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Selenium Nutrition of Green Plants. Effect of Selenite Supply on Growth and Selenium Content of Alfalfa and Subterranean Clover[1]

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Summary. Alfalfa and subterranean clover plants were grown in highly purified nutrient solutions to which selenite selenium had been added at 0, 0.025, 0.25, 2.5 or 25.0 μg-atoms/liter. In both species, yields of tops and roots were significantly less at 25.0 μg-atoms/liter than at lower selenium concentrations ($p < 0.01$). The results indicated that growth was adversely affected when the concentration of selenium in mature leaf tissue reached 0.2 to 0.8 μg-atom/g dry weight.

No beneficial effect of selenium was demonstrated on the growth of either species. If selenium is required by these species, the critical level will probably be below 0.001 μg-atom/g of dry plant material. Results are discussed in relation to earlier work on the selenium nutrition of plants.

It has been recognized for a long time that high concentrations of selenium in crop and range plants growing on seleniferous soils present a serious hazard to the health of both livestock and the human population (2, 13). Consequently, much of the research on effects of selenium on the growth and composition of plants has been oriented toward this problem, emphasis being placed on plant behavior at high levels of selenium supply.

More recently it has been shown that selenium is needed for healthy growth of animals, a deficiency of selenium leading to myopathic conditions such as White Muscle Disease (11, 12). Investigations with so called selenium accumulator species such as *Astragalus racemosus* suggest that selenium may be essential also for healthy growth of plants (15). Several researchers have reported varying degrees of growth increase when selenium was supplied to a number of non-accumulator species, although no convincing evidence of essentiality has been shown (13, p 86).

This paper presents results from solution culture experiments in which the growth of 2 non-accumulators, alfalfa and subterranean clover, was studied over a wide range of selenium concentrations, taking stringent precautions to prevent inadvertent selenium contamination of the cultures.

Materials and Methods

Nutrient Salt Purifications. All macronutrient salts were twice purified by acidic coprecipitation with copper sulfide as follows: 4 liters of 2 M solutions of KNO_3, $Ca(NO_3)_2$, or $MgSO_4$ were adjusted to pH 7.0 with ammonium hydroxide; $NH_4H_2PO_4$ at the same concentration did not require pH adjustment. Each of these salt solutions was treated with four 1-ml increments of 0.1 M cupric nitrate, and concurrently, water-scrubbed hydrogen sulfide gas was bubbled into the mixture. While continuing the H_2S treatment, which reduced the pH to 4 or 5, the temperature of the mixture was raised to boiling. Gas passage was then terminated, but boiling was continued for 30 minutes to remove excess H_2S and coagulate the precipitate. Solutions were allowed to cool, filtered through sintered glass, and the pH readjusted to 7.0. A second coprecipitation and filtration was then performed. The second filtrate was adjusted to pH 6.0 with isothermally distilled ammonia. These stock solutions were then used for plant cultures.

Tracer experiments with $H_2{}^{75}SeO_3$ of high specific activity demonstrated that this procedure removed approximately 95 % of the added selenite. Analysis of purified stock solutions for total selenium showed them to contain less than 1×10^{-8} g-atoms Se/mole.

The micronutrient salts were twice recrystallized. Water was double distilled using a Pyrex condenser and stored in Pyrex containers.

Plant Culture. Seeds of alfalfa (*Medicago sativa* L., var. African) and subterranean clover (*Trifolium subterraneum* L., var. Mt. Barker) were germinated in the dark at 20° on distilled-water washed cheesecloth suspended on a Pyrex glass frame over a dilute macronutrient solution. At

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the monofoliate leaf stage of development, selected seedlings were transferred to 4-liter polypropylene beakers filled with nutrient solution. The seedlings were secured in plaster of Paris lids with Dacron batting. All solutions were continuously aerated during these experiments. Plants grew under natural illumination in a well ventilated greenhouse; all incoming air was passed through activated carbon filters. Greenhouse temperatures varied within the range 18 to 38°.

In the first experiment, with alfalfa, the basal nutrient medium was that used earlier in chlorine studies (Johnson et al., 1957). Its composition at single strength level was (μg-atoms/liter): nitrogen (NO_3^-) 14,000; nitrogen (NH_4^+) 2000; potassium 6050; calcium 4000; magnesium 1000; phosphorus 2000; sulfur 1100; iron (as the EDTA salt) 90; chlorine 50; boron 25; manganese 5; zinc 2; copper 0.5; and molybdenum 0.1.

This medium was supplied to the plants at the beginning of culture, at three-quarters strength. After 4 weeks' growth, the plants were transferred to pots of fresh nutrient solution at single strength level for the final 2 weeks' growth.

Selenium as sodium selenite was added to the cultures to give the following initial concentrations (μg-atoms/liter): nil, 0.025, 0.25, 2.5, or 25.0. Each selenium treatment was replicated 5 times.

In the second experiment, the same amounts of selenium were supplied, with alfalfa and subterranean clover as the test plants, but the solution culture technique was modified. This was considered necessary because of the apparent sensitivity of subterranean clover to phosphate toxicity (3). The total amount of salts added to each culture was divided into a small initial application followed by larger additions given at the beginnings of each of the 5 subsequent weeks. As a further precaution, the full strength phosphate concentration in the subterranean clover cultures was reduced to 100 μg-atoms/liter.

Harvesting and Chemical Analysis. In experiment 1, plants were harvested after 7 weeks of growth. They were divided into roots and tops, and the tops subdivided into stems, petioles, and leaf laminae. The latter 2 fractions were further subdivided into young, mature, and old categories.

In experiment 2, alfalfa and subterranean clover plants were harvested after 5 and 7 weeks of growth, respectively, and fractionated as before except that stem and petiole fractions were combined in alfalfa.

In both experiments, the plant material was dried at 45° in a forced draft oven for approximately 36 hours. After dry weight determination, samples were ground and analysed for selenium by the fluorometric method of Allaway and Cary (1).

Results and Discussion

At the highest selenium level studied (25 μg-atoms/liter) the growth of alfalfa and subterranean clover was significantly less than in the other treatments (tables I, II). In experiment 1, the growth reduction was accompanied by a reddish-purple coloration of the older leaves of alfalfa, especially on the lower surface. These symptoms were not observed on either species in experiment 2. In both experiments the highest selenium application reduced root elongation, giving the lateral roots a shortened, stubby appearance. Results of a subsidiary experiment with alfalfa suggested that a selenite concentration as high as 100 μg-atoms/liter may be necessary to produce leaf chloroses similar to those reported by other researchers (13, p 121-22).

The selenite supply concentration found to be toxic to alfalfa and subterranean clover in this

Table I. *Effect of Selenium Supply on the Dry Matter Yields of Alfalfa Grown in Solution Culture (Experiment 1)*

The values are the means of 5 replications.

Selenium added μg-atoms/l	Tops	Roots	Total
	g/pot	g/pot	g/pot
Nil	13.8	3.7	17.5
0.025	16.1	4.2	20.3
0.25	15.1	3.8	18.9
2.5	16.3	3.9	20.2
25.0	8.8*	3.4*	12.2*

* Yield significantly less than other treatments ($p<0.01$).

Table II. *Effect of Selenium Supply on the Dry Matter Yields of Alfalfa and Subterranean Clover Grown in Solution Culture (Experiment 2)*

These values are the means of 4 replications.

Se added μg-atoms/l	Alfalfa			Subterranean clover		
	Tops	Roots	Total	Tops	Roots	Total
	g/pot	g/pot	g/pot	g/pot	g/pot	g/pot
Nil	22.1	4.5	26.6	5.2	5.0	10.2
0.025	21.1	4.5	25.6	5.3	5.1	10.4
0.25	23.8	5.1	28.9	5.4	5.1	10.5
2.5	22.2	4.9	27.1	5.0	5.3	10.3
25.0	12.6*	3.2*	15.8*	2.4*	2.5*	4.9*

* Yield significantly less than other treatments ($p<0.01$).

study is closely similar to that found to be toxic in other studies with alfalfa (13, table XXX), and tobacco and soybean (10). With wheat, concentrations 3 to 5 times as great have usually been needed to produce comparable growth reductions (6, 8) although this was not always so (9). With selenium accumulating species, much higher concentrations appear to have been necessary for the development of toxic effects. Thus, in *Astragalus racemosus* it was found that an initial selenite concentration in excess of 340 μg-atoms/liter was necessary to cause a 50 % growth reduction (14).

In the present study, high selenium decreased the growth of alfalfa tops relatively more than the growth of roots, even though the concentration of selenium in the roots was about 3 times higher than in the tops (tables III, IV). With subterranean clover, the concentration of selenium was again higher in roots than in tops, but the growth reductions were approximately equal. These results are in general agreement with those of Martin (9) and Hurd-Karrer (6) who found that high levels of selenite reduced the growth of tops and roots of wheat plants to about the same extent even though the concentration of selenium in the roots was much higher. These results would seem to indicate that either roots are less sensitive to the toxic effects of high selenium than are tops or that a substantial part of the selenium in roots has been converted to a relatively non-toxic form. The observation that root cells of wheat plants supplied with selenite contain granules of elemental selenium (6) would appear to be consistent with the latter explanation.

Growth reductions in alfalfa and subterranean clover were associated with selenium concentrations of 0.8 to 1.2 μg-atoms/gram dry weight; no adverse effects were observed at concentrations of 0.08 to 0.2 μg-atoms/gram dry weight. These results are in general agreement with those obtained by other researchers in experiments with *Astragalus crassicarpus* Nutt. (15), a non-accumulating *Astragalus* species. By contrast, *Astragalus racemosus* has been shown to tolerate a selenium concentration of at least 10 μg-atoms/gram and possibly as high as 50 μg-atoms/gram without obvious ill effect (15).

At applied selenite concentrations ranging from 0.025 to 2.5 μg-atoms/liter, plant growth was independent of selenium supply (tables I, II). The plants absorbed selenium roughly in proportion to the selenite supply. This was reflected by an approximately 10-fold increase in the concentration of selenium in the tissues with each 10-fold increase in the selenite concentration applied. As with the higher selenium treatment, selenium concentrations in roots were generally higher than in tops and often substantially so.

Table III. *Concentration of Selenium in Alfalfa (Experiment 1)*

Single analyses of pooled samples from 5 replications.

Se added μ-atoms/l	Roots	Stems	Petioles			Leaf laminae		
			Old	Mature	Young	Old	Mature	Young
	μg-atoms/g dry wt					μg-atoms/g dry wt		
Nil	0.00058	0.0011	0.00071	0.00096	...	0.00090	0.0025	0.0018
0.025	0.0020	0.00061	0.0006	0.00063	0.0013	0.0052	0.0014	0.0025
0.25	0.033	0.0089	0 0044	0 0070	0.0047	0.0073	0.0071	0.0075
2.5	0.38	0.39	0.049	0.043	0.043	0.051	0.075	0.081
25.0	3.60	0.56	1.44	0.72	0.95	0.73	1.18	1.34

Table IV. *Concentration of Selenium in Alfalfa and Subterranean Clover (Experiment 2)*

Single analyses of pooled samples from 4 replications.

Se added μg-atoms/l	Roots	Stems and petioles*			Leaf laminae		
		Old	Mature	Young	Old	Mature	Young
Alfalfa	μg-atoms/dry wt	μg-atoms/g dry wt			μg-atoms/g dry wt		
Nil	0.00049	0.0011	0.00037	0.00087	0.00025	0.0011	0.00028
0.025	0.0065	0.00063	0.00078	0.0015	0.0016	0.0024	0.0016
0.25	0.0760	0.0053	0.0082	0.016	0.015	0.015	0.011
2.5	0.55	0.052	0.080	0.14	0.11	0.12	0.11
25.0	2.58	0.29	0.53	0.98	1.14	0.80	0.79
Subterranean clover							
Nil	0.00039	0.00078	0.00078	0.0032	0.00097	0.0015	0.00048
0.025	0.0072	0.0023	0.0011	0.0058	0.0030	0.0019	0.0034
0.25	0.063	0.011	0.012	0.010	0.012	0.023	0.018
2.5	0.54	0.11	0.089	0.085	0.23	0.22	0.15
25.0	2.66	1.19	1.20	1.22	2.11	0.89	1.23

* Petioles only for subterranean clover.

In the control (nil) treatment, plant growth was not significantly different in magnitude from that of plants receiving non-toxic amounts of added selenium. Hence no beneficial effect of selenium on the growth of the species was demonstrated. The results suggest that if alfalfa or subterranean clover does have a requirement for selenium, the critical level must be very low indeed, probably less than 0.001 μg-atoms/gram dry tissue.

The selenium supply in the control treatment was estimated to be less than 0.0005 μg-atoms/culture, i.e. 0.005 of the next lowest selenium treatment (table V). Despite this difference, the selenium contents of the plants in the 2 treatments were of the same order of magnitude. Plants in the control treatment were found to contain considerably more selenium at final harvest than was originally present in the seed plus the nutrient solution. Thus, it appears that there was considerable inadvertent contamination, approximately 0.019 μg-atoms per culture, despite all the precautions taken to exclude selenium.

Although the nutrient salts used in the present study were shown by actual analysis to be very low in total selenium after copper sulphide coprecipitation, further work using $H_2{}^{75}SeO_4$ has shown that this procedure is relatively ineffective in removing selenium present as selenate. This problem can be obviated by treating the concentrated salt solution with 6 N HCl before coprecipitation (to reduce the selenate to selenite). However, later study has shown recrystallization to be preferable to coprecipitation since it is not only more convenient but is also highly effective in removing both selenite and selenate.

Research on chlorine, sodium, and sulfur nutrition has clearly demonstrated that substantial and physiologically significant amounts of these elements can be absorbed from aerial sources, when plants are in an urban area (4). In the present studies, analyses of the active carbon used to filter the air entering the greenhouses indicated that the atmosphere did contain measurable amounts of selenium; the selenium content of the filter carbon showed a 30-fold increase over a period of 4 years. Also, it has been shown that plants supplied with selenite release volatile selenium compounds (5, 7). Thus, even if the air filters were to completely remove selenium from the incoming air, the greenhouse air might still be contaminated. Where randomized experimental designs are used, aerial transfer of selenium from a plant in a high selenium treatment to an adjacent low selenium plant may be a significant source of experimental error. Preliminary results with ^{75}Se labelled selenite support this suggestion.

Table V. *Total Selenium Supply and Content in Alfalfa (Experiment 2)*

Total supply	Total Se in plants
μg-atoms/culture	
0.0005*	0.019
0.1	0.053
1.0	0.58
10.0	5.12
100.0	15.4

* Approximate values, includes selenium in salts, water, and seed.

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