

Selenium absorption by two-grooved milkvetch and western wheatgrass from selenomethionine, selenocystine, and selenite

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Abstract

Selenium (Se) occurs in various forms in soils, including inorganic selenite and selenate and organic selenomethionine. Plant uptake of the inorganic, but not the organic forms, has been studied extensively. Organic-Se uptake was therefore examined in two-grooved milkvetch (*Astragalus bisulcatus* (Hook.) Gray), a Se-accumulating forb, and western wheatgrass (*Pascopyrum smithii* (Rydb.) Löve), a non-Se accumulating grass. Plants were grown for 56 days in nutrient culture enriched with 1 or 2 mg Se liter⁻¹ as sodium selenite or 0.3 or 0.6 mg Se liter⁻¹ as Se-DL-methionine or Se-DL-cystine. Growth was not affected by the Se treatments. Selenium concentrations in shoots were proportional to nutrient-solution concentrations for both species grown in sodium selenite and selenocystine, and for wheatgrass when grown in selenomethionine. Selenium concentrations in milkvetch were

not increased by the higher concentration of selenomethionine. Shoots of milkvetch, growing in the low-Se treatment contained 243, 283, and 47 $\mu\text{g Se g}^{-1}$, for the sodium selenite, selenomethionine, and selenocystine treatments, respectively, whereas values for the wheatgrass were 20, 32, and 17. Shoot:root Se concentrations were 1.2, 0.7, and 0.4 in milkvetch and 0.1, 0.5, and 0.1 in wheatgrass for the sodium selenite, selenomethionine, and selenocystine, respectively. Selenium is more readily transported to shoots in the accumulator plant, or conversely; there is a barrier to Se movement to shoots in the nonaccumulator plant. Wheatgrass contained sufficient Se to be of concern in animal toxicosis and because of greater dry matter yield accumulated as much or more Se than did the milkvetch.

Key Words: *Astragalus bisulcatus*, *Pascopyrum smithii*, hydroponics, nutrient culture, selenosis, uptake

Plant species differ in the amounts and concentrations of Se which they potentially absorb. Thus, they are classified as excluders, passive absorbers, or accumulators, if they usually absorb less

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than 50, 50 to 100, or more than 100 $\mu\text{g Se g}^{-1}$, respectively (Mayland et al. 1989, 1990). Often, the plants are simply referred to as nonaccumulators or accumulators. The actual Se uptake is controlled not only by the plant species, but also by the activity of the various Se forms in the soil and the amount of soil water present.

Most soils contain no more than 0.1 $\mu\text{g Se g}^{-1}$, but those derived from the Cretaceous shales may contain 1 to 2 $\mu\text{g g}^{-1}$ and some may have values as high as 500 $\mu\text{g g}^{-1}$ (Mayland et al. 1989). Soil Se exists in several chemical forms that differ widely in their solubility and availability to plants (Mayland et al. 1989, 1991). These forms include selenide (Se^{2-}), elemental Se (Se^0), selenite (Se^{4+}), selenate (Se^{6+}), and organic forms. Most of the plant-available soil Se occurs as selenate and selenite. Plant uptake from these 2 sources has been investigated extensively and results have been summarized by Mayland et al. (1989, 1991) and Mikkelsen et al. (1987, 1989).

Accumulator plants retain the absorbed Se as water-soluble selenite and nonprotein organic forms (Brown and Shrift 1981). Nonaccumulator plants metabolize much of the Se into protein-bound selenomethionine or selenocystine (Olson et al. 1970, Yasumoto et al. 1988). Organic Se may be volatilized from shoots, actively excreted from roots of growing plants, or mineralized from decaying vegetation (Abrams et al. 1990a). Thus it is not surprising that organic Se forms have been found in soils (Abrams et al. 1990a).

Plant uptake of organic Se has rarely been reported. Hamilton and Beath (1963a, 1963b) noted that many plant species absorbed 'organic' Se from *Astragalus* plants which had been dried, finely ground, and mixed with the potting soil. The organic forms were not verified and may have been largely inorganic (Mayland et al. 1991). Abrams et al. (1990b) reported that selenomethionine was absorbed by wheat (*Triticum aestivum*) via an active metabolic process. The present study was conducted to learn more about organic-Se availability to plants. The specific objective was to

determine Se uptake by both an accumulator and nonaccumulator plant when the Se was provided as selenomethionine or selenocystine.

Materials and Methods

The uptake of several Se compounds was determined in both a passive absorber, western wheatgrass, and an active accumulator, two-grooved milkvetch. The wheatgrass seed was obtained from the USDA-ARS Forage and Range Laboratory, Logan, Ut., and milkvetch seed was obtained from the USDA-ARS Western Regional Plant Introduction Station, Pullman, Wash. Seeds from each source were germinated in vermiculite in the greenhouse.

Seedlings were transferred to 4-liter pots containing continuously aerated Hoagland's (Hoagland and Arnon 1950) solution, modified by the addition of iron chelate HEDTA. Plants were held upright by styrofoam plugs placed in each of 2, 2-cm I.D. holes cut into each polyethylene pot cover. The pots and lids were covered with aluminum foil to exclude light. After a two-week establishment period, the nutrient solution was changed and Se treatments added. The study was conducted in a greenhouse without supplementary lighting.

The first experiment, conducted in February and March, included the following treatments: (1) Hoagland's as control, (2) Hoagland's plus 1 or 2 mg Se liter⁻¹ (12.7 or 25.3 μM) as sodium selenite, and (3) Hoagland's plus 0.3 or 0.6 mg Se liter⁻¹ (3.8 or 7.6 μM) as seleno-DL-methionine. Seedlings of both species were in the 2- to 4-leaf stage.

The second experiment was conducted like the first, but during April and May. The wheatgrass seedlings were again at the 2-leaf stage, while the milkvetch seedlings were at 4- to 6-leaf stage. This experiment included the following treatments: (1) Hoagland's as control and (2) Hoagland's plus 0.3 or 0.6 mg Se liter⁻¹ (3.8 or 7.6 μM) as seleno-DL-cystine.

Pots containing wheatgrass were spatially separated from those containing milkvetch, but all were on the same bench in the green-

Table 1. Least-squares arithmetic means of dry matter yield, selenium concentration and selenium uptake and selected treatment contrasts for two-grooved milkvetch and western wheatgrass grown in nutrient solutions containing sodium selenite (Na_2SeO_3) or selenomethionine.

Source	Dry matter yield		Se conc.		Se uptake		
	Tops	Roots	Tops	Roots	Tops	Roots	Total
	----- g 4-plants ⁻¹ -----		----- μg g ⁻¹ -----		----- μg 4- plants ⁻¹ -----		
<u>Two-grooved milkvetch</u>							
Control	1.51	1.44	0.4	0.3	0.6	0.5	1
Na ₂ SeO ₃ , 1 mg SeL ⁻¹	0.88	0.89	243	202	222	189	411
Na ₂ SeO ₃ , 2 mg SeL ⁻¹	1.25	1.18	510	407	622	481	1100
SeMeth., 0.3 mg SeL ⁻¹	1.49	1.39	283	350	392	423	815
SeMeth., 0.6 mg SeL ⁻¹	1.53	1.28	274	428	416	529	945
<u>Contrasts</u>							
Control vs others	ns	ns	***	***	***	***	***
SeO ₃ 1 vs SeO ₃ , 2	ns	ns	**	***	**	**	**
SeMeth., 0.3 vs SeMeth., 0.6	ns	ns	ns	ns	ns	ns	ns
SeO ₃ vs SeMeth.	ns	ns	ns	*	ns	*	ns
<u>Western wheatgrass</u>							
Control	34.1	4.86	0.2	0.5	8.0	2.5	10
Na ₂ SeO ₃ , 1 mg SeL ⁻¹	36.8	5.60	20.2	187	727	1010	1740
Na ₂ SeO ₃ , 2 mg SeL ⁻¹	26.3	3.90	55.1	647	1450	2520	3970
SeMeth., 0.3 mg SeL ⁻¹	32.9	7.38	31.5	81	807	512	1320
SeMeth., 0.6 mg SeL ⁻¹	35.9	4.56	92.8	161	3200	724	3920
<u>Contrasts</u>							
Control vs others	ns	ns	***	***	***	***	***
SeO ₃ , 1 vs SeO ₃ , 2	ns	ns	***	***	*	***	***
SeMeth., 0.3 vs SeMeth.,	ns	ns	***	***	***	*	***
SeO ₃ vs SeMeth.	ns	ns	*	***	ns	***	ns

* $P \leq .05$, ** $P \leq .01$, *** $P \leq .001$, ns = not significant ($P > .05$)

house. The experimental design for each species was therefore a randomized complete block with 5 replications and 5 (Expt. I) or 3 (Expt. II) treatments. There were 2 pots (4 plants) per treatment. Nutrient solution volumes were regularly restored by adding the modified Hoagland's solution.

After 8-weeks growth in the selenium cultures, plants were harvested by cutting the foliage just above the crown. The shoots and roots plus crowns were washed thoroughly in 1 g liter⁻¹ Prell detergent and rinsed with distilled water. The plant samples were dried at 100° C for 1 hour followed by 50° C for 24 hours in forced-air. Samples were ground by a Wiley mill to pass a 20-mesh sieve and stored in capped vials.

Plant samples were digested in 3:1 nitric:perchloric acid and Se was determined fluorometrically (Olson et al. 1972). Laboratory recovery and precision were characterized as 1.02 ± 0.08 mg Se kg⁻¹ for NIST wheat flour #1567 (certified at 1.1 ± 0.02) and 0.72 ± 0.02 mg Se kg⁻¹ for NIST Bovine Liver #1577 (certified at 0.71 ± 0.07), respectively. These values are provided to verify our laboratory analysis of Se.

Data for each experiment were tested by analysis of variance using GLM and orthogonal contrasts (SAS PC version 6.03). Values were widely divergent and variances were proportional to means. Therefore raw data were transformed as log (Xi × 100) to normalize the data and equalize variances (Box et al. 1978). The transformation met the Cochran test for variance homogeneity at *P* < 0.06. Tests of significance were carried out on the transformed data and inferences were made on the arithmetic means.

The selenocystine salt was represented as 90% selenocystine and possibly 10% as other forms, unknown to the manufacturer. These were possible breakdown products appearing during manufacture or purification. Under conditions of this experiment a small, but undetermined amount of selenocystine oxidized to form elemental Se⁰ and alanine (more about this in the discussion section).

Results

Milkvetch and wheatgrass grew well in the Hoagland's nutrient solution even when it included 2 mg Se liter⁻¹ as sodium selenite or 0.6 mg Se liter⁻¹ as selenomethionine or selenocystine (Tables 1, 2). Visual symptoms of toxicosis were not observed (Mikkelsen et al. 1989) and dry matter yields of the 2 species were not significantly (*P* < 0.05) affected by the 3 Se sources.

Plants grown in the selenium cultures had higher Se concentrations in both shoot and root tissues (*P* < 0.001) and took up more (*P* < 0.001) Se than plants grown in control solutions (Tables 1, 2). Both species had greater (*P* < 0.01) Se concentrations and greater (*P* < 0.05) Se uptake when grown in the 2 than 1 mg Se liter⁻¹ sodium selenite solution. Wheatgrass absorbed more (*P* < 0.05) Se from the high than from the low selenomethionine culture and more Se was concentrated in shoots and the total plant (*P* < 0.01) from the high than from the low selenocystine culture. Milkvetch did not differentiate between the low and high levels of either selenomethionine or selenocystine.

The shoot:root Se concentrations in milkvetch were about 1.2, 0.7, and 0.4 for selenite, selenomethionine, and selenocystine sources, whereas for wheatgrass they were 0.1, 0.5, and 0.1, respectively (computed from data in Table 1, 2). The Se in shoots of milkvetch was 55, 46, and 44% of total uptake from the selenite, selenomethionine, and selenocystine sources, whereas for wheatgrass the portions were 40, 72, and 41%.

When grown in the selenite and selenomethionine solutions, wheatgrass produced 15 times more mass and absorbed 3 times more Se than did milkvetch (Table 1). In the second experiment, wheatgrass plants produced only 1.6 times the mass and absorbed about one-half the amount of Se as did milkvetch at the 0.3 mg Se liter⁻¹ concentration, but about the same at the 0.6 mg Se liter⁻¹ concentration.

Milkvetch absorbed less than 10% of the inorganic Se, and 23 to 48% of the organic Se added to the nutrient solution (Table 3). Wheatgrass absorbed a larger portion of the selenite and selenomethionine from the nutrient culture than did milkvetch.

Discussion and Conclusions

We have demonstrated that both two-grooved milkvetch and western wheatgrass absorb Se from organic sources of selenomethionine and selenocystine. This finding corroborates that of Abrams et al. (1990b) who reported that cereal wheat absorbed Se-L-methionine. They showed that this was a metabolically active process which was linear to solution concentrations as high as 0.08 mg Se liter⁻¹ (1.0 μM). Our data on selenomethionine uptake by wheatgrass extends that linearity to solution concentrations of 0.6 mg Se liter⁻¹. However, the selenomethionine-uptake mechanism

Table 2. Least-squares arithmetic means of dry matter yield, selenium concentration and selenium uptake and selected treatment contrasts for two-grooved milkvetch and western wheatgrass grown in nutrient solutions containing selenocystine.

Source	Dry matter yield		Se conc.		Se uptake		
	Tops	Roots	Tops	Roots	Tops	Roots	Total
	---- g 4-plants ⁻¹ ----		----- μg g ⁻¹ -----		----- μg 4- plants ⁻¹ -----		
<u>Two-grooved milkvetch</u>							
Control	7.91	3.32	1.5	1.9	15	6	20
SeCyst., 0.3 mg Se/L	9.88	4.40	46.8	124	454	550	1010
SeCyst., 0.6 mg Se/L	5.86	3.32	95.2	222	547	689	1240
<u>Contrasts</u>							
Control vs SeCyst.	ns	ns	***	***	***	***	***
SeCyst., 0.3 vs SeCyst., 0.6	ns	ns	ns	ns	ns	ns	ns
<u>Western wheatgrass</u>							
Control	16.4	3.08	.8	1.4	13	5	18
SeCyst., 0.3 mg Se/L	12.7	1.88	17.4	158	181	277	458
SeCyst., 0.6 mg Se/L	17.4	3.06	28.6	220	484	657	1140
<u>Contrasts</u>							
Control vs SeCyst.	ns	ns	***	***	***	***	***
SeCyst., 0.3 vs SeCyst., 0.6	ns	ns	ns	ns	***	ns	**

P* ≤ .05, *P* ≤ .01, ****P* ≤ .001, ns = not significant (*P* > .05)

Table 3. Relative selenium uptake by plants from nutrient solution.

Source	Se Amount added	Relative recovery	
		Two-grooved milkvetch	Western wheatgrass
	μg nd†	—	—
Control			
Na ₂ SeO ₃	7,000	6	25
Na ₂ SeO ₃	14,000	8	28
Se Methionine	2,100	39	63
Se Methionine	4,200	23	93
Se Cystine	2,100	48	22
Se Cystine	4,200	29	27

†not detectable

for milkvetch was saturated at 0.3 mg Se liter⁻¹. Unlike the non-accumulators, milkvetch and other accumulator plants do not synthesize Se-methylselenomethionine and do not incorporate Se into protein (summary by Mayland et al. 1989, 1991). Perhaps the accumulator plants do not have an active process for selenomethionine absorption or the process may be saturated at lower concentrations than for nonaccumulator plants.

Milkvetch and wheatgrass had about twice the Se concentration when grown in the 0.6 mg Se liter⁻¹-selenocystine, as when grown in the 0.3 mg Se liter⁻¹ culture solutions. Selenocystine is subject to decomposition, forming red Se⁰ and alanine. Traces of the red Se⁰ were observed in milkvetch roots, but not on wheatgrass roots nor on any of the solution-culture containers. The wash and rinse process removed little of the red Se⁰ and it subsequently became a component of total-root Se. However, root-Se concentrations in both milkvetch and wheatgrass were similar.

Selenite is the second most common form of Se absorbed by plants under most field situations. Selenium concentrations in plants grown on selenite treated soils are generally an order of magnitude less than plants grown on selenate treatments (Banuelos and Meek 1990, Broyer et al. 1972). When growing in the selenite solution, the roots and shoots of both plant species contained Se concentrations that were roughly proportional to those in the 2 nutrient solutions.

Selenium concentrations in shoots are often substantially lower than in roots when Se is supplied in the culture medium of plants (Johnson et al. 1967). The shoot:root ⁷⁵Se values for a large number of nonaccumulator plants ranged from 0.04 to 0.33 (Johnson et al. 1967). Peterson and Butler (1962) examined ⁷⁵Se-selenite uptake by 4 nonaccumulators and 1 accumulator and found that the Se concentrations in shoots:roots ranged from 0.02 to 0.47. Both studies utilized ⁷⁵Se and were of limited duration (minutes or hours). If the growth period is extended to days or weeks, Se concentrations in shoots might exceed those found in roots. That apparently was the case reported by Rosenfeld and Beath (1964) for 11 accumulator plants grown in the field where shoot/root Se values ranged from 0.33 to 44 with a value of 9 for the two-grooved milkvetch.

In our study, plants were grown in the selenium cultures for 56 days. By then Se concentrations in shoots vs that in roots were approximately 1.2, 0.7, and 0.4 in milkvetch and 0.1, 0.5, and 0.1 in wheatgrass for sodium selenite, selenomethionine, and selenocystine, respectively. This illustrates that Se is more readily transported to shoots in accumulator plants, or conversely, that there is some restriction to Se transport from roots to shoots in non-accumulators. This study was conducted with a depleting supply of Se in the culture solution. The net accumulation of Se might have been higher if a constant supply of Se had been maintained in the culture solution.

The experimental design did not allow an F-test for an overall or robust comparison of Se sources and plant species. The several

experiments are not statistically comparable. However, we believe that the bio-availability of Se was best determined as selenomethionine > selenocystine = sodium selenite for wheatgrass and two-grooved milkvetch. The greater availability of selenomethionine is supported by Besser et al. (1989), who showed a preferential bioaccumulation of selenomethionine to selenite and selenate in aquatic systems.

The 2 species were spatially separated, because it was initially assumed that milkvetch plants would volatilize considerable amounts of Se which would be absorbed by the wheatgrass foliage (Zieve and Peterson 1984a, 1984b). The volatile-dimethylselenide aroma was smelled in the experimental area, especially near the milkvetch plants. Some volatile Se was likely absorbed through the foliage and likely contributed to background-Se levels measured in the control plants of both species. The potential contamination led us to spatially separate the two-grooved milkvetch from the wheatgrass. In retrospect, randomizing the 2 species would have likely resulted in much less biological confounding than anticipated, and would have allowed for a valid statistical comparison among species.

Another, and perhaps more serious concern, is that manufactured forms of seleno-DL-methionine and seleno-DL-cystine were used in this study. The 2 amino acids contained equimolar amounts of the 2 stereoisomers, as measured by zero optical rotation (Personal communication, Sigma Chemical Technical Services). Unlike chemical syntheses which lead to mixtures of D and L forms, biosynthetic processes produce predominately the L isomer (Adelberg and Magee 1987). However, more recent evidence has demonstrated the existence of enzymes that employ both stereoisomers of some amino acids as substrate (Robinson 1976, Kavanaugh et al. 1990). Thus both stereoisomers of selenocystine and selenomethionine might be absorbed, or the D isomer could undergo racemization to the L isomer and then be fully metabolized by processes previously thought restricted to the L isomer. Wheatgrass absorbed and transported a large amount of Se from the selenomethionine source to the plant shoots. A minimal 30% of this was provided as Se-D-methionine in the nutrient solution. Selenocystine uptake by both plants and selenomethionine uptake by milkvetch accounted for less than half of the Se provided. In this latter case, it is not known whether any of the absorbed Se was associated with the D-amino acid. Considerable research must be done before these processes can be fully understood.

In summary, both the non-Se accumulator and the Se accumulator absorbed Se from the inorganic selenite and the organic selenomethionine. The Se was transported to shoots of two-grooved and selenocystine milkvetch at greater rates than found for wheatgrass. We interpreted this as some interference at the root:shoot interface of non-Se accumulators which restricted Se transport to shoots.

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