SELENIUM UPTAKE BY FOUR-WING SALTBUSH AND YELLOW SWEET CLOVER AS INFLUENCED BY VESICULAR-ARBUSCULAR MYCORRHIZAE[1]

Patsy L. Wanek, George F. Vance, and Stephen E. Williams[2]

Abstract. Both four-wing saltbush and sweet clover have been shown in previous studies to accumulate moderate amounts of selenium when grown in seleniferous soils. Because these plants are used as forage by both wildlife and domestic livestock, and four-wing saltbush is a shrub used in reclamation plantings, knowledge of factors which influence the uptake of selenium by these plants would be useful. A greenhouse study was undertaken to measure the effect of root infection by vesicular-arbuscular mycorrhizae (VAM) on plant selenium uptake. Yellow sweet clover easily forms associations with VAM fungi; four-wing saltbush has been shown to become mycorrhizal as well. Both plant species are capable of growing without VAM, so the effect of the VAM can be measured in a controlled setting. A greenhouse pot experiment used a seleniferous soil containing native mycorrhizal propagules obtained near Laramie, Wyoming. Additional selenium was added to the pots as sodium selenite or sodium selenate. Four-wing saltbush and yellow sweet clover were grown in the soil, using steamed soil as a VAM control. Extent of establishment of vesicular-arbuscular mycorrhizal associations, levels of plant selenium, and levels of soil selenium were measured. The presence of mycorrhizal associations are expected to enhance plant uptake of selenium from seleniferous soils by the increased root absorption area created by the associated mycelia, and demonstration of the association of mycorrhizae with elevated plant selenium levels can then possibly elucidate some of the selenium bioaccumulation behavior of these plant species.

Additional Key Words: reclamation, establishment

Introduction

Selenium was identified at least as early as the 1930's as the element responsible for toxic syndromes known as "alkali disease" and "blind staggers" found in Wyoming livestock (Knight and Beath, 1937; Beath et al., 1953). Since then, many studies have tried to elucidate the soil-plant relationships of selenium, because plant accumulation of selenium from the soil is ultimately responsible for the animal toxicity potential of selenium. Selenium is analogous to sulfur and consequently is associated with sulfur both geologically and biologically. The chemical separation of selenium and sulfur occurs as a result of their different melting points, boiling points, and oxidation potentials (McNeal and Balistrieri, 1989). Selenium, like iron, is very sensitive to pH changes, and, like iron, it occurs in higher oxidation states as the alkalinity and oxidation state of the environment increase. In the Se^{4+} form, selenium is found as the selenite anion (SeO_3^{2-}), which has a high adsorption affinity for clay minerals, iron oxides, and negatively charged organic compounds. As the result of weathering processes, selenium is concentrated mostly in secondary minerals, such as those found in sedimentary rocks. Selenite is therefore found in fine-grained sediments such as shales and often in coal-bearing formations (Mayland et al., 1989).

When oxidized to the selenate ion (SeO_4^{2-}), selenium adsorption decreases, and the element becomes mobile, potentially becoming an environmental hazard. Soluble selenium mobility is known to increase with increasing alkalinity (McNeal and Balistrieri, 1989; Tisdale et al., 1985). As the concentration of soluble selenate increases, the plant availability of selenium proportionally increases. Plant uptake of selenium generally depends on the plant type and several soil chemical factors: the selenium concentration in the soil; availability; soil pH and salinity; and the soil's chemical content, such as calcium carbonate, sulfate, or phosphorous levels (McNeal and Balistrieri, 1989; Tisdale et al., 1985).

---

[1]Paper presented at the 1995 National Meeting of the American Society for Surface Mining and Reclamation, Gillette, Wyoming, June 5-8, 1995.

[2]Patsy L. Wanek, Graduate Assistant, George F. Vance, Associate Professor, Stephen E. Williams, Professor, Department of Plant, Soil and Insect Sciences, University of Wyoming, Laramie, WY 82071-3354.

Proceedings America Society of Mining and Reclamation, 1995 pp 309-316
DOI: 10.21000/JASMR95010309

Selenium can substitute biologically for sulfur in organic molecules such as the amino acids cystine and methionine. These selenium analogs can subsequently be used to build proteins in some plants (Mayland et al., 1989). Within the biological pH range, ionization states of sulfur and selenium differ. This difference contributes to selenium analogs' toxicity and dysfunctional ability to compete with sulfur in normal biological metabolism (Combs and Combs, 1986).

Researchers have categorized plants into three groups of plants according to their tendency to concentrate selenium in their tissues (Mayland et al., 1989). Genera identified as having in excess of 1000 mg kg^{-1} are termed "primary indicators". These genera are *Astragalus, Machaeranthera, Haplopappus, Xylorhiza,* and *Stanleya.* However, not all member species of the above-named genera are such accumulators. Plants which typically concentrate selenium from 50-100 mg kg^{-1} are termed secondary selenium absorbers; genera include *Aster, Astragalus, Atriplex, Castilleja, Grindelia, Gutierrezia, Machaeranthera,* and *Mentzelia.* Plants in the third group, tending to accumulate <50 mg kg^{-1} selenium, are *Trifolium*, grains, and grasses. Plants in the genus *Melilotus*, including the yellow sweet clover used in this study, are considered within the third group, based on data from an Abandoned Mine Lands study at the University of Wyoming (unpublished). For non-accumulators of selenium, the element is toxic to the plant to varying degrees (Beath et al., 1953).

In addition to plant taxonomic variability for concentrating selenium, there is variability between plants. Expected causes of differential plant selenium uptake are variation in selenium availability, spatial variability of adsorbing minerals, the nutritional status of the plant, or physical plant factors such as root depth.

Types of rhizosphere microflora might also explain individual plant variability. A key factor in this regard would be the presence of a plant-root association known as vesicular-arbuscular mycorrhizae (VAM). VAM associations have long been known to influence the uptake of phosphorous from the soil (Gianinazzi-Pearson and Gianinazzi, 1981). More recently, their role in water uptake has been reported (Ellis et al. (1985) as reported in Sylvia and Williams (1992)). In addition, VAM have been shown to increase the uptake of non-nutrient elements such as cesium and cobalt (Rogers and Williams, 1986). Mycorrhizae may also play a role when the plant is physiologically stressed (Sylvia and Williams, 1992). Therefore, a possible relationship between VAM colonization of the root and plant selenium uptake may exist. If there is such a relationship, at least one component of plant bioaccumulation behavior and variation in selenium uptake might be explained.

Our research tested two plant species that differed in physiology, extent of bioaccumulation of selenium, and facility for becoming colonized by vesicular-arbuscular mycorrhizal fungi. Four-wing saltbush (*Atriplex canescens* (Pursh) Nutt.) is a halophytic, or salt-loving, shrub characteristic of semi-arid sandy hills and plains. It is a member of the family Chenopodiaceae, a family which some references list as entirely non-mycorrhizal (Koch (1961) as reported in Williams and Aldon (1976)). Yet, Williams and Aldon (1976) reported *Atriplex canescens* to be endomycorrhizal in field conditions. The second plant species tested was yellow sweet clover (*Melilotus officinalis* (L.) Pallas). Yellow sweet clover readily forms symbiotic relationships with VAM fungi, as well as with a nitrogen-fixing bacterium.

The root zone is very complex, involving interactions of the root, microflora such as bacteria and saprophytic fungi, microarthropods, protozoa, and VAM fungi. Population interactions exist that complicate measurement of single component effects, such as the VAM fungi, without perturbing the rest of the system. This study tested the separation of single component effects. To keep perturbation of the soil ecosystem to a minimum, the indigenous soil components were used as much as possible in the research.

Materials and Methods

The experiment consisted of two soil treatments (steamed and not steamed), two levels of VAM (with and without a root propagule supplement), two plants (yellow sweet clover and four-wing saltbush), a no-plant control, and three levels of selenium (additional selenite, additional selenate and a control with no added selenium) for a combined total of 36 treatments, replicated five times.

Soil was collected from an area 3 meters on a side and to a depth of 40 cm from a pasture site across the highway from the Laramie, Wyoming, airport. The site was selected because tine-leafed milk vetch (*Astragalus pectinatus*), a plant known to grow optimally in seleniferous soils, was growing on the site, indicating the presence of native soil selenium. The soil was transported to the laboratory, where plant material, mostly grasses, was removed. Rocks, gravel, and large chunks of plant material were discarded. All soil was sieved while partially damp to pass a 2 mm sieve. The fraction slightly greater than 2mm, consisting mostly of soil aggregates and plant roots, was collected separately to serve as part of a VAM inoculum.

The sieved soil was split into two portions with the aid of a riffle box. One portion was steamed in a Pro-Soil floor steamer with a temperature setting of 200°F (93°C) for 14 hours to destroy any VAM propagules (Garbaye et al., 1992). Actual temperatures measured during steaming varied from 65 to 80°C. All utensils used in subsequent handling of the steamed soil were rinsed with a 1:10 dilution of bleach. Both steamed and non-steamed soils were again split into two portions each.

Plastic pots (20 cm dia.), previously plugged with polypropylene cloth to prevent soil leakage, were filled with 5 kg of the appropriate soil. The filled pots, placed on plastic saucers to prevent loss of irrigation water, were randomly arranged on a greenhouse bench.

Stock solutions of selenium as sodium selenite and sodium selenate were prepared at a concentration of 1.9 x 10^{-2} M selenium. A 10-mL portion of the appropriate solution was added to approximately 300 mL of deionized water in a beaker. This solution was slowly poured onto the surface of air-dried soil in pots to be treated with additional selenium. The beaker was rinsed with an additional 200 mL of deionized water, and the rinsate was also poured onto the soil surface. Total selenium added was 15 mg Se/pot, or 3 mg Se kg^{-1} soil. Deionized water (500 mL) was added in the place of the selenium solution to one-third of the pots to serve as a control.

A mixture of the greater than 2mm aggregate-plant mixture was mixed with deionized water in the proportion of 5 kg soil to 4.25 liters water to create a slurry. Roots were removed by skimming, and the slurry was poured through a 53-mm (270 mesh) sieve. This sieve size would retain VAM spores, while allowing microorganisms such as bacteria and spores of saprophytic soil fungi to pass through. The sieved slurry was mixed, and 500-mL portions were added to all pots containing steamed soil, in an effort to restore the original microflora of the soil, with the exception of the VAM population.

An additional source of viable VAM propagules (roots of plant species known to form mycorrhizal associations) was collected from an area proximal to the original collection site to ensure microbial adaptation to similar edaphic and seleniferous conditions. The presence of VAM spores was confirmed by staining a sample of collected roots, using the lactic acid-carbol fuchsin method (Kormanik et al., 1980). The freshly collected and air-dried roots were combined with skimmed, washed, and air dried roots from the slurry preparation in order to increase the total volume of available root inoculum. The final root collection consisted of approximately two-thirds washed and one-third freshly collected roots. The root mixture was ground through a 20-mesh screen in a Wiley mill. Roots treated in this way retained their VAM propagule viability potential (Menge and Timmer, 1982). Individual 7.5-g portions of the ground roots were added to the appropriate pots as a VAM supplement.

De-winged fruits (commonly referred to as "seeds") of *Atriplex canescens* v. panaca from a Utah source were used in this experiment. An inoculum of one hundred seeds per pot was used to compensate for the low germination rate of this shrub. Individual polypropylene bags containing the 100-seed inoculum were then closed by stitching the open end. Bags were immersed for 10 minutes in a 1:5 dilution of bleach solution to surface-sterilize the seeds, then were thoroughly rinsed with sterile, deionized water. The bags were placed on sterile filter papers in sterile Petri dishes to dry at room temperature prior to planting. A drying period of 7 days was used, as recommended by Springfield (1970).

Yellow sweet clover seeds were soaked in similar bags (150 seeds per bag) in a 1:5 bleach solution for 1 minute, followed by a sterile, deionized water rinse. The bags were placed in sterile Petri dishes. Sweet clover seeds were removed individually for planting with forceps rinsed in 75% alcohol.

At the time of planting, two portions of soil, of approximately 1 liter and 200 mL each, were removed from each pot that was to receive ground root (VAM supplement) inoculum. The pre-weighed portion (7.5 g) of the roots was placed on top of the soil remaining in the pot and the 1-liter portion of soil replaced. A 500-mL portion of water was added to the pot. Then the seeds (100 saltbush seeds or 10 sweet clover seeds per pot) were placed on the soil surface and were lightly pressed into the soil. The remaining 200-mL portion of soil was used as a seed cover and provided a seed burial depth of about 0.5 cm. Only the 200-mL portion of soil was removed from pots which were not to receive the root inoculum. The seeds were then planted as described above. All pots received a total of 500 mL of water.

After planting, all pots (including the no-plant controls) were watered daily with deionized water. Water was added to cover the soil, but an attempt was made to avoid leaching. Additional lighting (6000W provided by mercury vapor lamps) of 3 hours per day was provided at three weeks after planting to assure 12 hours per day of light. The pots were randomized and positions changed weekly to nullify differences due to various microenvironments in the greenhouse.

Results and Discussion

Results reported in this paper are based primarily on observable plant responses to the various treatments. Some plant responses currently observed are indicative of plant selenium uptake and toxicity. Plants have been harvested and their tissues are being analyzed for selenium content and roots stained to assess the presence of mycorrhizae. The selenium content of the soils, including both the reduced selenite and selenate forms, are also being analyzed.

First, the germination rates of the two plants, yellow sweet clover and four-wing saltbush, did not appear to be affected by added selenium. Table I lists germination rates for four-wing saltbush, tabulated as the maximum number of plants present in a pot at any time during the one-month period after planting. Table II shows similar data for yellow sweet clover. There appears to be no substantial difference in germination rates among the 12 treatments in either plant species, although non-steamed soil may offer a slight germination advantage for the saltbush, and addition of roots may enhance the yellow sweet clover germination.

Table I. Germination percentage rates of four-wing saltbush at one month after planting.

	Selenium Added		
Treatment	0	Se^{4+}	Se^{6+}
		---per cent---	
Steamed			
w/roots	5.4	13.4	8.0
w/o roots	10.8	7.8	7.4
Not Steamed			
w/roots	10.0	15.4	10.2
w/o roots	9.2	11.4	9.2

After a three-week growing period from planting date, it was apparent that the selenate-treated soil was toxic to yellow sweet clover. Although seedlings had appeared, they were arrested in growth at a two- to three-leaf stage

and eventually they died. At approximately six weeks following planting, toxic signs appeared in the saltbush seedlings growing in selenate-treated soil. They too showed arrested growth and many died. The survivors were stunted, had dried-looking leaf edges, and sometimes had the vestiges of the fruiting structures attached instead of shedding them as the leaves opened. No chlorosis was observed in either plant species.

Table II. Germination percentage rates of yellow sweet clover at one month after planting.

	Selenium Added		
Treatment	0	Se^{4+}	Se^{6+}
		---per cent---	
Steamed			
w/roots	58	52	50
w/o roots	52	42	52
Not Steamed			
w/roots	58	44	54
w/o roots	46	48	50

Tables III and IV list survival rates for four-wing saltbush and yellow sweet clover, respectively, in percentage of viable plants at harvest, 17-20 weeks after plant date, compared to the maximum number of seedlings per pot during the experiment. Although there appears to be a survival advantage associated with the root inoculum for sweet clover in the presence of selenite, there seems to be no such advantage afforded the saltbush. The highest percentage of survivors of either plant type grown in selenate-amended soil was found in steamed soil with the root supplement.

Table III. Survival rates for four-wing saltbush at harvest.

	Selenium Added		
Treatment	0	Se^{4+}	Se^{6+}
		---per cent---	
Steamed			
w/roots	100.0	97.8	23.7
w/o roots	98.0	97.1	3.3
Not Steamed			
w/roots	100.0	100.0	12.9
w/o roots	100.0	99.8	15.3

Table IV. Survival rates for yellow sweet clover at harvest.

Treatment	Selenium Added		
	0	Se^{4+}	Se^{6+}
		---per cent---	
Steamed			
w/roots	89.3	97.1	11.7
w/o roots	96.0	66.0	0.0
Not Steamed			
w/roots	97.5	100.0	0.0
w/o roots	85.5	93.3	0.0

The average heights of all saltbush and clover plants for each treatment at the termination of the experiment are listed in Tables V and VI.

Table V. Average heights (cm) for four-wing saltbush at harvest.

Treatment	Selenium Added		
	0	Se^{4+}	Se^{6+}
Steamed			
w/roots	23.3 ± 4.70	22.7 ± 2.94	2.3 ± 0.74
w/o roots	24.6 ± 3.13	22.2 ± 3.88	0.3
Not Steamed			
w/roots	13.3 ± 1.73	10.7 ± 2.22	1.6 ± 0.86
w/o roots	15.1 ± 0.52	12.2 ± 0.91	1.8 ± 0.37

By the time plant heights were measured, all sweet clover plants that had received the selenate treatment had died, except for three plants in steamed soil which also had received the root inoculum. The saltbush plants that had received the selenate treatment were stunted and reduced in number after eight weeks. However, there were some survivors in each treatment category. This result indicates that the saltbush plants are less susceptible to selenium toxicity than are the sweet clover plants. The extent of the vigor loss was unexpected. Davis (1972) grew 15 species of *Atriplex* in a greenhouse study to measure selenium bioaccumulation. A total of 18 mg Se kg^{-1} soil was added as sodium selenate to the pots over the course of the experiment, without any evidence of toxicity. However, the selenium was added to six-week-old plants growing in a non-seleniferous soil, in a possibly less sensitive part of their growth cycle.

Table VI. Average heights (cm) for yellow sweet clover at harvest.

Treatment	Selenium Added		
	0	Se^{4+}	Se^{6+}
Steamed			
w/roots	5.4 ± 1.85	4.6 ± 1.36	2.9 ± 0.071
w/o roots	3.7 ± 1.09	4.4 ± 1.31	0.0
Not Steamed			
w/roots	7.3 ± 1.27	6.1 ± 0.97	0.0
w/o roots	7.9 ± 3.13	7.5 ± 1.21	0.0

We expected, based on the reported selenite adsorption to soil and decreased availability to plants, that plants grown in soil amended with the selenite form of selenium would be more vigorous than plants grown on selenate-treated soils. This indeed was the case. Saltbush plants grown in soils with added selenite were less vigorous, based on plant height, than their corresponding no-selenium-added controls for the non-steamed soil or the steam-treated soil without root supplements; however, in the pots containing added root inoculum and either steamed or non-steamed soil, the plants in soil with selenite grew as well or better than their control counterparts. The noticeable difference between response in steamed vs. non-steamed soil with no selenium addition or with added selenite could be due either to loss of native selenium during the steaming process or to destruction of a root pathogen, killed by steaming, but not added back with the slurry. Comparison of treatments in the selenate-amended soils cannot be evaluated, since so few plants survived.

There was essentially no difference in growth response of yellow sweet clover in the selenite-amended soil compared with its no-selenium-added control.

These results pose many unanswered questions as to the involvement of mycorrhizal associations in the uptake of selenium. Some answers may become evident after the soils and plant shoots are analyzed for selenium content and the roots are evaluated for existence of mycorrhizal colonization.

The root-soil environment is complex. This experiment does not directly address another important plant-microbial relationship, namely, that of the symbiosis between a legume such as yellow sweet clover and nitrogen-fixing bacteria. Wu et al. (1994) demonstrated that there is a relationship with selenium between nitrogen fixation symbiotic activity and *Melilotus indica*, an annual legume species. An investigation of the three components -- plant, nitrogen-fixing bacteria, and VAM -- should be the subject of a future study.

References

Beath, O. A. , C. S. Gilbert, H. F. Eppson, and I. Rosenfeld. 1953. Poisonous plants and livestock poisoning. Agricultural Experiment Station Bull. 324, University of Wyoming., Laramie, WY.

Combs, G. F., Jr. & S. B. Combs. 1986. The Role of Selenium in Nutrition. Academic Press, New York, NY.

Davis, A. M. 1972. Selenium accumulation in a collection of *Atriplex* species. Agron. J. 64:823-824.
http://dx.doi.org/10.2134/agronj1972.00021962006400060037x

Ellis, J. R., H. J. Larsen, and M. G. Boosalis. 1985. Drought resistance of wheat plants inoculated with vesicular-arbuscular mycorrhizae. Plant Soil 86:369-378.
http://dx.doi.org/10.1007/BF02145457

Garbaye, J, J. L. Churin, and R. Duponnois. 1992. Effects of substrate sterilization, fungicide treatment, and mycorrhization helper bacteria on ectomycorrhizal formation of pedunculate oak (*Quercus robur*) inoculated with *Laccaria laccata* in two peat bare-root nurseries. Biol. Fertil. Soils 13:55-57.
http://dx.doi.org/10.1007/BF00337239

Gianinazzi-Pearson, V., and S. Gianinazzi. 1981. Role of endomycorrhizal fungi in phosphorous cycling in the ecosystem. In Wicklow, D. T., and G. C. Carroll (eds). The Fungal Community, Chap. 33. Marcel Dekker, Inc., New York.

Koch, H. 1961. Untersuchungen uber die Mycorrhiza der Kulturpflanzen unter besonderer Beruchsichtigung von *Althaea officinalis* L., *Helianthus annuus* L. und *Solanum lycopersicum* L. Gartenbauwissenshaft 26:5-32.

Knight, S.H. and O. A. Beath. 1937. The occurrence of selenium and seleniferous vegetation in Wyoming. Agricultural Experiment Station Bulletin 221, University of Wyoming, Laramie, WY.

Kormanik, P. P., W. C. Bryan, and R. C. Schultz. 1980. Procedures and equipment for staining large numbers of plant root samples for endomycorrhizal assay. Can. J. of Microbiol. 26:536-538.
http://dx.doi.org/10.1139/m80-090

Mayland, J. F., L. F. James, K. E. Panter, and J. L. Sondregger. 1989. Selenium in seleniferous environments. In L. W. Jacobs (ed.) Selenium in Agriculture and the Environment, Chap. 2. Soil Sci. Soc. Amer. Spec Publ. No. 23, Am. Soc. of Agron., Madison, WI.

McNeal, J. M. and L. S. Balistrieri. 1989. Geochemistry and occurrence of selenium: An overview. In L. W. Jacobs (ed.) Selenium in Agriculture and the Environment, Chap. 1 . Soil Sci. Soc. Am. Spec Publ. No. 23, Am. Soc. Agron., Madison, WI.

Menge, J. A. and L. W. Timmer. 1982. Procedures for inoculation of plants with vesicular-arbuscular mycorrhizae in the laboratory, greenhouse, and field. In N. C. Schenck (ed.) Methods and Principles of Mycorrhizal Research, Chap. 6. The Am. Phytopathological Soc., St. Paul, MN.

Rogers, R. D., and S. E. Williams. 1986. Vesicular-arbuscular mycorrhiza: influence on plant uptake of cesium and cobalt. Soil Biol. Biochem. 18:371-376.
http://dx.doi.org/10.1016/0038-0717(86)90040-4

Springfield, H. W. 1970. Germination and establishment of fourwing saltbush in the southwest. USDA Forest Service Res Paper RM-55. Rocky Mtn. Forest and Range Exp. Sta. Forest Service, US Dept of Agri., Fort Collins, CO.

Sylvia, D. M. and S. E. Williams. 1992. Vesicular-arbuscular mycorrhizae and environmental stress. In Mycorrhizae in Sustainable Agriculture, Am. Soc. Agron. Spec. Publ. no. 54, ASA/SSSA, Madison, WI.

Tisdale, S. L., W. L. Nelson, and J. D. Beaton. 1985. Soil Fertility and Fertilizers, 4th Edit., Macmillan Publ. Co., NY.

Williams, S. E., and E. F. Aldon. 1976. Endomycorrhizal (vesicular arbuscular) associations of some arid zone shrubs. The Southwestern Naturalist 20:437-444.
http://dx.doi.org/10.2307/3669860

Wu, L., A. Emberg, and J. A. Biggar. 1994. Effects of elevated selenium concentration on selenium accumulation and nitrogen fixation symbiotic activity of *Melilotus indica* L. Ecotox. Environ. Safety 27:50-63.
http://dx.doi.org/10.1006/eesa.1994.1006