Selenate Uptake by Symphyotrichum ascendens Western Aster

Nathan Hankes

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SELENATE UPTAKE BY SYMPHYOTRICUM ASCENDENS (WESTERN ASTER)

by

Nathan B. Hankes

A thesis submitted in partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE

in

Soil and Water Chemistry

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ABSTRACT

SELENATE UPTAKE BY SYMPHYOTRICHUM ASCENDENS (WESTERN ASTER)

by

Nathan Hankes, Master of Science
Utah State University, 2016

Major Professor: Dr. Paul Grossl
Department: Plants, Soils and Climate

Symphyotrichum ascendens is a native forb species responsible for selenium poisoning of livestock grazing on mine wastes in the American Intermountain West. While S. ascendens has long been assumed to be a Se accumulator species, this report details the first investigation into its Se uptake, affinity, partitioning, and growth in response to selenate fertilization. The study was performed in a hydroponic growth environment at selenate concentrations of 0.1, 0.25, 0.5, and 1.0 mM selenate. Symphyotrichum ascendens absorbed selenate in percentage concentrations, with the highest tissue concentration of 3.6% Se. Symphyotrichum ascendens exhibited signs of stress, producing 69.2% less biomass at the 1.0 mM Se treatment than in the control. Water use efficiency decreased with increased exposure to selenate while the transpiration stream concentration factor increased.

Also included is a popular audience-style article exploring the impact that S. ascendens has had on an entire rural community, while communicating the scientific principles as they pertain to the ecologic context at large. And finally, this report contains a work of poetry, included in the spirit of academia,
acknowledging the joys and wonder of scientific inquiry in a Universe as mystical as our own. As a whole, this report is a commentary on the range of possibilities within the field of science communication while, at the same time, introducing novel findings of an original scientific investigation. The information gleaned within this investigation will serve as a foundational cornerstone for future mine land reclamation efforts as they pertain to *S. ascendens*. 

(46 pages)
I would like to extend my sincere gratitude to the members of my committee. Each of you has served as an inspiration in your own way. From you I have learned being through non-being, of humility in action, and of finding balance amidst the external demands of life. For this, I am a better person.

I've enjoyed my time in Utah. The valley, with its surrounding ranges, invites a reverence for the natural systems that we've all chosen to study. The canyons and trails are where I did my best work and internalized the principles I learned in the classroom. For these natural gifts, I give thanks.

I would also like to extend my gratitude to Zane Davis for his support, enthusiasm, and guidance. To Dave Powelson, for his untiring attention to detail in teaching me proper lab etiquette and techniques. To Sandra, Justin, and Micah for their help in the lab and the greenhouse. And finally to Alec Hay, for his maverick exploits in the greenhouse.

Nathan B. Hankes
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CHAPTER 1
INTRODUCTION

Selenium (Se), a chalcogenic metalloid found in Group 16 of the periodic table, is a naturally occurring element that can be fatal to animals if ingested in high concentrations. Selenium is essential for animal health, but has one of the narrowest ranges between deficiency and toxicity of all the essential elements (Fordsyce, 2005). In Southeast Idaho’s phosphate mining region, earth-moving activities have disturbed seleniferous geologic formations, exposing previously immobile Se to oxidative weathering, thereby mobilizing Se into the environment (Knotek-Smith et al., 2006). Once mobile, the Se leaches into surface waters and soils, freely entering the biosphere where it can concentrate in toxic levels. Researchers in this region have linked fatal cases of selenium toxicity in livestock to these seleniferous mine wastes (Piper et al., 2000). The vast majority of these livestock deaths involve the ingestion of Western Aster (Symphyotrichum ascendens), a native perennial forb documented to hyper-accumulating Se.

Phosphate deposits have been mined in Idaho since the early twentieth century, but the associated selenium pollution was only discovered in 1996 after six horses were diagnosed with chronic selenosis (Knotek-Smith et al., 2006). Prior to this discovery, seleniferous waste rock was left on the surface and in some cases used as a growth medium, where it was left exposed to oxidative weathering. As of the time of this writing, these exposed seleniferous waste rocks continue to freely leach Se in regional surface and soil-pore waters.
However, many of the offending mines are now managed as Superfund sites under Administrative Orders of Consent and will one day undergo remediation. The process is slow to progress as industry and land management agencies move toward a workable remediation solution. Livestock continue to die while these entities work through the legal aspects of these remediation efforts. In the meantime, researchers at Utah State University are investigating stopgap measures that will mitigate selenium poisoning in livestock until large-scale remediation becomes a reality.

Chapters Two and Three address this issue directly. Chapter Two does so for the lay audience, instilling, hopefully, a sense of the inherent ecologic and social impacts as well as an interest in earth science. Chapter Three reports the findings of a hydroponic greenhouse study, in which the effect of selenate absorption in *S. ascendens* were investigated at selenate concentrations of 0.1, 0.25, 0.5, and 1.0 mM selenate. This was the first study to investigate the relationship between *S. ascendens* and selenium, and will serve as a foundational cornerstone for future mine land reclamation efforts as they pertain to *S. ascendens*.

Chapter Four is my swing for the fences. And it’s no less than an act of love. The scientific training I received at Utah State University taught me to view the world in a radically different way, and changed my relationship to the world. I now relate to the world in a sort of animist spirituality. I firmly believe that the arts are a viable means of instilling the wonder and the magnitude of scientific achievement to the masses. I view Chapter Four as my contribution to the sciences and humanity at large.


CHAPTER 2
SELENIUM IN THE WEST

He sets a boning knife and hacksaw on the top of his truck’s custom veterinarian box. His mood is solemn, but his demeanor suggests that if asked, he could offer a list of places he’d rather be. As he slides open a pull out drawer, I see rows of vials and syringes, mineral supplements and scalpels. He grabs a box and pulls out half a dozen shoulder-length plastic gloves, balling them into a pocket of his workmen’s overalls.

We’re standing on the side of a private ore haul road, just northeast of Soda Springs, Idaho. The mine manager and mining department lead are already in the pasture, waiting for the veterinarian to gather his tools and get on with it. Their shadows are long against a backdrop that is so quintessentially American West. Detracting from the mountainous skyline and sublime beauty of the sagebrush steppe is the bawling of hungry calves and the corpses of three cows lying where they died early that morning.

The mining personnel look nervous from their position just upwind of the bloating cows, talking strategy in muffled tones, already anticipating the negative publicity should the veterinarian’s findings confirm their suspicions. The autopsy won’t take more than a couple hours, but the implications of its findings threaten a tenuous way of life for a Southeastern Idaho mining community. We all suspect the cows got into a patch of Western aster (Symphyotrichum ascendens), a native forb known regionally to hyper-accumulate selenium brought to the surface by area
mining operations. But only a liver biopsy and tissue analysis will confirm if these deaths are yet another fatal case of acute selenosis.

***

The land will tell its history if you slow down enough to listen. And some of the earliest chapters in Idaho’s Upper Blackfoot Watershed history are the easiest to hear. The landscape here commands your attention. Mountainous ridgelines, fractures, folds, and faults tell of a past marked by tectonic upheaval and intense geologic pressures. Basaltic lava domes and flows dot the landscape, harkening back to a time when geologic growth outpaced weathering and erosion.

The sparse vegetation tells of arid summer nights. Douglas fir and Lodgepole pine congregate on north and east facing slopes, unable to survive just over the ridgeline where the sun hits at its most radiant hours. Mountain big sagebrush covers most everything that hasn’t been converted to pasture or grain production. And from a distance, the Blackfoot River is most easily identified as a serpentine corridor of maroon willows.

It’s the human imprint on the land that tells the story of a culture’s tenacity to survive. Barley, rolling underneath a crisp autumn wind, grows tight to the road’s edge. Plow lines nip at the border of lava outcrops. Fences cut through forests and over ridgelines as neat and predictable as the property lines on the Caribou County plat map. Barley and dry land wheat bring a welcome flush of green to an otherwise desiccated landscape. Forgotten homesteads rot to dust in the middle of grain fields. These hard-won wooden structures, artifacts of the earliest European pioneers, slowly return to the soil from which they originated—
a nutrient cycle delayed by decades of protective paint coatings and human interference.

Driving down the Blackfoot River Road or north along Highway 34, you’ll notice bands of pale green grass cutting through the mountainsides. You might catch a glimpse of a bare limestone cliff. These are the ruins of large-scale mechanized surface mining operations, yet another artifact of the human story as told by the watershed. The pale green strips are the result of reclamation work on exploration pads and roads, the non-native grasses standing out like tourists in a foreign land. The limestone cliffs are the remnants of each mine’s final pit high walls. All told, there are twelve such mines within the watershed (Mebane et al., 2015), two of which are currently active. The others have been mined and largely forgotten. Of these inactive mines, the state of restoration and reclamation varies widely depending on the years in which they were mined and the corresponding state of environmental regulation at the time of their closure. Of the stories the watershed can tell, mining is an obvious chapter. But this landscape holds on to some stories more tightly than others.

An astute hiker might notice a marine fossil along one of the watershed’s many Permian-era rock outcrops, an allusion to the region’s history as an ancient seabed. This area, along with the other major phosphate concentrations of the Western Phosphate Patch, was once a warm, shallow sea. Nutrient-rich runoff, processed by bacteria, precipitated phosphate-rich minerals to the sea floor where they concentrated and turned into the phosphate ore mined today (Hiatt et al., 2001).
If you’re out after a heavy rain, you might be lucky enough to find an arrowhead poking out of an eroded streambed. The human story of such a find can only be informed by the landscape out of which it was carved. British writer, Lawrence Durrell, believes that every place has an invisible constant that informs its culture (Durrell, 1997). If this is true, the Shosone flint-knapper responsible for that arrowhead relied on the same tenacity for survival that the modern day farmers, ranchers, and miners must.

The elk and deer tracks skirt fence lines and carve impossible paths through the sagebrush sea. Hunting forms the core of a man’s identity in much of the population here, and these men live for the brief season where they’re allowed to scratch the deep primal itch that modern living never will. And a walk along the watershed’s primary drainage, the Blackfoot River, reveals foot-worn pads where countless fishermen have stood to test their skill catching the river’s native Yellowstone cutthroat trout. The landscape here is alive.

There is another story told by the land, one made evident by the five-strand barbwire fences adorned by skull-and-crossbones placards. The mines in this watershed are toxic. Selenium, another artifact of the ancient warm, shallow sea, is inextricably tied to phosphate mining in this region. Between two geologic bands of rich phosphate ore lays a narrow, black band of seleniferous shale. The selenium bearing shale, once brought to the surface and exposed to aerobic conditions by mining processes, changes form from the immobile (-2) and (0) valence states to the environmentally and biologically mobile (+4) and (+6) forms (Tiwary, 2004). In this way, phosphate mining opens a pathway for the seleniferous shales to
weather and contaminate the underlying Wells Formation aquifer (Myers, 2013; Mars and Crowley, 2003). Not only that, but surface water, recharged by the aquifer or receiving runoff from the abandoned mine lands, continues to carry selenium downstream; the USGS has recorded as much as 24 kilograms of selenium entering the Blackfoot Reservoir, the terminus of the Upper Blackfoot Watershed, in a single day—though most of this contamination is limited to the spring rainy season (Mebane et al., 2015). This is concerning because in the early to mid-eighties, researchers investigating the impact of agricultural subsurface irrigation to wetland impoundments in California’s Kesterton National Wildlife Refuge found that selenium had bio-accumulated to such a degree that the health and reproductive success of wetland birds had become severely degraded, with reproductive impacts including deformities in offspring (Ohlendorf, 2002).

Selenium is an essential element for the production of many enzymes and proteins; it also supports immunity and reproduction, but can quickly become toxic, having the narrowest range between deficiency and toxicity of any element essential to mammals (Pfister et al., 2014). Water and aquatic ecosystems are not the only environmental resources imperiled by selenium’s presence in the environment.

In Idaho’s phosphate patch, no one in the mining industry or the regulatory agencies knew that selenium was a part of the mining arithmetic until the death of six horses in 1996, a full ninety years after the mountains were first exploited for their phosphate reserves (Fessler, 2003; Mebane et al., 2015). Until then, the seleniferous shale was considered a viable growth medium for reclamation and
purposely left at the surface to help establish vegetation, a decision that haunts the industry today.

The horse deaths were a simple curiosity for the miners who first reported the sickly horses. A stream, after flowing through an inactive mine, wound through the horse pasture. As the grazing season progressed, the horses had to rely more heavily on the plants thriving in the moist soils around the stream—plants that had accumulated selenium in their tissues. The veterinarian called to the scene had to euthanize the animals, diagnosing the animals with chronic selenosis (Vice, 2012). And with that, mining in Southeast Idaho was forever changed.

Since those first deaths, an estimated thousand+ head of livestock have died from selenium poisoning directly related to phosphorous mining activities (Panter, 2015). In 2012, ninety-five sheep died within twelve hours of being pushed into an inactive mine for forage and water; the foreign sheepherder hadn’t heeded the warning signs and only thought of the food and water needs of his herd (Mebane et al., 2015; Vice, 2012). Western aster was the culprit.

Western aster has been collected from mine wastes with selenium concentrations as high as 13,000 parts per million. A thimbleful of this vegetation will cause pulmonary edema in livestock. The animals suffocate to death as their lungs fill with fluids (Vice, 2012). An entire herd can be lost in a matter of hours.

What’s worse, livestock may prefer Western aster at certain stages of its life cycle, though this idea is still only a theory among researchers at Logan, Utah’s USDA Poisonous Plant Research Lab. There is a case that supports this theory: In 2009, sixteen yearling steer died of acute selenosis in a five hundred acre pasture
where only five acres of that pasture contained reclaimed mine land. Western aster was to blame in those deaths.

Deer and elk hunters fear massive die-offs of the herd should the game animals graze through an inactive mine. In October 2013, the United States Fish and Game investigated the case of an elk exhibiting signs of a loss of neuromuscular coordination near the inactive Lanes Creek Mine; a post-euthanasia liver analysis showed elevated Selenium concentrations (Mebane et al., 2015). Despite this isolated case, researchers at the USDA Poisonous Plant Lab have observed that elk can distinguish between feed containing varying levels of selenium, ignoring forages with the highest concentration in favor of those with the lowest levels of selenium (Pfister et al., 2015).

Western aster threatens the way of life for the entire community. Ranchers lose their independence as they find themselves dependent on corporate payoffs to compensate for their fallen livestock. The Soda Springs community is nothing more than a bedroom for the mining industry’s personnel. Should the selenium issue raise the environmental obligations of mine land reclamation beyond the level where phosphate mining is economical, the city would cease to exist. Soda Spring’s entire economy, built around catering to the needs of the area’s mining operations, would collapse.

While current reclamation practice mitigates against future selenium releases into the environment, the problem remains the inactive mine sites. The United States Geological Survey has evaluated just nineteen of over eighty legacy sites, focusing their efforts on the large-scale offenders, and has documented
twenty-two square miles of surface disturbance directly related to phosphate mining activities (Blanchard et al., 2002). That’s twenty-two square miles that, by definition, leave seleniferous shale exposed to weather into soil, leach into water, and accumulate in plant matter.

Mining companies and the government are working through the protocol outlined in the Comprehensive Environmental Response, Compensation, and Liability Act (Superfund Protocol) to investigate potential remedial actions for the inactive mines, but progress is slow. No one is willing to accept the financial responsibility of a failed remediation project, so the process has stalled in the research phase. And until remedial actions are complete, fences dotted by skull-and-crossbones signs are a generally accepted approach to protect the public and wildlife. The locals learn to live with a new normal and carry on surviving with the same tenacity the land commands.

***

We hear back from the veterinarian. The tissue analysis confirms our suspicions. The cows died of selenium poisoning. Two days later, area poisonous plant researcher, Dr. Zane Davis, comes out to investigate the case and finds the smoking gun: A knee high stand of Western aster growing in seleniferous shales just across a loose fence line, the leaves clipped by unsuspecting grazers.

Months later, I kneel at the bank of the Blackfoot River. I unhook a Yellowstone cutthroat trout from a mangled Elk hair caddis fly, releasing it back into the slow-moving water. The fish takes off with a flash, no doubt terrified and disoriented. Despite the fish’s qualms, I find our interaction a meditative and
somewhat cathartic experience. I sit down and think about the fate of the river and the wilderness surrounding me. In 2012, The J. R. Simplot Company conducted a biological risk assessment and submitted photographs of two-headed trout embryos as part of their findings. The study, which acknowledged a 20% deformity rate among the fry, concluded that the selenium coming off their inactive mine site was not an environmental concern. Researchers at the U.S. Fish and Wildlife Service disagreed, believing that the data was biased and found the methods for assessing deformities wanting, believing instead that deformities reach levels as high as 70% (Kaufman, 2012). I throw a rock into the water, watching the ripples bend and fade in the lazy currents.

Rich natural treasure is often a double-edged blade, where land stewards must balance economic realities with more ethereal ecological sensibilities. And, as a result, the selenium issue demands no less than a nuanced understanding. There are no bad guys in this story, just companies and regulatory agencies scrambling to absolve the ignorance of the past. What is clear is that there are no winners. Everyone feels this, whether it is the miner and regulators or the ranchers and outdoorsmen. Livelihoods and lifestyles are at stake. It’s hard to predict the fate of the phosphate mining industry in Southeast Idaho, whether a weed’s impact is enough to tip the scales to where ecologic health outweighs the health of an entire economy, but in a place where survival is so hard-won, economics almost have to take precedent.
LITERATURE CITED


CHAPTER 3
THE SELENATE UPTAKE, AFFINITY, PARTITIONING, AND GROWTH RESPONSE OF SYMPHYOTRICHUM ASCENDENS (WESTERN ASTER): A HYDROPONIC INVESTIGATION

ABSTRACT

*Symphyotrichum ascendens* is a rangeland species implicated in the selenium poisoning of livestock grazing on mine wastes in the phosphate-mining region of the American Intermountain West. The effects of selenate absorption in *S. ascendens* were investigated in a hydroponic greenhouse trial at selenate concentrations of 0.1, 0.25, 0.5, and 1.0 mM selenate. This was the first study to investigate the relationship between *S. ascendens* and selenium. *Symphyotrichum ascendens* absorbed selenate in percentage concentrations, with the highest observed tissue concentration of 36,011 mg Se kg⁻¹ DM. *Symphyotrichum ascendens* exhibited signs of stress, producing 69.2% less biomass at the 1.0 mM Se treatment than in the control. Water use efficiency decreased with increased exposure to selenate while the transpiration stream concentration factor increased.
INTRODUCTION

*Symphyotrichum ascendens*, commonly referred to as Western Aster, has fatally poisoned grazing livestock in the phosphate-mining region of the Intermountain West (USA). The toxicity of *S. ascendens* has been linked to its selenium (Se) content (Pfister et al., 2013). Pfister et al. (2013) reported *S. ascendens* tissues reaching concentrations as high as 4,455 mg Se kg\(^{-1}\)DM, though unpublished field samples collected from mine wastes have tested as high as 13,000 mg Se kg\(^{-1}\)DM. A recent review on Se accumulators by White (2016) lists only two Se accumulator species as reaching tissue concentrations higher than 13,000 mg Se kg\(^{-1}\)DM: *Astragalus bisulcatus* (13,685 mg Se kg\(^{-1}\)DM) and *Astragalus racemosus* (14,920 mg Se kg\(^{-1}\)DM).

Selenium can be found in four inorganic states in soil systems: selenate (Se\(^{VI}\)), selenite (Se\(^{IV}\)), elemental Se (Se\(^{0}\)), and selenide (Se\(^{-II}\)). Physical, chemical, and biological processes affect Se speciation, and Se speciation influences bioavailability, mobility, and reactivity in soil systems (Sharma et al., 2015; Schilling et al., 2015). Elemental Se and selenide are immobile. Selenate and selenite are mobile, with selenate being the most bioavailable form of Se in the environment (Goodson et al., 2003). Phosphate mining in the Intermountain West has exposed previously reduced Se to oxidative weathering, releasing bioavailable Se into the environment (Myers, 2013). Despite having been mined since the early twentieth century, the associated selenium pollution was only discovered in 1996 after six horses were diagnosed with chronic selenosis (Knotek-Smith et al, 2006). Prior to this discovery, seleniferous waste rock was
left on the surface and in some cases used as a growth medium. *Symphyotrichum ascendens* has colonized many of these sites, creating concern among area ranchers, environmental activists, regional community members, and the phosphate mining industry.

While Se is an essential physiological component of healthy animal, archaea, and some microorganism function (Köhrle, 2012), it becomes toxic in concentrations exceeding biological necessity, which varies from species to species (Rosenfeld and Beath, 1964). Symptoms of selenium toxicity, or selenosis, in livestock occur when feed reaches concentration greater than 2-5 mg Se kg\(^{-1}\)DM (Terry et al., 2000). In mammals, chronic Se toxicity results in dermatitis, cracking of nails, and hair loss, while acute Se toxicity results in respiratory distress, heart attack, renal failure, and death (White, 2016).

Selenium accumulation can be categorized into one of three ecologic tiers: primary accumulation, secondary accumulation, or non-accumulation (White, 2016). The majority of plant species are non-accumulators, and will rarely accumulate foliar concentrations above 100 mg Se kg\(^{-1}\)DM, even when grown on high Se soils (White, 2016; Ellis and Salt, 2003). Secondary and primary accumulator species tolerate Se and can colonize seleniferous soils; Secondary accumulator species assimilate Se concentrations below 1,000 mg Se kg\(^{-1}\)DM, whereas primary accumulators generally exceed foliar concentrations of 1,000 mg Se kg\(^{-1}\)DM (White, 2016). Primary and secondary accumulators can be found in the Amaranthaceae, Asteraceae, Brassicaceae, Fabaceae, Orobanchaceae, and Rubiaceae families (El Mehdawi et al., 2014; White, 2016). Plants uptake Se as
selenate (as $\text{SeO}_4^{2-}$), selenite (as $\text{SeO}_3^{2-}$, $\text{HSeO}_3^-$, or $\text{H}_2\text{SeO}_3$), and certain organo-
selenium compounds, such as selenocysteine and selenomethionine (White and
Broadly, 2009). Se accumulating species are believed to uptake selenium via
sulfur transporters, reducing selenate to selenide prior to incorporation into
selenocysteine and selenomethionine (Yasin et al., 2015; White, 2016).

Of the primary accumulators in the American West, species in the
Astragalus and Stanleya genus are two of the earliest discoveries and are the most
widely studied (Rosenfeld and Beath, 1964). White (2016) lists 35 Astragalus
species as having been identified as primary accumulators. In contrast, this list
contains only three Symphyotrichum species. Compared to the Astragalus
literature, few studies have investigated the Symphyotrichum genus (Oram et al.,
2008; Oram et al., 2011; El Mehdawi et al., 2011; El Mehdawi et al., 2014; El
Mehdawi et al., 2015; El Mehdawi et al, 2015). And of these, none investigated S.
ascendens.

There is interest in developing cost-effective remediation strategies to
limit grazing livestock access to seleniferous S. ascendens. Currently, cattle
fencing is the major barrier preventing livestock from entering areas colonized by
S. ascendens, though this strategy has failed on multiple occasions. Therefore,
additional protections are of interest to the phosphate mining industry and the
community at large. The objective of this study was to characterize the effects of
selenate concentration on S. ascendens using an environmentally controlled
hydroponic study in order to eliminate the confounding factors associated with
soils. The results of this study will lend themselves to further investigation into
future remediation strategies.

**MATERIALS AND METHODS**

**Treatment and Growth Conditions**

There were five Se treatments in this hydroponic greenhouse study: 0.0 (control), 0.1, 0.25, 0.5, and 1.0 mM Se (Fig. 3-1). The 0.1 mM Se level was chosen because it matched water extractable Se soil levels in the mine wastes associated with the study. The concentrations above 0.1 mM were chosen to evoke an exaggerated response to Se uptake. Lower Se concentrations will likely be investigated in future work with *S. ascendens*. An individual treatment consisted of eight *S. ascendens* plants suspended over 50L of a shared nutrient solution. Each treatment had automatic pH control holding pH at 5.5 (HANNA Instruments BL 931700 controller, Oakton meter, Cole-Parmer 800 323-4340 solenoid, 0.1 M HNO₃) and was continuously aerated at a rate of seven L min⁻¹. In addition to ambient light, three Gavita Pro 1000W HPS lamps lit the study area sixteen hours daily. The daily light integral was 30 mols of photons m⁻² day⁻¹. Humidity and temperature within the greenhouse fluctuated with the outdoor conditions, but averaged 40% and 27°C.

As nutrient solution reservoirs were depleted, nutrients were replenished from a shared stock solution, and Se was added separately from a 1M sodium selenate (Na₂SeO₄) stock solution alongside the bulk nutrient refill. Water use was recorded, serving as a proxy for transpiration, with any evaporation equal across treatments due to the regulated airflow. Evaporation was equivalent to
~0.1 L per day and was accounted for in all calculations. Selenium was allowed to concentrate in the nutrient solution over time (Fig. 3-2) as early dilution efforts proved unsatisfactory as water use outpaced lab results. However, the 1.0 mM solution was gradually diluted to the equivalent 0.91 mM Se and the 0.5mM treatment to 0.46 mM Se. Selenium mass balance values can be found in Table 3-1. All graphs with a Se-concentration-in-solution axis use the values for the mean Se concentration for the study period.

The nutrient solution supplied all plant essential elements as proportioned in the Utah State University Crop Physiology Laboratory Dicot Nutrient Solution formulation (USU Crop Physiology Lab, 2015). Sulfur content was 0.5 mM in the initial 50L of nutrient solution and 1.0 mM in refill solution. Water samples were collected weekly and sent to the Utah State University Veterinary Diagnostics Laboratory (UVDL) where Se levels were quantified via inductively coupled plasma mass spectrometry (ICP-MS).

**Plant Material**

*Symphyotrichum ascendens* seeds were collected from Se-contaminated mine land during mid-October of 2016 in the Soda Springs, Idaho region. Seeds were germinated in a clear, plastic germination box on a vermiculite substrate at 25°C. Seedlings were transplanted to a Profile® soilless planting media within three days of germination and allowed to grow until they were large enough to transplant to the hydroponic tubs (~ 30 days). Seedlings were fertilized with a 21-5-20 Peters Excel Water Soluble Fertilizer after the first true leaves emerged.
Prior to transfer to the hydroponic tubs, roots were rinsed with distilled water in order to clean off any residue.

**Tissue Sampling and Analyses**

Plants were harvested forty-five days after transplanting. Roots were double-rinsed in DI water, pat dried, and divided into root, leaf, and stem tissue. Plant tissues were then dried in an oven at 80°C for 48 hours and ground. One half gram of plant tissue was than added to an Oak Ridge Teflon tube (Nalge Nunc International) and digested overnight (~18 hrs) in a 67-70% HNO₃ solution. The digest tubes and their contents were then heated to 90°C for an additional 1.5 hour digestion period. These samples were then submitted to the UVDL for ICP-MS elemental diagnostics.

**Water Sampling and Analyses**

Upon termination, nutrient solution samples were collected from each treatment, filtered by vacuum through a 1 micron filter paper, and submitted to the UVDL for a full elemental analysis via ICP-MS. Separate nutrient solution samples were collected from each treatment, filtered via syringe through a 0.45 μm filter, and submitted to Brooks Applied Lab (Bothell, WA). Brooks Applied Labs then performed a selenium speciation analysis via ion chromatography coupled to an inductively coupled plasma collision reaction cell mass spectrometer (IC-ICP-CRC-MS).
Calculations

The transpiration stream concentration factor (TSCF) is the ratio between the concentrations of a compound in xylem tissue and the concentration of the same compound in the nutrient solution and is calculated as:

\[
\frac{g \text{ Se in above ground tissue}}{g \text{ Se in nutrient solution}} = \frac{L H_2O \text{ transpired}}{L \text{ nutrient solution}}
\]

The equation is modified from Dettenmaier et al. (2008) in that the denominator was based on the mean.

RESULTS AND DISCUSSION

Selenate was the dominant Se species in the nutrient solution at the termination of the study, accounting for 96.6% or more of the Se in each treatment. Therefore, we conclude that selenate was the primary Se species absorbed by \textit{S. ascendens} in this study and that the observed effects were primarily due to selenate uptake. This is important to note, because the results can reasonably be attributed to selenate and not other selenium species.

\textit{Symphyotrichum ascendens} accumulated high levels of Se and began to preferentially partition Se to leaf tissue as Se exposure in the root zone increased (Fig. 3-3). The highest observed leaf tissue concentration was found in the 1.0 mM treatment and measured 36,011.36 mg Se kg\(^{-1}\) DM, or 3.6% Se. However, there
was considerable variability in tissue Se concentrations amongst individual plants within a given treatment. In the same 1.0 mM Se treatment, the lowest observed leaf concentration was 13,653 mg Se kg$^{-1}$DM, a 2.6-fold difference. Statwick et al. (2016) reported similar variability while investigating selenate uptake in the primary accumulator *Astragalus bisulcatus* and the non-accumulator *Astragalus cicer* grown in soil, finding a 10-fold and 5-fold difference, respectively, within a given selenate treatment.

This variability may be due to differences in gene expression relating to Se uptake mechanisms amongst individuals. Such variability in the field would allow for a broad response to varying natural selection pressures, such as herbivory. For instance, Se accumulation in some primary accumulating species has been found to protect against prairie dog herbivory (Quinn et al., 2008). In the presence of such a selective pressure, high accumulating individuals would survive where low accumulating individuals may not complete their life cycle. Conversely, in the absence of herbivory, the highest accumulating individuals may well suffer a selective disadvantage if Se accumulation exacted a metabolic cost or a negative growth response. Within the 1.0 mM treatment, individual plants with higher Se concentrations in the leaf tissues tended to produce less biomass.

*Symphyotrichum ascendens* showed signs of stress when supplied selenate. Biomass production, water use, and water use efficiency decreased as selenate concentrations increased in the nutrient solution (Fig. 3-4, 3-5, 3-6). The 1.0 mM selenate treatment produced 69% less biomass than the control, while
transpiration decreased 63.6%. This observation differs from the primary accumulator *A. bisulcatus*, which Statwick et al. (2016) found exhibited a positive growth response up to 0.53 mM selenate, their experimental maximum. Within the *Symphyotrichum* genus, El Mehdawi et al. (2014) found *S. ericoides* to have a positive growth response up to 80 μM selenate fertilization when compared to an Se-deprived control. In the same study, *Machaeranthera tanacetifolia* and *Astragalus bisulcatus* showed positive growth responses, though to a lesser degree, and only the secondary Se accumulator *Brassica juncea* exhibited signs of stress. The differences in growth response may indicate differences in selenate assimilation and storage; however, the selenate concentrations used in the present study were higher than those by El Mehdawi et al. (2014). It is interesting that *S. ascendens*, despite its capacity to accumulate Se, is stressed by the accumulation.

Selenium concentrations increased in the nutrient solution over time (Fig. 3-2), with increases of 100% (0.1 mM), 72% (0.25 mM), 29% (0.5 mM), and 28% (1.0 mM). This observation suggests that *S. ascendens* excludes selenate. Indeed, the transpiration stream concentration factor (TSCF) of each treatment supports this (Fig. 3-7). The TSCF is the ratio between the concentrations of a compound in xylem tissue and the concentration of the same compound in the nutrient solution and describes how readily a plant takes up a compound. A TSCF greater than 1.0 is indicative of active uptake, while a value below one indicates exclusion. A TSCF value of 1.0 would indicate passive uptake. The TSCF values of selenate uptake by *S. ascendens* all fall below the 1.0 threshold, though increase
with increasing exposure to selenate. This observation may be indicative of an imperfect affinity in the sulfate transporter mechanisms of *S. ascendens* in the root membranes. Sulfur and selenium are both in Group 16 of the periodic table and both form oxygenated plant available anions with a negative two charge (SeO$_4^{2-}$ and SO$_4^{2-}$, respectively). For this reason, it has been proposed that selenate uptake competes with that of sulfate (Terry et al., 2000) and the subsequent literature has bolstered this theory as DNA analyses have shown increased gene expression for high affinity sulfate transporters (HASTs) in the presence of selenate in plant tissue and nutrient solutions (White, 2016). White (2016) mentions a hypothesis that Se primary accumulators have HASTs that are selective to selenate, and the HASTs of non-accumulating angiosperms have HASTs selective to sulfate. But in the case of *S. ascendens*, the TSCF below 1.0 implies active exclusion of selenate.

Yet the TSCF values increased as the rooting zone was exposed to increasing concentrations of selenate. This relationship could be explained by an imperfect sulfate affinity and the increased gene expression of sulfate transporting mechanisms in the presence of selenate, and might be better represented as Figure 3-8. If a plant is scavenging for sulfate ions, but the HASTs grab the wrong ion, the plant is likely up regulating the genetic expression of its HASTs to increase its ability to get at the sulfate in solution—not to scavenge more selenate. In fact, White (2016) reports on a trial in which non-accumulator and secondary accumulators exhibited enhanced sulfate transporter gene expression when deprived of sulfate (El Kassis et al., 2007; Rouached et al., 2008;
Shinmachi et al., 2010; Schiavon et al., 2015). As we’ve shown in this study, selenate did not increase the growth of *S. ascendens*, so up regulating HASTs to get more selenate seems to defy the inherent logic of biology.

So, for *S. ascendens*, it is possible that grazing pressure selected for an imperfect sulfate affinity, with selenate uptake serving as a deterrent to grazing pressure. At natural levels of selenium in soils, this might not be a growth inhibiting factor, but in unusually high concentrations of soil selenium this evolutionary advantage suddenly becomes a distinct disadvantage.

**CONCLUSIONS**

*Symphyotrichum ascendens* accumulated selenate at concentrations above the 1,000 mg Se kg⁻¹ primary Se accumulator threshold, though exhibits a stress response as a result. This study found that increasing exposure to selenate in the rooting zone decreased total biomass, cumulative water use, and water use efficiency in *Symphyotrichum ascendens*. *Symphyotrichum ascendens* began to preferentially partition Se to leaf tissue as Se exposure in the root zone increased. Transpiration stream concentration factor data and the increase in Se concentrations in the nutrient solution over time suggest *S. ascendens* actively excluded selenate from root uptake. Further work is needed to develop meaningful strategies for land managers attempting to prevent further livestock deaths.
LITERATURE CITED


Figure 3-1: A photo of the five Se treatments prior to harvest. Eight *S. ascendens* plants were grown in each treatment. Each treatment had an automatic pH control, set at 5.5, adjusted with 0.1 M HNO₃. Airflow was set at 7 L min⁻¹, metered by individual rotometers. Plants were grown for 45 days. The photo was taken immediately prior to harvest.

Figure 3-2: The nutrient solution Se concentration increased over the study period. Initial efforts to maintain Se concentrations at constant levels proved untenable as plant water requirements outpaced lab-processing speeds. Increases in Se concentrations over time suggest Se exclusion within the rooting zone. The refill solutions were gradually diluted to 0.91¹ mM Se and 0.46² mM Se prior to ceasing dilution efforts early in the study.
Figure 3-3: Selenium concentration in plant tissue as a function of Se concentration in the nutrient solution. Root, leaf, and stem tissues were separated and analyzed individually. In some treatments, not all plants developed stems by harvest: 0.25 mM Se: n=5; 0.05 mM Se: n=2; 1.0 mM Se: n=1. The x-axis values are based on the mean over the study period. (Mean ± Std Dev of individual plants within a treatment.)
Figure 3-4: The effect of the average nutrient solution selenium concentration on whole plant dry mass. Plants exposed to higher concentrations of selenate produced less biomass. The x-axis values are based on the mean over the study period. (Mean ± Std Dev of individual plants within a treatment.)

Figure 3-5: The cumulative transpiration over the study period. Trends across treatments remained consistent over time, suggesting that the treatment effects were constant throughout the life cycle of the plants. Water use was quantified by tracking volume of nutrient solution refilled over the course of the study. Approximately 0.1 liters of water was lost as evaporate per day.
Figure 3-6: Water use efficiency is a measure of aboveground biomass produced per liter of H₂O consumed by the plant. Water use efficiency decreased as the nutrient solution Se concentration increased. These values were calculated per tub, based on total aboveground biomass production and total transpiration. The x-axis values are based on the mean over the study period.

Figure 3-7: Effect of nutrient solution Se concentration on the transpiration stream concentration factor (TSCF). TSCF is a measure of how effectively a plant removes an ion from the nutrient solution. A value greater than 1.0 is indicative of active uptake, while a value below 1.0 suggests exclusion. TSCF was calculated as \((\text{g Se in aboveground tissue/ L H}_2\text{O transpired})/(\text{g Se in nutrient solution/ L nutrient solution})\). TSCF is the ratio of Se concentration in leaf and shoot tissue over the Se concentration in the nutrient solution. The x-axis values are based on the mean over the study period.
Figure 3-8: The effect of the nutrient solution [Se]/[S] ratio on transpiration stream concentration factor (TSCF). The shape of the relationship is similar to that of Michaelis-Menton enzyme kinetics, suggesting that Se uptake was mediated by an active transport mechanism. TSCF is a measure of how effectively a plant removes an ion from the nutrient solution. A value greater than 1.0 is indicative of active uptake, while a value below 1.0 suggests exclusion. TSCF was calculated as \((\text{g Se in aboveground tissue/ L H}_2\text{O transpired})/(\text{g Se in nutrient solution/ L nutrient solution})\). TSCF is the ratio of Se concentration in leaf and shoot tissue over the Se concentration in the nutrient solution.

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Table 3-1: Selenium mass balance values (mg).
“Look deep into nature, and you will understand everything better.”

-Albert Einstein, 1951

Plant Physiology got me praying like a pagan

I.

A beauty I see:
tall, curvaceous, and searching for the light.

What lessons you teach.

II.

It can make for an undignified repose,
what with such greedy neighbors.

But you lend it such grace.

III.

What one rejects
is often their best asset.

A gift even for the astronauts.

Thanks, friend!
Einstein my Guru

Opportunity is the legacy of evolution.
The inevitable draw, specialization.
A sharp beak!

And all from light colliding with light.
The death of a star,
a needle point expansion.

A dimple in the fabric with all that cosmic baggage.
And from this:

Feast & Famine.
War & Peace.

We’re so blessed.

Impulse, inevitable.
And from this, vast libraries.
Leather bound collections where

Rationalism

Justifies

Attraction.

For oil.
For admiration.
For atomistic ambitions.

That’s a sharp beak, indeed!

Entropy!

Rise up or brace for impact!

But how?
Buddha might know, 
or 
the nuns praying up a psychedelic

hit
of
bliss.

Some call it the way things are. 
Some call it the Tao.

Smile! 
Feel the $-\Delta H$.
Notice the trees.
Some say it’s a moment, 
some say awareness and they

Train, 
Train, 
Train.

Like Olympians.
Like Samurai.

They alone, gnawed upon by 
the 
Sense of Urgency

Bye-
bye

Forced Ambitions

Bye-
Bye

Blue Mondays

&

War & Greed®

Bliss in the Now
might be the sharpest beak, indeed!
All the American Saints knew:

    Turn on,
    Tune in,
    &
    Drop out, man.

Excess and adoration, such exhausting preoccupations.

All the Mystics knew.
Analogies they offered to grasp.

    Like a father!
    Like love!

Some grasp the analogies.

Albert was a poet.

    Maybe he missed
or was the most concise in history.

Even the neuroses of the Samurai occasionally produce the
Nobel-est of Medals

    Maybe that’s why,
the value on Diamonds & Gold.

    Glucose on reserve,
man’s starch.

What’s the redox potential

    of a dollar bill?

But in that Tao headspace,
so silly in deed.
Just trust in that neurotic genius.

Matter
will always
drill for Energy.

It’s a Holy Marriage,
an eternal, cosmic courtship.

We love the e’, but we forget that source.

It’s just magnets
blindly building beaks—
of both carbon and thought.

And Albert felt it, there in the depths.

The Jews
have
that

Cosmic guilt.

Just read the book of Job.

That fear touches places beyond language.

Descartes had it.
Galileo transcended it,
if just for that brief moment.
Heidegger knew it when he saw it,
that neuroses to merge with the bliss.

There’s truths, some elemental & some man-made,
that only the mad ones and the mystics must reconcile.
That regardless how special the beak,
in the logic of energies
of microbes
and men

\[ E \]

will
always
equal

\[ mc^2 \]

\[ \infty \]
CHAPTER 5

CONCLUSION

*Symphyotrichum ascendens* accumulates selenate at concentrations above the 1,000 mg Se kg\(^{-1}\) primary Se accumulator threshold, though exhibits a stress response as a result. The original scientific work contained within this thesis report found that increasing exposure to selenate in the rooting zone decreased total biomass, cumulative water use, and water use efficiency in *S. ascendens*. *Symphyotrichum ascendens* began to preferentially partition Se to leaf tissue as Se exposure in the root zone increased. Transpiration stream concentration factor data and the Se concentration of the nutrient solution suggest *S. ascendens* actively excluded selenate from root uptake, though this capacity waned as selenate concentrations increased in the nutrient solution.

Additionally, Chapters Two and Four have provided examples of alternative forms of scientific communication. Chapter Two is a traditional form of popular science communication and was written with the goal of instilling a sense of the inherent ecologic and social impacts of the issues surrounding *Symphyotrichum ascendens* as well as instilling an interest in earth science for prospective students and conscientious citizens alike. Chapter Four is born of a belief that the arts are a viable means of instilling the wonder and the magnitude of scientific achievement to the masses. It’s the scientific worldview that brought me to a life of spirituality. I view Chapter Four as my contribution to the sciences and humanity at large.