing known supply. The loss from the nil treatments (Table 2) is explicable, since, although only small amounts were supplied from salts and water, a relatively large provision occurred from the seeds derived from the plants which had grown on soils in their native habitat.

Replicate *Astragalus* plants were allowed to grow to maturity on repurified solutions, from seed previously obtained from plants on soil in their native habitat. First generation seeds were then collected. Seeds from each generation and species were compared as suggested by Rosenfeld and Beath⁸. Data are shown in Table 2. The selenium concentrations per gram dry weight of seed and per seed were greatly decreased in the first generation. Plants were then cultured from the first generation seeds, as before with native seeds. A supply: recovery balance from the second generation plants revealed an inadvertent accretion of selenium, where no further selenium was applied, over that of the measureable contaminations. Such accretions have been demonstrated for chlorine and sodium^{2 3} and selenium⁴ with alfalfa and other plant species. These acquisitions probably were from air-borne sources⁴. Losses and accretions of organo-selenium volatiles have been dramatically demonstrated where plant cultures of low and high known supply were in random

Selenium supply and recovery

Treatment	Source	Weight, mg	Seed		Total Supply, ng-at	Salt Supply, ng-at	Water Supply, ng-at	Total		Gain or loss, ng-at
			Se concentration					Supply, ng-at	Recovery, ng-at	
			µg-at/kg	ng-at/ seed						
<i>A. bisulcatus</i>										
<i>Experiment I</i>										
Se nil **	F***	4.7	63,900	300	1,500	2.7	1.3	1,504	640	- 864
<i>Experiment II</i>										
Se nil	G	4.9	6.1	0.03	0.15	2.7	1.3	4.15	27.4	+23.25
SeO ₃ 0.25	G	4.9	6.1	0.03	0.15	8,003	1.3	8,004	7,910	- 94
<i>A. crotolariae</i>										
<i>Experiment I</i>										
Se nil	F	6.6	34,500	228	912	1.9	1.6	915	790	- 125
SeO ₃ 1	F	6.6	34,500	228	912	19,000	1.6	19,915	13,400	- 6,515
SeO ₃ 10	F	6.6	34,500	228	912	175,000	1.6	170,915	126,000	- 44,915
SeO ₃ 50	F	6.6	34,500	228	912	536,000	1.6	536,915	471,000	- 65,915
SeO ₃ 100	F	6.6	34,500	228	912	569,000	1.6	569,915	389,000	- 180,915
SeO ₃ 50 + P&M (-Fe)	F	6.6	34,500	228	912	507,000	1.6	507,915	440,000	- 67,915
SeO ₃ 100 + P&M (-Fe)	F	6.6	34,500	228	912	661,000	1.6	661,915	493,000	- 168,195
<i>Experiment II</i>										
Se nil	G	7.4	7.0	0.05	0.25	2.7	1.3	4.25	33.0	+28.75
SeO ₃ 0.25	G	7.4	7.0	0.05	0.25	8,003	1.3	8,004	5,344	- 2,660

* Data are on a per pot culture basis unless otherwise specified; 5 plants for *A. bisulcatus* and 4 for *A. crotolariae* per 4 liters.

** Maintained selenium supply $\mu\text{g-atom/liter}$.

*** F = seed from natural soil habitat plants; G = first generation seed from purified culture plants.

close proximity¹. Inter-plant transfer of Se^{75} was evident there. Since such transfer of selenium was anticipated here, the various treatments were placed upon adjacent tables separated by an air curtain.

Selenium essentiality considerations

Plants cultured from first generation seeds were provided much less selenium in the controls than those from seeds derived under natural conditions. One statistically significant increasing effect of selenium on growth is apparent for *A. bisulcatus* (Table 1). The plant yield was significantly increased, between the nil (control) and 0.25 Se supply treatments of Experiment 11, from 26.4 to 29.9 grams. This yield difference implies a favorable effect of selenium on growth. The observation of a flower production difference in favor of selenium addition also suggests possible essentiality for development. However, for a greater than 250-fold increase in plant concentration of Se, there was only a 13 per cent increase in the measured yield among the five comparable replicates. Considering the usual diagnostic relations between yield and internal plant concentration (Fig. 4), the disparity between the yields here would suggest either that (1) if Se is essential for growth of this species, then the concentration of 1.04 μg -atoms Se per kg dry weight is probably close to the critical, or (2) the possibility is that we have dealt here with a 1 in 20 chance that there was no beneficial effect of the higher Se concentration on growth. Certain considerations should be made before deducing that a benefit has or has not been revealed here from advertent selenium application. These include the relatively large inherent genetic variability in growth and development of indigenous species, and in these experiments the treatment arrangement and the observations of floral initiation.

Selenium concentrations in the second generation control plants were less than one μg -atom Se per kg dry weight for both *A. bisulcatus* and *A. crotolariae* (Table 1). Therefore, if either of these species has a requirement for selenium, the critical level probably would be about one μg -atom Se per kg dry plant tops. This value is of the same order reported similarly for alfalfa and/or subterranean clover⁴. Comparable figures for *A. racemosus* heretofore were 3,000¹³ and 15,000 (Rosenfeld and Beath⁸, Table 17) μg -atoms/kg or 3×10^3 and 15×10^3 times greater than our results would suggest.

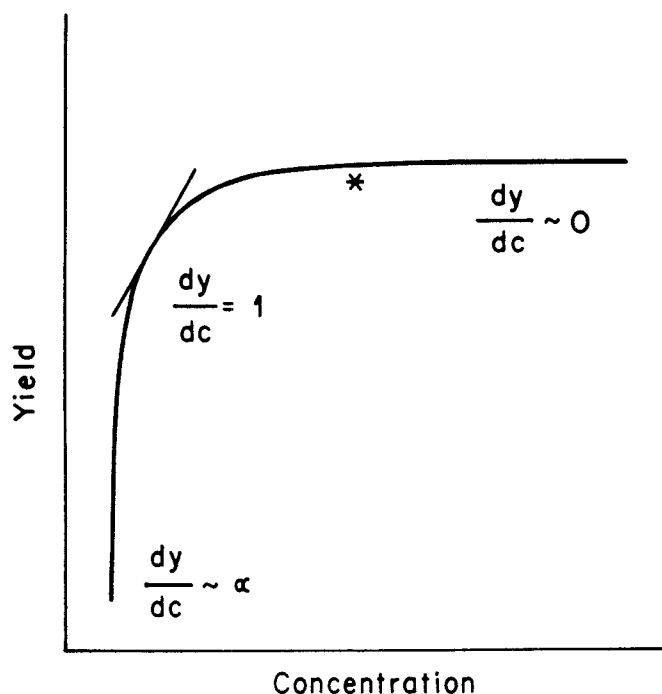


Fig. 4. Schematic diagnostic relationship between yield and the internal concentration of the predominantly growth-limiting nutrient element. Near *, Δy is small, but not zero; Δc is large, but not infinite. Therefore, $\Delta y/\Delta c$ is quite small here (see yield and plant concentration differences between the nil and 0.25 $\mu\text{g-at Se/l}$ treatments in Expt. II for *A. bisulcatus* in Table 1).

With the second generation plants, the inadvertent selenium contamination per culture exceeded the advertent control supplies by more than a factor of ten (Table 2). The seeds were particularly low in Se concentration; about one thousandth that in soil-grown native seeds. Before it would be practicable to carry the seed generations to lower selenium concentrations, to further study its possible essentiality for growth⁸, it will be necessary to substantially decrease the inadvertent contamination, which probably has been air borne.

Recently, Ziebur and Shrift have reported the lack of evidence for the essentiality of selenium for growth of callus cultured from hypocotyls of either indicator or non-indicator species of *Astragalus*¹⁶.

Minimal concentrations in the callus approximated those found in plants in the present studies. Physiological differentiation with respect to sorption of salt was not evident between calluses of accumulator and non-accumulator species, as has been found, however, with whole plants.

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