Alberta Innovates Technology Futures

Development of an Ecological Contact Soil Selenium Guideline

Prepared by

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1.0 Rationale

The purpose of this research was to assess Selenium (Se) toxicity on a variety of plant species and to provide the empirical evidence required to potentially re-define the soil Se ecological toxicity guideline for Alberta and Canada. Current soil Se guidelines in Canada and Alberta are 1.0 mg/kg and are set to protect ecological and human health in natural, agricultural, and residential/parkland land uses (Alberta Environment, 2010). There are a few flaws with the current guideline of 1.0 mg Se/kg. First, the current guideline acknowledges that the background concentration of soils in some areas can be greater than Tier 1 guidelines. The regional database of naturally occurring soils suggests background concentration of Se frequently range from 2.3 to 4.7 mg/kg in Canada (CCME, 2009 and Penny, 2004). Second, the guideline was developed based on only two studies, both of which were conducted using the selenate [+6] valence form of Se (Carlson, et al 1991 and Singh and Singh 1979), resulting in a potential overestimation of toxicity as Se also exist in the less toxic form of selenite [+4]. Lastly, the selenate-based guideline of 1 mg/kg is likely overprotective if sulfate is present in the soil (Prediger et al., 2012). This is further supported by Dhillon & Dhillon (2000); Mikkelsen et al. (1988); and Terry et al. (2000). Sulfate (S) occurrence is wide-spread across Alberta. In east-central Alberta Na_2SO_4 dominates most soils while $MgSO_4$ is dominant in the Peace River region.

This study is the continuation of the PTAC funded project: "Phase 1 (2013-2014) Development of EcoContact Soil Se Guideline" (Woosaree et al. 2014), and extends the toxicity testing study to six plant species under various Se and S concentrations in fine, coarse and an artificial soil substrate. The overarching goal of this PTAC project is to assess and potentially redefine the Alberta and Canadian soil Se guideline for the ecological contact pathway. This would benefit industry in terms of reduced remediation costs as well as improved environmental performance by decreasing the amount of remediation required because of guideline exceedance.

1.1 Background

1.1.1 Selenium in the Environment

Se is a naturally occurring trace metal, commonly found in Cretaceous marine sedimentary rocks or seleniferous rocks (Zhao et al. 2005). Other natural sources of Se include coal and other fossil fuel deposits (CCREM 1987; US-EPA 1988; Haygarth 1994). Naturally occurring soil Se concentrations range up to 3.7 mg/kg in Canada (CCME 2009) and up to 2.3 mg/kg in Alberta (Penny 2004).

Anthropogenic sources of Se in soil include coal ash from coal-fired power plants, irrigation waters from seleniferous soils and wastewaters from some industrial processes (CCREM 1987; US-EPA 1988). Se is also released during the manufacturing of glass, metals and electronics (Guidotti et al. 1999; CCME 2009) and has been known to be associated with drilling waste from oil and gas activities. In 2003, Canada was the second largest producer of Se; the primary source was identified to be copper refining (CCME 2009).

The threshold at which Se is toxic to plants (>1 mg/kg) and the level at which it has no effect and/or a beneficial effect (<1 mg/kg) are comparatively close in value (Bronkinkowski et al. 2000). Because of this small range, Se has been referred to as an "essential toxin' or a "double-edged sword" (Bailey et al. 2012), justifying the extensive researcher required to determine toxicity.

1.1.2 Selenium Guideline Levels

The existing soil quality guideline in Canada for selenium proposed by various jurisdictions is presented in Table 1. Compared to B.C and Ontario, Alberta has a much stricter Se guideline across the different end land uses. The intention of the 1.0 mg/kg Se threshold guideline was to reduce the cumulative effects of inter-species contamination and toxicity through interactions (i.e. herbivory, predation) across Canada. This proposed threshold may be limited in scope, as certain areas in Alberta are known to naturally have soil Se concentrations exceeding the current guideline level. As noted above, the 1.0 mg Se/kg guideline is based on the selenate [+6] valence form of Se (Carlson, et al 1991 and Singh and Singh 1979), resulting in a potential overestimation of toxicity as Se exist in the less toxic form of selenite [+4].

Table 1. Soil quality guideline proposed by various jurisdictions for selenium.

Jurisdiction	Description	Maximum Concentration (mg/kg)		Reference
Canada	Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health	1 1 3.9	Agr R/P C/I	Environment Canada, 2001
British Columbia	Generic Numeric Soil Standards for Contaminated Sites	2 3 10	Agr R/P C/I	BCMOE, 2005
Ontario	Generic Numeric Criteria for Soils	1.2 1.5 5	Agr R,P, C I	MOE, 2009
Alberta	Alberta Tier 1 Soil and Groundwater Remediation Guidelines	1 1 2.9	Agr R/P C/I	Alberta Environment, 2010

A: Background concentrations; B: moderate soil contamination which requires additional study; C: threshold value that requires immediate cleanup. Agr: agricultural land use: R: residential land use; P: parkland land use; C: commercial land use; I: industrial land use.

1.1.3 Selenium Uptake by Plants

Although Se is not generally considered an essential element for plants (Brady and Weil 2008; Ellis and Salt 2003; El Mehdawi et al. 2011), it has been claimed to be a beneficial element in a number of *Fabraceae* and *Brassicaceae* species that are hyperaccumultors of Se (El Mehdawi et al. 2011; Zhu et al. 2009). Some species utilize Se for allelopathy, others take up Se and release it through volatilization (Barillas et al. 2011; Terry et al. 2000); the capacity for Se utilization has been observed to be species-dependent (Brown and Shrift 1982; White et al. 2004). There may be other plant species that seek out Se in the soil through their root systems, as was inferred from a study that examined prince plume (*Stanleya pinnata)* and mustard greens (*Brassica juncea)* (Goodson et al. 2003). This feature has been a driver behind the use of certain plant species for remediation (i.e. phytoextraction) in Se-contaminated soils (Raskin et al. 1997).

The physiologic response of plants to Se, as well as the range between beneficial and harmful levels of Se, varies greatly depending on the species of interest. Plant species differ in their ability to accumulate Se and have been divided into three categories: 'non-accumulator,' 'Se-indicator' and 'Se-accumulator'. Plants that contain less than 25 ppb Se in their tissue are non-accumulators, and are incapable of tolerating high Se environments (Rosenfeld and Beath, 1964; Shrift, 1969; Brown and Shrift, 1982; and Wu 1998). Se-accumulator species are those that may contain up to 20-40 ppb Se in their tissue when grown under natural conditions (Rosenfeld and Beath, 1964; Shrift, 1969; Brown and Shrift, 1982; and Wu 1998). These plants typically like to grow in seleniferous soils (soils naturally high in Se). Species that can grow adequately in seleniferous and non-seleniferous soils and can contain up to 1000 ppb Se in their tissue are those 'Se-indicator' plants.

The accumulatory effect, in turn, can affect other biota; animals grazing on accumulator plants may develop symptoms of Se toxicity (Pichtel 2007; Sors et al. 2005). This bioaccumulation and toxicity effects on the biota will depend on the soil Se concentration found in the area; the amount of Se accumulated within a preferred or available forage species; and, the amount of affected vegetation consumed through browsing/grazing. Trophic transfer through accumulatory effects (Hopkins et al. 2005) is one vector for cross contamination of elements such as Se. Plant species that are Se accumulators become vectors to other biota that consume them. The result is Se toxicity within grazer/browser species, (Hartikainen 2005) also known as selenosis when accumulator plants are consumed in excess (Tiwary et al. 2006). Effects have been listed as chronic or acute (Żarczyńska et al. 2013) and occasionally fatal in cattle (Davis et al. 2012), sheep (Tiwary et al. 2006) and farmed white-tailed deer (Al-Dissi et al. 2011).

Since the utilization of Se is species-dependent, it is likely that the tolerance of high or increased Se concentrations in soils may also be species-specific. Some agronomic species, such as wheat, have been found to be reasonably tolerant of elevated levels (>1.5 ppm) (Lyons et al. 2005), whereas others, such as barley (*Hordeum vulgare),* exhibit stress resulting from increased Se concentrations (>1 ppm) (Molnárová and Fargašová 2009). Research related to aquatic ecosystems has noted that toxicity effects may increase in secondary heterotrophs, notably birds and fish, more than invertebrate prey species (deBruyn and Chapman 2007). Similar findings have been recorded in terrestrial reptile species (Hopkins et al. 2005).

1.1.4. Effects of Sulfate on Selenium Toxicity

Sulfur is essential for many growth functions in plants including nitrogen metabolism, enzyme activity, and protein and oil synthesis (Hirai and Saito 2004; Dubuis et al. 2005; Leustek et al. 2000). Sulfur deficient plants have short and/or spindly stems and chlorosis of the young (top) leaves (Hirai and Saito 2004). Concentrations of sulfur (>1 ppm) is often correlated with high concentrations of dissolved organic carbon such as plant litter, compost materials, etc. (Fenell and Bentley 1988) and the presence of sulfur may aid in plant resistance to certain fungal pathogens (Dubuis et al. 2005).

Se uptake in plants follows the same metabolic pathway as S because of their structural similarities (Leggett and Epstein, 1956 and Sors et al. 2005). As a result, the presence of S in soil was found to reduce the uptake of Se by plants, improving tolerance and survivability in non-favorable conditions (Kaur et al. 2014; Mackowiak and Amacher 2008). It is unknown whether native Alberta plants can grow under increased Se concentrations in the presence of S. The relationship between S and Se toxicity in plants as a function of co-exposure requires more clarification, especially in Alberta where the parent material is made up of Cretaceous marine sedimentary rocks or seleniferous rocks which are known to have elevated levels of $MgSO_4$ and Na_2SO_4.

1.1.5 Summary of Preliminary Research to Date

In 2011 and 2012, Prediger, Knafla, Woosaree, and Cook conducted preliminary Se toxicity testing. The research involved spiking artificial soils with Se (as selenate) and growing alfalfa in a controlled environment following Environment Canada (2007) methodology. Additionally, soils were spiked with S to assess the previously reported antagonistic relationship where S reduces plant toxicity to Se. Results indicated that the effective concentration to cause a 25%

adverse effect (EC25) without S ranged from 0.49 mg/kg to 0.86 mg/kg for the endpoints measured. However when soils were spiked with S, an apparent hormetic effect was observed where low concentrations of Se caused a stimulatory response and decreased Se toxicity. Results indicated that when S was present in the soils, Se plant toxicity was reduced by more than 139-fold, pending the S concentration. Although other research (White et al., 2004 and Mackowiak and Amacher, 2007) has previously reported the antagonistic relationship, it is believed that this is the first research to observe a hormetic effect. This research highlighted the need to reassess current soil Se guidelines in Alberta and Canada and to make considerations for the presence of S.

2.0 Materials and Methods

2.1 Soils

Coarse and fine textured test soils were provided by Equilibrium Environmental Inc. Soils were dried, homogenized, saturated at 20% moisture as per Environment Canada (2007) and placed in a cooler until ready to use. An artificial soil was also prepared according to Environment Canada (2007) using 10% Sphagnum sp., 20% kaolinite clay and 70% silica sand. Reagent-grade $CaCO_3$ was added (~20 g $CaCO_3$/kg peat) to the artificial soil to attain a pH within 6 –7.5. The soils were mixed in their dry form first and gradually hydrated with de-ionized water. Soils were mixed until they were visibly homogenous in color, texture and degree of saturation. Baseline physiochemical properties were determined prior to spiking the soils to the targeted Se (in the form of Na_2SeO_4) and S levels (Table 2). The mass of Na_2SeO_4 required to reach the targeted Se levels was calculated and weighed using an analytical scale. It was then dissolved in 250 mL of distilled water and mixed in with the soil until it reached a homogeneous, crumbly consistency with clumps approximately 3-5 mm in diameter. To achieve the targeted S levels while maintaining Ca:Na ratios within 10 – 20 and Ca:Mg ratios within 2.5 – 5, a combination of $CaSO_4$, $MgSO_4$ and Na_2SO_4 were used. The quantity of $CaSO_4$, $MgSO_4$ and Na_2SO_4 required to reach targeted S levels were calculated and weighed using an analytical scale. It was then dissolved in 500 mL of distilled water and mixed in with the soil until it reached a homogeneous, crumbly consistency with clumps approximately 3-5 mm in diameter.

Table 2. Baseline chemical properties were determined prior to spiking the test soils and artificial soil to the targeted Se and S levels.

Soil Type	Baseline properties				
	Ca:Mg	Ca:Na	pH	EC (ds/m)	SAR
Artificial	5.1	17.9	7.4	1	0.2
Coarse	3.1	57.2	7.2	0.93	<0.1
Fine	3.6	34	7.5	0.75	<0.1

2.2 Plant Species Selection and Growing Conditions

To appropriately assess toxicity on relevant species and Se/S concentrations it was important to consult with ESRD (now Alberta Environment and Parks (AEP)). Plant species selection and Se concentrations levels were finalized after discussions between Equilibrium Environmental Inc. and AEP. AEP suggested the use of common monocot and dicot agricultural species and some common garden species due to their sensitivity to Se (Banuelos and Schrale, 1989). A total of six plant species were selected for the trial (Table 3), all recommended by Environment Canada (2007).

Table 3. Plant species selected for the Se toxicity study.

Species Name		Plant Se Accumulator Type	Type of species
Scientific	Common		
Daucus carota	Garden carrot	Non-accumulator	Dicot
Elymus lanceolatus	Northern wheatgrass	Non-accumulator	Monocot
Medicago sativa	Alfalfa	Non-accumulator	Dicot
Festuca rubra	Creeping red fescue	Non-accumulator	Monocot
Hordeum vulgare	Barley	Non-accumulator	Monocot
Cucumis sativus	Cucumber	Non-accumulator	Dicot

Prior to the start of the study, seeds from a recognized supplier were obtained and tested for seedling emergence as per Environment Canada (2007) to ensure seeds of each species had 80% or higher emergence.

Five seeds (barley, cucumber) or ten seeds (alfalfa, Northern wheatgrass, red fescue and carrot) were planted into coarse, fine or artificial soil; each soil had 16 treatments (four levels of Se and four levels of S), and each treatment was replicated four times (Table 3). The test was terminated after 14 days of exposure for barley and cucumber and after 21 days of exposure for alfalfa, carrot, northern wheatgrass and red fescue (Environment Canada 2007).

Testing vessels with seeds were set to grow in a greenhouse at 24/15°C, day/night temperature and 18 hour photoperiod. Approximately 500 mL weight equivalent of soil was used in each pot. Pots were 1 L polypropylene food containers with lids. All pots were labelled, seeds chosen and sown individually and then watered in accordance with Environment Canada's contaminated soils test. Lids were placed on pots for the first seven days or until plant leaves reached the lids. Watering was done when necessary, about every three days. Visual assessments were recorded on the condition of the emerged plants on a weekly basis (i.e. good, delayed emergence, impaired development, necrosis, defoliation, desiccation, malformation, mottling, staining, wilting, discoloured, or chlorosis).

2.3 Study Design

2.3.1 Se and S treatments

The study consisted of spiking two field-collected soils (fine and coarse textured) and an artificial soil with, four concentrations of Se (Se0, Se1 (~1 ppm), Se2 (~2.5 ppm) and Se3 (~6 ppm)), four concentrations of S (S-Control, S-Low, S-Mid, and S-High) (Table 4), and growing six test species, including garden carrot, northern wheatgrass, creeping red fescue, alfalfa, barley and cucumber. The targeted concentrations for Se and S were determined based on the results from Phase 1 (2013-2014) PTAC funded Development of EcoContact Soil Se Guideline project (Woosaree et al., 2015) (Table 4).

Table 4. Targeted concentrations for Se and S for each soil type.

Soil type	Se treatments and targeted concentrations	S treatments and targeted concentrations			
		S-Control (%S)	S-Low (%S)	S-Mid (%S)	S-High (%S)
Artificial	Se0 (ppm)	<0.3, 0*	<0.3, 0.05	<0.3, 0.1	<0.3, 0.2
	Se1 (ppm)	1.15, 0	1.15, 0.05	1.15, 0.1	1.15, 0.2
	Se2 (ppm)	2.65, 0	2.65, 0.05	2.65, 0.1	2.65, 0.2
	Se3 (ppm)	5.65, 0	5.65, 0.05	5.65, 0.1	5.65, 0.2
Coarse	Se0 (ppm)	0.325, 0	0.325, 0.05	0.325, 0.1	0.325, 0.2
	Se1 (ppm)	1.15, 0	1.15, 0.05	1.15, 0.1	1.15, 0.2
	Se2 (ppm)	2.65, 0	2.65, 0.05	2.65, 0.1	2.65, 0.2
	Se3 (ppm)	5.65, 0	5.65, 0.05	5.65, 0.1	5.65, 0.2
Fine	Se0 (ppm)	0.625, 0	0.625, 0.05	0.625, 0.1	0.625, 0.2
	Se1 (ppm)	1.40, 0	1.40, 0.05	1.40, 0.1	1.40, 0.2

Se2 (ppm)	2.95, 0	2.95, 0.05	2.95, 0.1	2.95, 0.2
Se3 (ppm)	5.95, 0	5.95, 0.05	5.95, 0.1	5.95, 0.2

*The first value is for Se concentration and the second value is for S concentration.

Spiked soil was submitted to EXOVA (Edmonton, AB) for analysis prior to the start of the experiment. Se determination was completed using acid digestion (USEPA Method 3051a) and total S was determined using a colorimetric analysis (method detection limit is 0.01% or 100 ppm). Post experiment soil analysis remains to be completed, due to budgetary constraints.

2.3.2 Growth Endpoints

The biological endpoints for the test were: percent (%) seedling emergence (Day 7), root and shoot length and root and shoot dry weight at the end of the test (Day 14 or Day 21, depending on the test species). Root and shoot length and weights were collected by separating individual plants from both the test soil and from each other. This was achieved by gently loosening the soil and root matrix from the test vessel and removing soil that could be easily dislodged without disturbing the root matrix. The remaining soil and plant mass were placed into a 1 mm sieve and held under a gentle stream of tap water to gently dislodge as many of the remaining soil particles as possible. Once all the soil particles were removed from the plants, plants were placed onto a moistened paper towel and covered until measurements were made and recorded. Measurement of root and shoot length and weight were done according to Environment Canada (2007).

2.3.3 Data Analysis

The mean (± standard deviation) values for % emergence, shoot and root length as well as shoot and root dry weight were reported. A statistically significant reduction in the growth endpoints was considered indicative of an adverse toxic effect. Differences between means were completed using One-way ANOVA in R Statistical Software R-3.2.3 for Windows (32/64 bit) (https://cran.r-project.org/bin/windows/base/, 2015).

3.0 Results

Prior to the start of the study, seeds from a recognized supplier were obtained and tested for seedling germination as per Environment Canada (2007). The germination test result for the test species are deemed satisfactory and are summarized in Table 5.

Table 5. Pre-trial seedling emergence test of selected test species.

Species Name			
Scientific	Common	Germination (%)	Germination Time (days)
Daucus carota	Garden carrot	93	6
Elymus lanceolatus	Northern wheatgrass	86	6
Medicago sativa	Alfalfa	90	6
Festuca rubra	Red fescue	93	6
Hordeum vulgare	Barley	90	3
Cucumis sativus	Cucumber	99	6

Photographs of each species were taken at the end of the test period to visually record the concentration-response relationship in the above-ground biomass and can be found in Appendix A.

3.1 Soil analysis

Despite the textural differences, the three soils used in this study had relatively similar baseline chemical properties prior to spiking with Se and S (Table 6). The addition of Se and S did increase the EC and SAR of the soils, particularly the EC post spiking, which was classified as Fair according to the Alberta Tier 1 Soil Remediation Guidelines (Alberta Environment, 2010). This was not expected to affect plant growth, especially for those plants grown in the artificial and fine texture soils as these soils have more exchange sites available to capture free ions. However, it was expected that plants grown in the coarse texture soils may experience water stress due to the osmotic potential differences between soil pore water and water stored in the plants.

Table 6. Soil chemical properties pre and post spiking with Se and S.

Soil Type	Pre-spiking					Post-spiking				
	Ca:Mg	Ca:Na	pH	EC (ds/m)	SAR	Ca:Mg	Ca:Na	pH	EC (ds/m)	SAR
Artificial	5.1	17.9	7.4	1	0.2	3.2	9.75	7.29	2.78	0.89
Coarse	3.1	57.2	7.2	0.93	<0.1	2.5	18.05	6.98	3.01	0.92
Fine	3.6	34	7.5	0.75	<0.1	3.16	15.6	7.46	2.42	0.65

The baseline Se and S analysis indicated that the spiked soil did achieve the targeted concentrations (Table 7). The variability between samples was relatively small and the majority of samples were within ± 10% of targeted concentrations.

Table 7. Concentration of Se and S of spiked soils prior to the start of the experiment.

Soil type	Se Treatments	Targeted Se (ppm)	Actual Se (ppm)	S Treatments	Targeted S (%)	Actual S (%)
Artificial	Se0	<0.30	<0.3±0.00	S-Control	0.00	0.01±0.01
	Se1	1.15	1.00±0.14	S-Low	0.05	0.07±0.02
	Se2	2.65	2.45±0.30	S-Mid	0.10	0.11±0.02
	Se3	5.65	6.05±0.70	S-High	0.20	0.21±0.04
Coarse	Se0	0.33	0.33±0.05	S-Control	0.00	0.02±0.01
	Se1	1.15	0.95±0.10	S-Low	0.05	0.05±0.01
	Se2	2.65	2.63±0.21	S-Mid	0.10	0.09±0.01
	Se3	5.65	5.73±0.39	S-High	0.20	0.16±0.02
Fine	Se0	0.63	0.63±0.05	S-Control	0.00	0.03±0.03
	Se1	1.40	1.40±0.08	S-Low	0.05	0.06±0.01
	Se2	2.95	3.25±0.17	S-Mid	0.10	0.11±0.04
	Se3	5.95	8.05±1.25	S-High	0.20	0.22±0.04

3.2 Criteria for Valid Test

According to the Environment Canada's Biological Test Method: Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soil (2007), a toxicity test is only valid if each of the following five test criteria is achieved:

1. The mean % emergence for individual plant species grown in negative control soil (Se0 S-control) for the duration of the test must be: ≥ 60% for carrot, cucumber and ≥ 70% for alfalfa, barley, northern wheatgrass and red fescue.
2. The mean survival for emerged seedlings grown in negative control soil (Se0 S-control) for the duration of the test must be ≥ 90%.

3. The mean % of seedlings grown in negative control soil (Se0 S-control) for the duration of the test that exhibit phytotoxicity and or developmental anomalies must be < 10%.
4. The mean root length for individual plant species grown in negative control soil (Se0 S-control) for the duration of the test must be: ≥ 70mm for red fescue, ≥ 80 mm for carrot, ≥ 110 mm for northern wheatgrass, ≥ 120 mm for alfalfa and cucumber ≥170 mm for barley.
5. The mean shoot length of individual plant species grown in negative control soil (Se0 S-control) for the duration of the test must be: ≥ 40 mm for alfalfa, ≥ 45 mm for carrot, ≥60 mm for cucumber, ≥ 80 mm for red fescue, ≥ 100 mm for northern wheatgrass and ≥150 mm for barley.

All six plant species passed criteria #2 and #3 in the artificial, coarse and fine soil (data not shown). Criteria #1, #4 and #5 are summarized in Tables 8, 9 and 10 for the artificial, coarse and fine soils, respectively. The majority of the species passed criteria #1 and #5, with the exception of barley in artificial soil; carrot, cucumber and red fescue in coarse soil for criteria #1 and carrot and cucumber in artificial and fine soil, respectively for criteria #5. Majority of the species, with the exception of barley and alfalfa failed criteria #4 for all three soil types. It is likely that fine roots were broken during root washing; the fine roots are very fragile and delicate especially for carrots, northern wheatgrass and red fescue. However, this did not affect the root weight data since each sample was washed using a 1 mm sieve, capturing any broken root pieces.

Table 8. Valid test requirements for criteria 1 - mean % emergence, criteria 4- root length and criteria 5 - shoot length observed in the artificial soil.

Species	Mean % emergence		Mean Root Length		Mean Shoot Length	
	Criteria for Valid Test	Test results	Criteria for Valid Test	Test results	Criteria for Valid Test	Test results
Alfalfa	≥ 70%	83%	≥ 120 mm	**115.7**	≥ 40 mm	53.4
Barley	≥ 70%	**50%**	≥ 170 mm	277	≥ 150 mm	207
Carrot	≥ 60%	88%	≥ 80 mm	**63.3**	≥ 45 mm	**34.4**
Cucumber	≥ 60%	65%	≥ 120 mm	**95.04**	≥ 60 mm	72.7
Northern wheatgrass	≥ 70%	95%	≥ 110 mm	129.8	≥ 100 mm	131.1
Red fescue	≥ 70%	75%	≥ 70mm	**68.1**	≥ 80 mm	101.6

Bolded values are those below the criteria for valid test.

Table 9. Valid test requirements for criteria 1 - mean % emergence, criteria 4- root length and criteria 5 - shoot length observed in the coarse soil.

Species	Mean % emergence		Mean Root Length		Mean Shoot Length	
	Criteria for Valid Test	Test results	Criteria for Valid Test	Test results	Criteria for Valid Test	Test results
Alfalfa	≥ 70%	78%	≥ 120 mm	**84.4**	≥ 40 mm	71.1
Barley	≥ 70%	75%	≥ 170 mm	173.1	≥ 150 mm	214.9
Carrot	≥ 60%	**43%**	≥ 80 mm	**63.4**	≥ 45 mm	51.7
Cucumber	≥ 60%	**30%**	≥ 120 mm	**30.5**	≥ 60 mm	**46.4**
Northern wheatgrass	≥ 70%	90%	≥ 110 mm	**85.3**	≥ 100 mm	158
Red fescue	≥ 70%	**60%**	≥ 70mm	**56.9**	≥ 80 mm	89.0

Bolded values are those below the criteria for valid test.

Table 10. Valid test requirements for criteria 1 - mean % emergence, criteria 4- root length and criteria 5 - shoot length observed in the fine soil.

Species	Mean % emergence		Mean Root Length		Mean Shoot Length	
	Criteria for Valid Test	Test results	Criteria for Valid Test	Test results	Criteria for Valid Test	Test results
Alfalfa	≥ 70%	83%	≥ 120 mm	139.5	≥ 40 mm	50.0
Barley	≥ 70%	100%	≥ 170 mm	**161.6**	≥ 150 mm	200.5
Carrot	≥ 60%	63%	≥ 80 mm	**35.9**	≥ 45 mm	48.1
Cucumber	≥ 60%	95%	≥ 120 mm	**64.2**	≥ 60 mm	95.1
Northern wheatgrass	≥ 70%	95%	≥ 110 mm	**104.7**	≥ 100 mm	162.05
Red fescue	≥ 70%	90%	≥ 70mm	**46.8**	≥ 80 mm	106.4

Bolded values are those below the criteria for valid test.

3.3 Treatment Effects on % Emergence

The mean % emergence ± standard deviation at the end of the test for the individual species and soil type are summarized in Appendix B (Figures B-1 to B-6). One-way ANOVA was used to compare mean % emergence between the various Se and S treatments for each species and soil type, those with p-values ≤ 0.05 have treatments with significantly different mean % emergence (Table 11).

Table 11. One way ANOVA p-value for % emergence between the Se and S treatments for each species and soil type.

Soil Types	Plant Species					
	Barley	Red fescue	Alfalfa	Carrot	Cucumber	Northern wheatgrass
Artificial	0.19	0.06	0.80	0.65	0.07	0.08
Coarse	0.06	0.01*	0.00*	0.04*	0.00*	0.00*
Fine	0.63	0.24	0.99	0.04*	0.01*	0.47

*Those with p values ≤ 0.05 (or with an asterisk) have mean % emergence significantly different between the Se and S treatments

The effect of increasing Se concentration under various levels of S on the emergence of all six plant species are depicted in Figures 1, 2 and 3 for artificial, coarse and fine soil, respectively.

3.3.1 Artificial Soil

There were no significant differences in % emergence between the S and Se treatments in the six plant species used in the study (Figure 1). The emergence of barley was amongst the lowest (23% to 70%) followed by cucumber (60-95%) in the Se0 treatments. Increasing Se did not have a consistent effect on % emergence in the S-Control treatments; the % emergence was decreased for cucumber, carrot, and northern wheatgrass with increasing Se concentration, while % emergence was increased for barley and fescue at increasing Se concentration. The addition of S at all three levels did have a positive (not significant) effect on the emergence of barley and cucumber, especially at the highest Se concentration (Se3). This effect was also observed for Northern wheatgrass, alfalfa and carrot but only in Se3 and S-High treatments.

3.3.2 Coarse Soil

The % emergence data for the coarse soil was more variable compared to the other two test medium. There were significant differences found in % emergence between the S and Se treatments in five out of six plant species; the exception was barley (Figure 2). Carrot consistently had higher % emergence with the addition of S, especially for Se2 and Se3 treatments. The addition of S decreased % emergence of alfalfa at all Se concentrations. The effect of S on carrot was positive only at the Se3 treatment but it was not consistent at the lower Se concentrations. The effect of Se was positive for cucumber under no or low S concentration; However, when S and Se were both added at higher concentrations, the % emergence decreased significantly.

3.3.3 Fine Soil

% Emergence in fine soil was the highest out of the three test medium used for all six plant species (Figure 3). Overall, the addition of Se and S did not significantly impact the % emergence, only carrot and cucumber saw significant differences in % emergence between the various S and Se treatments. The emergence of carrot seeds has a positive relationship with Se concentration at all S levels, while the emergence of cucumber responded negatively to the Se2 S-High treatment only.

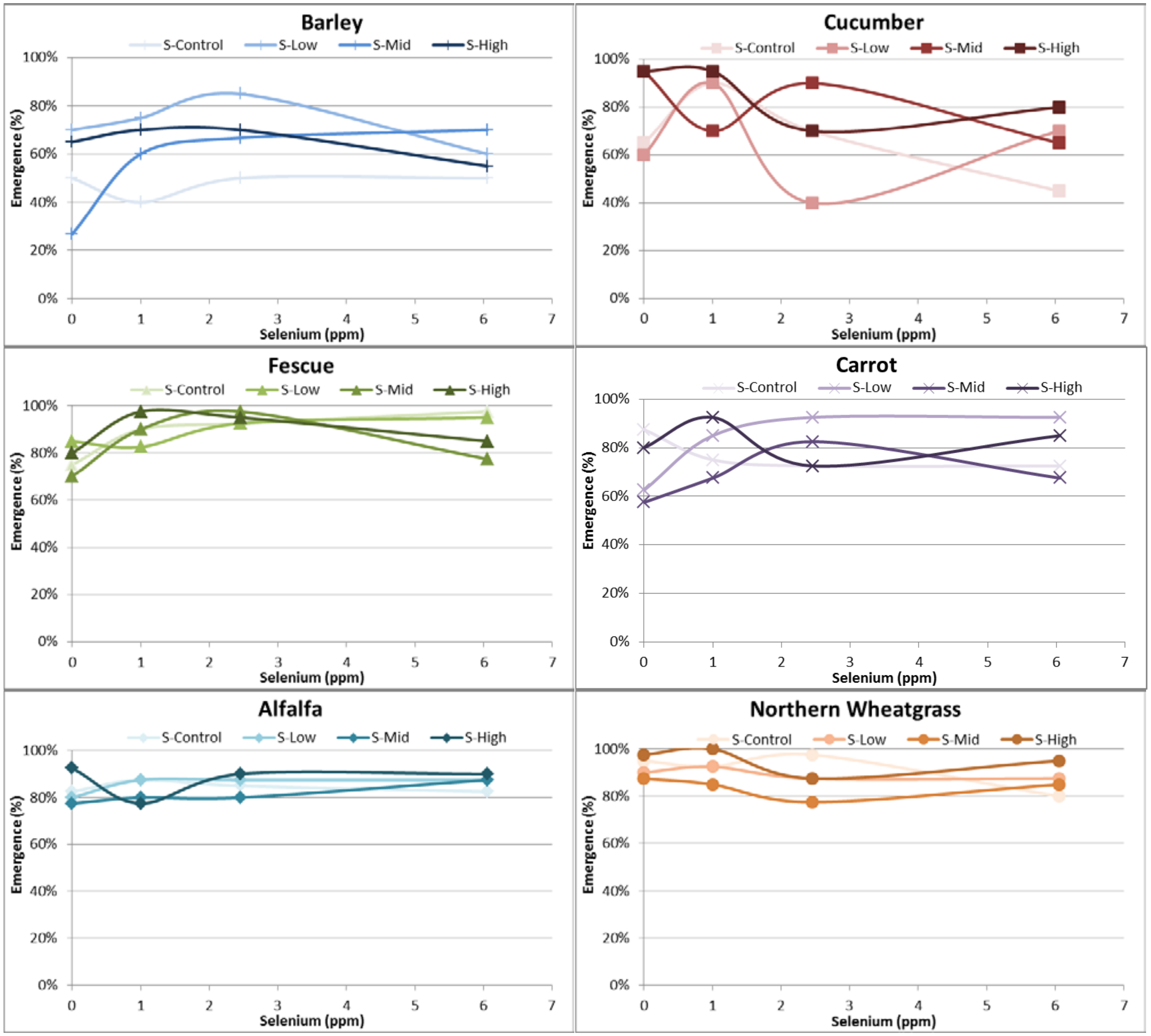

Figure 1. Plant species emergence response curves for artificial soils spiked with increasing levels of selenium under 4 sulfur concentrations.

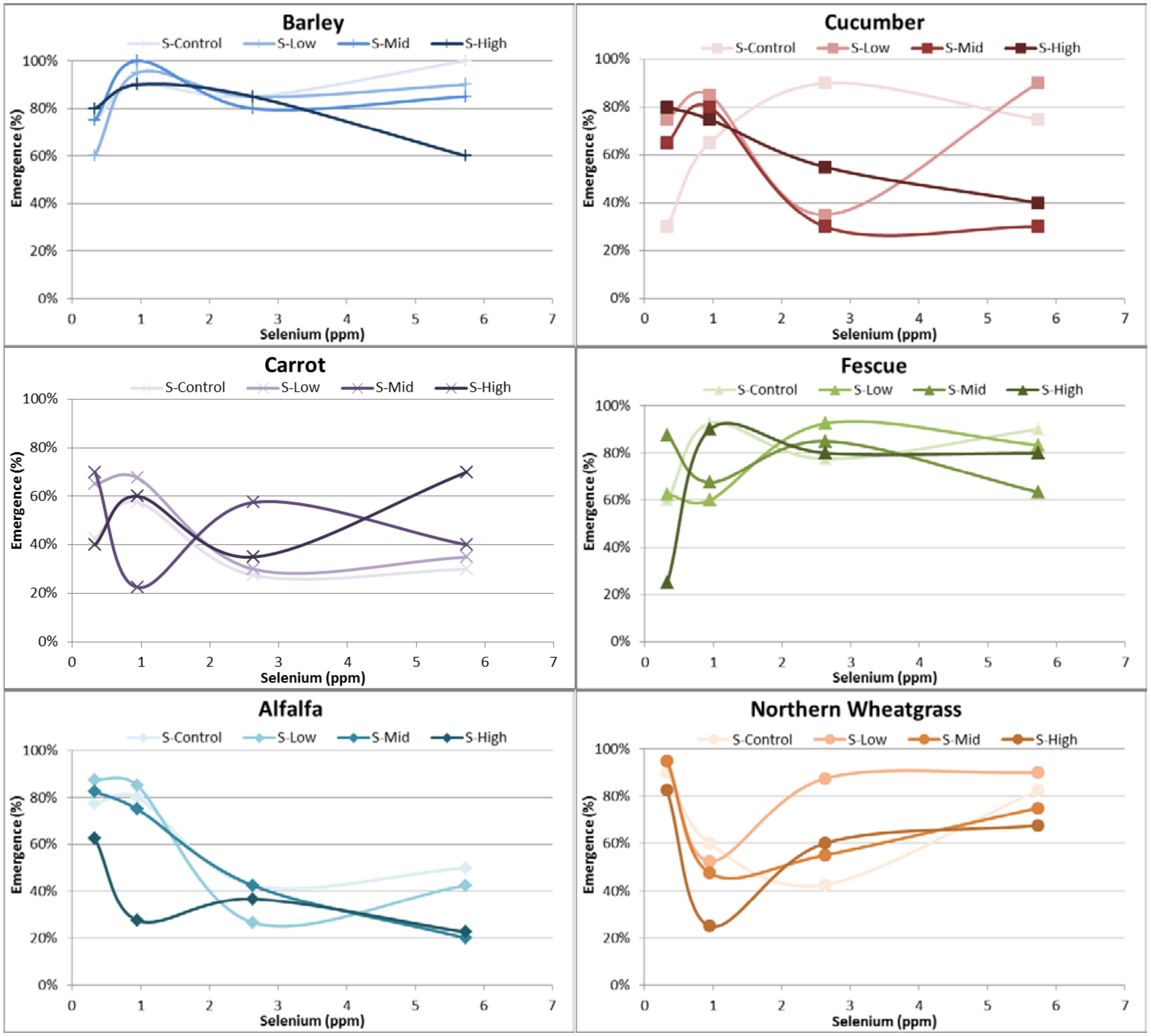

Figure 2. Plant species emergence response curves for coarse soils spiked with increasing levels of selenium under 4 sulfur concentrations.

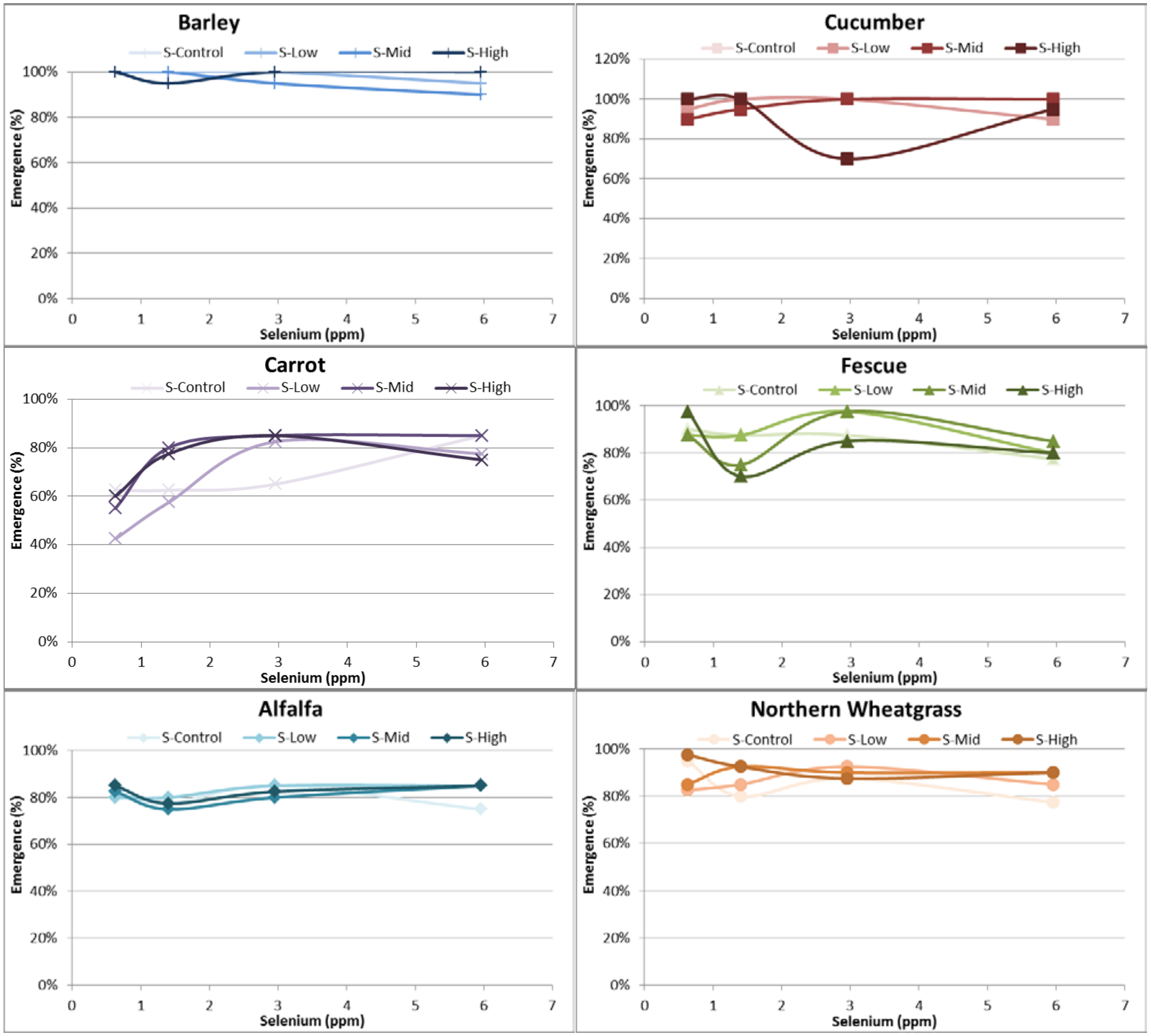

Figure 3. Plant species emergence response curves for fine soils spiked with increasing levels of selenium under 4 sulfur concentrations.

3.4 Treatment Effect on Growth Endpoints

3.4.1 Belowground biomass

The mean root length and weight ± standard deviation at the end of the test for the individual species and soil type are summarized in Appendix B (Figures B-7 to B-12). One-way ANOVA was used to compare root length and weight between the various Se and S treatments for each species and soil type, those with p-values ≤ 0.05 have treatments where the measured endpoints were significantly different (Tables 12).

Table 12. One way ANOVA p-value for root length and root weight between the Se and S treatments for each species and soil type.

	Artificial Soil		Coarse Soil		Fine Soil	
	Root length	Root weight	Root length	Root weight	Root length	Root weight
Barley	0.044*	0.025*	0.00053*	0.0046*	0.0087*	0.0091*
Red fescue	0.0012*	0.018*	0.014*	4.4E-05*	0.28	0.039*
Alfalfa	0.023*	0.00022*	3.8E-10*	1.6E-05*	0.00047*	6.0E-06*
Carrot	1.8E-06*	0.14	0.00088*	0.0046*	0.00022*	0.0023*
Cucumber	0.0081*	0.0072*	0.12	0.0033*	0.022*	0.0063*
Northern wheatgrass	4.6E-07*	0.0053*	0.0024*	3.1E-06*	1.5E-05*	0.0021*

* Those with p values ≤ 0.05 (or with an asterisk) have mean root length significantly different between the Se and S treatments.

3.4.1.1 Artificial Soil

There were significant differences found in root length between the S and Se treatments in the artificial soil for all six plant species (Table 12 and Figure 4). The response to the toxicity of Se was inconsistent amongst the species; in fact, root lengths were significantly increased for fescue and carrot at the 1 and 2.63 ppm Se concentration. The hormetic effect of Se was observed here where low concentrations of Se caused a stimulatory response (higher root length) and decreased Se toxicity. The antagonistic effect of S on Se toxicity was inconsistent at the lower Se concentrations in all species. However, for all plant species (with the exception of barley) the addition of S increased root length compared to the S-Control under elevated Se concentrations (Se3=6.05ppm) (Figure 4).

All species, with the exception of carrot, also saw a significant increase in root biomass within Se spiked treatments when additional S was added (Figure 5). The most dramatic increase in root biomass was observed in barley, which is opposite of what was observed in the corresponding root length data (Figure 5). The data suggest that under elevated Se concentrations, the addition of S minimized the negative effect on root biomass in barley, fescue, alfalfa, and cucumber and northern wheatgrass. The hormetic effect of Se was observed in barley, fescue, alfalfa and northern wheatgrass, where low concentrations of Se (Se1) caused a stimulatory response (higher root biomass). For both root length and weight, the antagonistic effect of S on Se toxicity was most obvious when Se level was the highest (Se3).

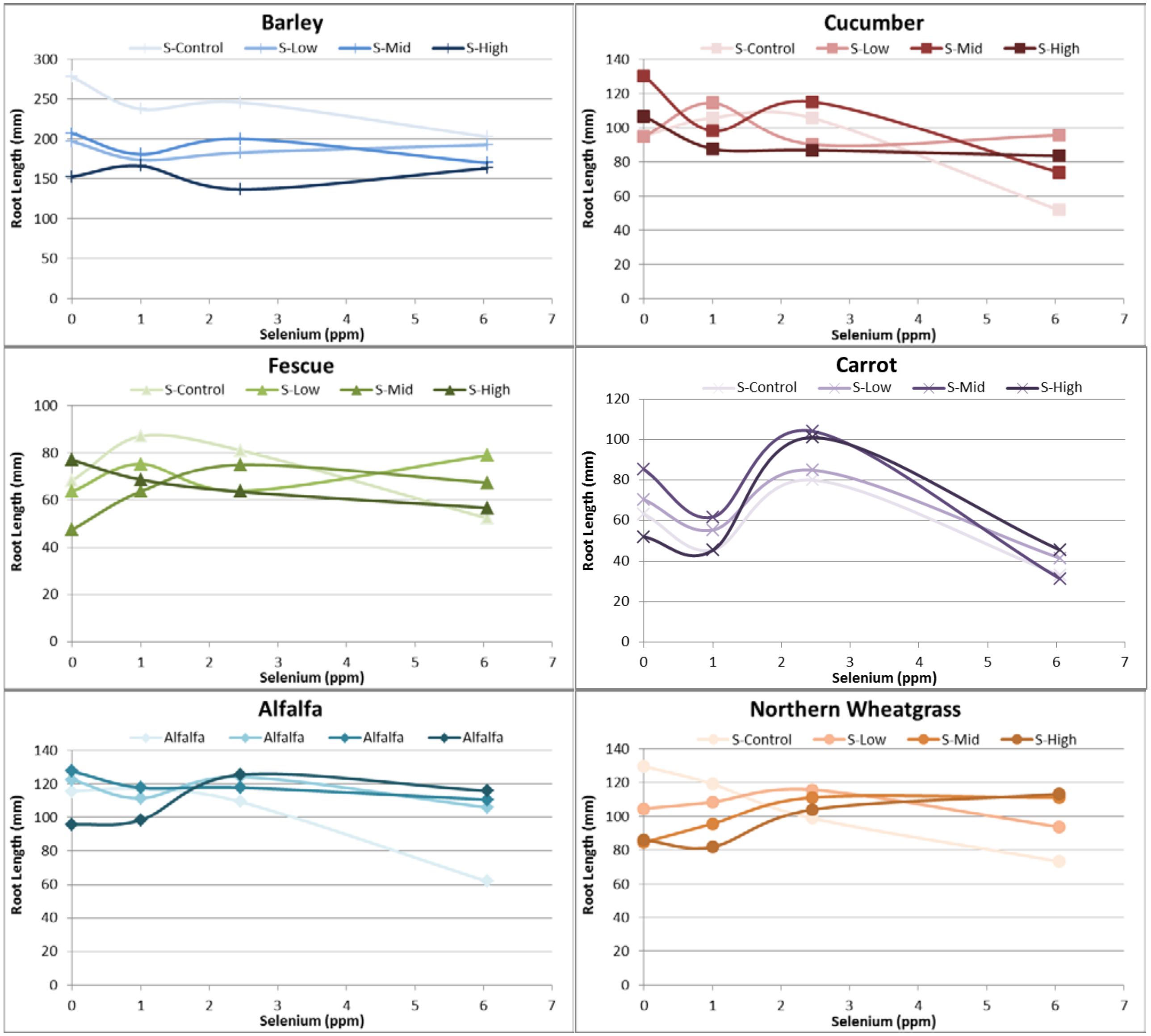

Figure 4. Plant species root length response curves for artificial soils spiked with increasing levels of selenium under 4 sulfur concentrations.

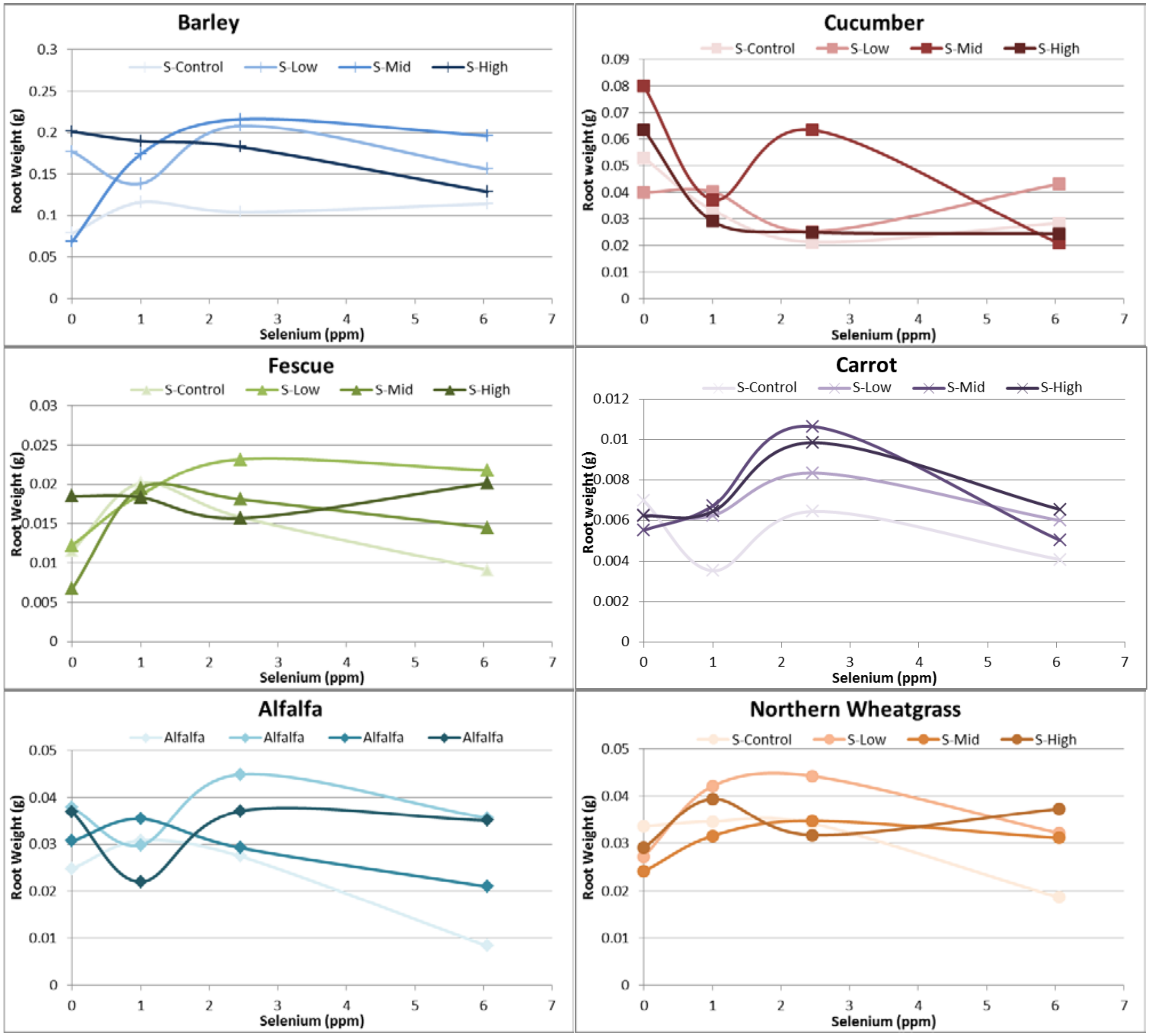

Figure 5. Plant species root weight response curves for artificial soils spiked with increasing levels of selenium under 4 sulfur concentrations.

3.4.1.2 Coarse Soil

The toxicity of Se was more consistent in the coarse soil; the root length and weight for all species (except cucumber at Se1 concentration) significantly decreased with increasing Se under S-control treatments (Figures 6 and 7). The addition of S significantly increased root length for Se spiked treatments in five out of six species at the highest Se concentration (Se3) (Figure 6). Cucumber was the only plant species where the response in root length was not statistically significant. On the other hand, barley had the greatest increase in root length; 155% increase in root length was observed for the highest Se concentration when 0.05% S was added to the soil (Figure 6).

Barley and northern wheatgrass saw the greatest increase in root weight when S was added to the Se spiked soils (Figure 7). The antagonist effect of S and Se on root length and weight was most evident at S-Low (0.05%) level for most species. Due to the lack of exchange sites in the coarse soil, the addition of S, especially at the S-High treatment, created water stress on the plants because of the higher salt content, thus possibly negating any positive benefits from S addition.

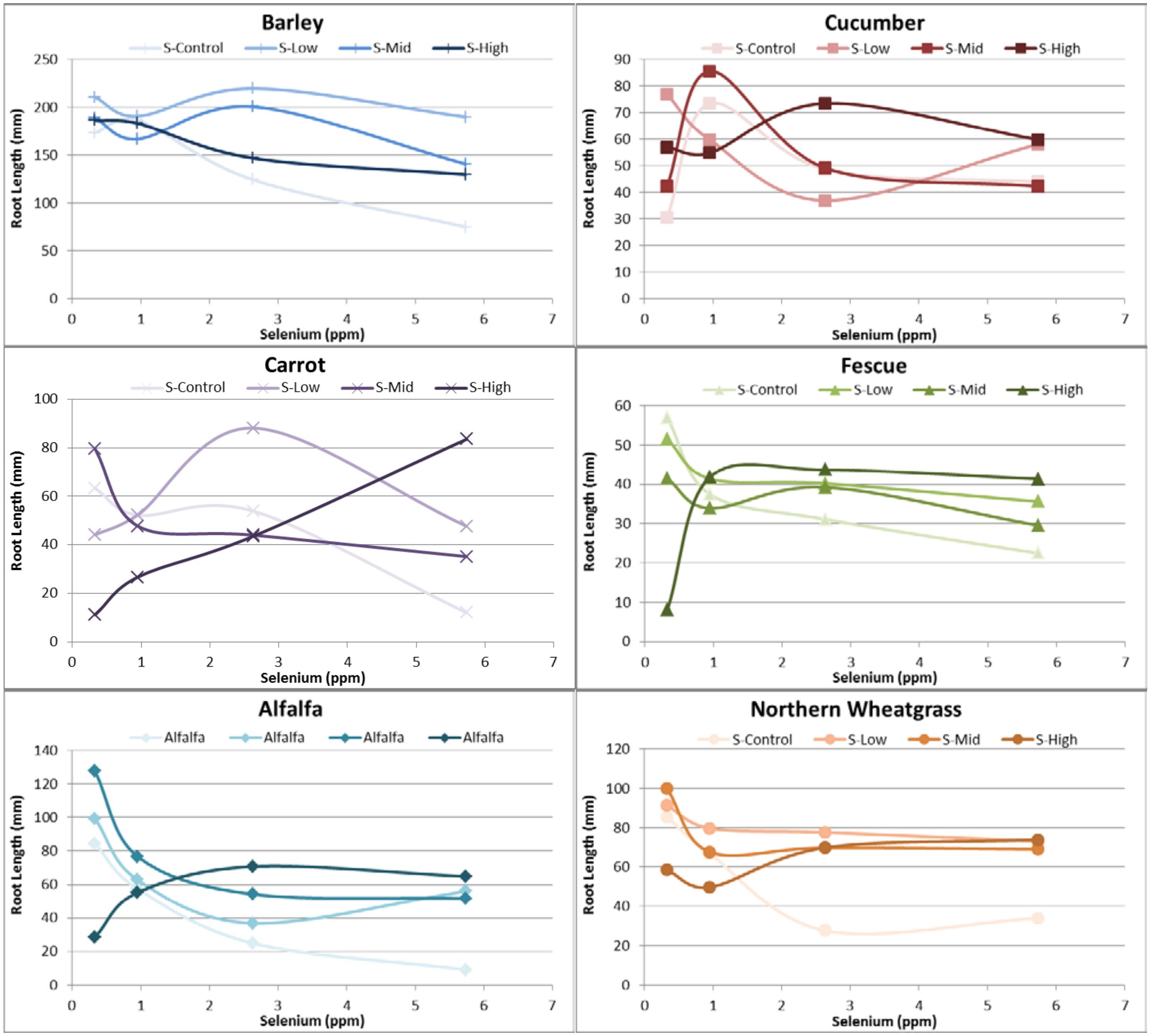

Figure 6. Plant species root length response curves for coarse soils spiked with increasing levels of selenium under 4 sulfur concentrations.

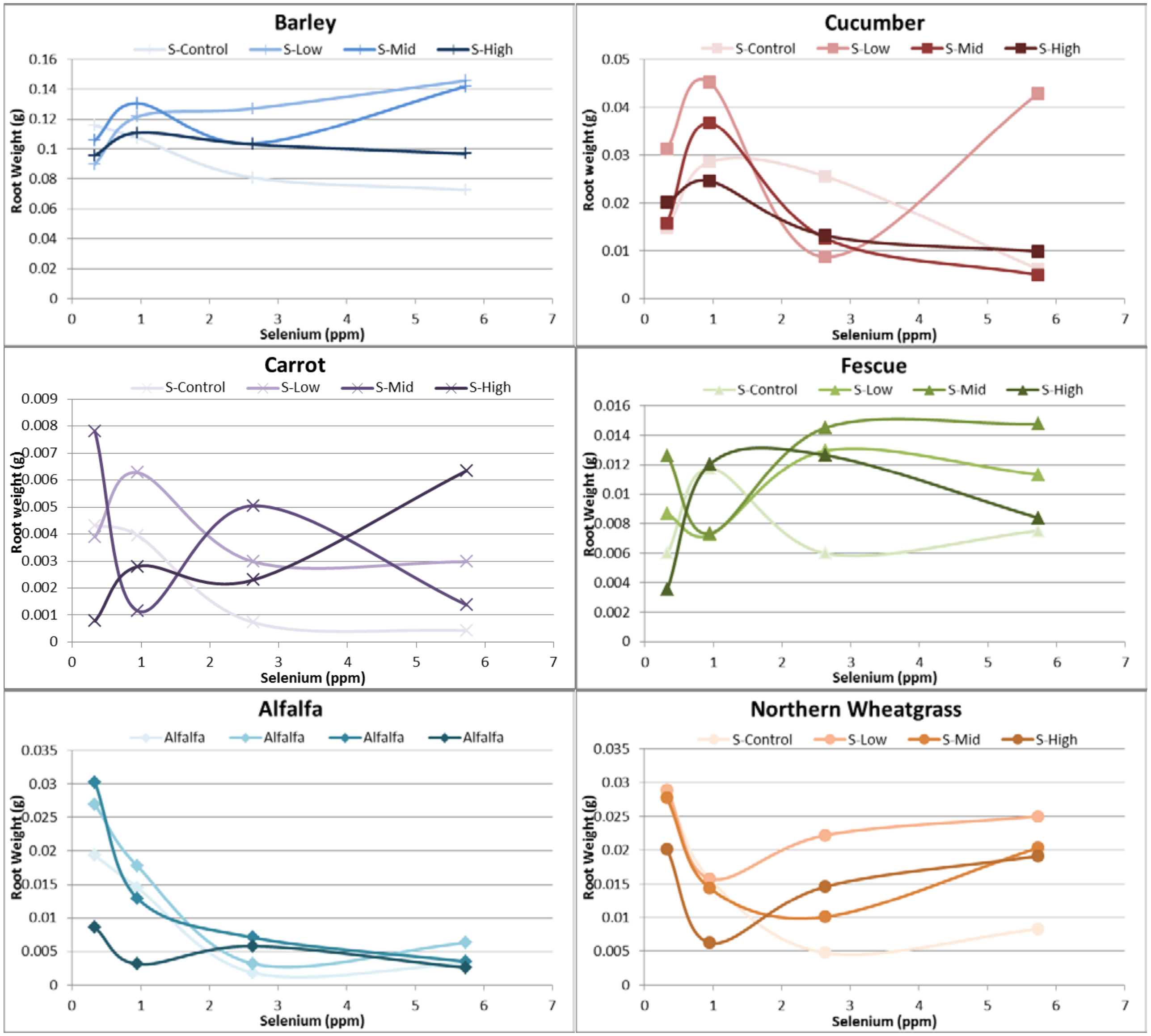

Figure 7. Plant species root weight response curves for coarse soils spiked with increasing levels of selenium under 4 sulfur concentrations.

3.4.1.3 Fine Soil

Similar to the artificial soil, the response to the toxicity of Se was inconsistent amongst the species (Figure 8). Although the increase in root length may not be statically significant, hormetic effect of Se was observed in four species (barley, carrot, cucumber and fescue), where under Se1 and/or Se2 concentration, Se caused a stimulatory response (higher root length). At the highest Se concentration (Se3), all species (except cucumber) had a significantly lower root length compared to Se0 for the S-Control treatments. The antagonistic effect of S and Se was most evident in alfalfa and barley, where the length of roots increased most significantly (Figure 8). The increase in alfalfa root length ranged from 317% (S-Low) to 438% (S-Mid), while the increase in barley root length ranged from 102% (S-Low) to 142% (S-High). There was no difference in root length between treatments for red fescue, while the effect of S addition in Se spiked soil was inconsistent in carrot. Overall, the toxic effect of Se on root length was most evident at the highest Se concentration (Se3) for all species except cucumber, and the antagonistic effect of Se and S was also most evident at that concentration.

For all species, the toxic effect of Se on root weight resulting in the greatest reduction was observed at the highest Se concentration (Se3); hermetic effect of Se was obvious in barley and cucumber at Se2 and Se1 concentration, respectively (Figure 9). Although not statically significant for all species, the addition of S (at all 3 concentrations) increased root weight in every Se3 treatments. The antagonist effect between Se and S was not consistent amongst all species at S-Low and S-Mid levels.

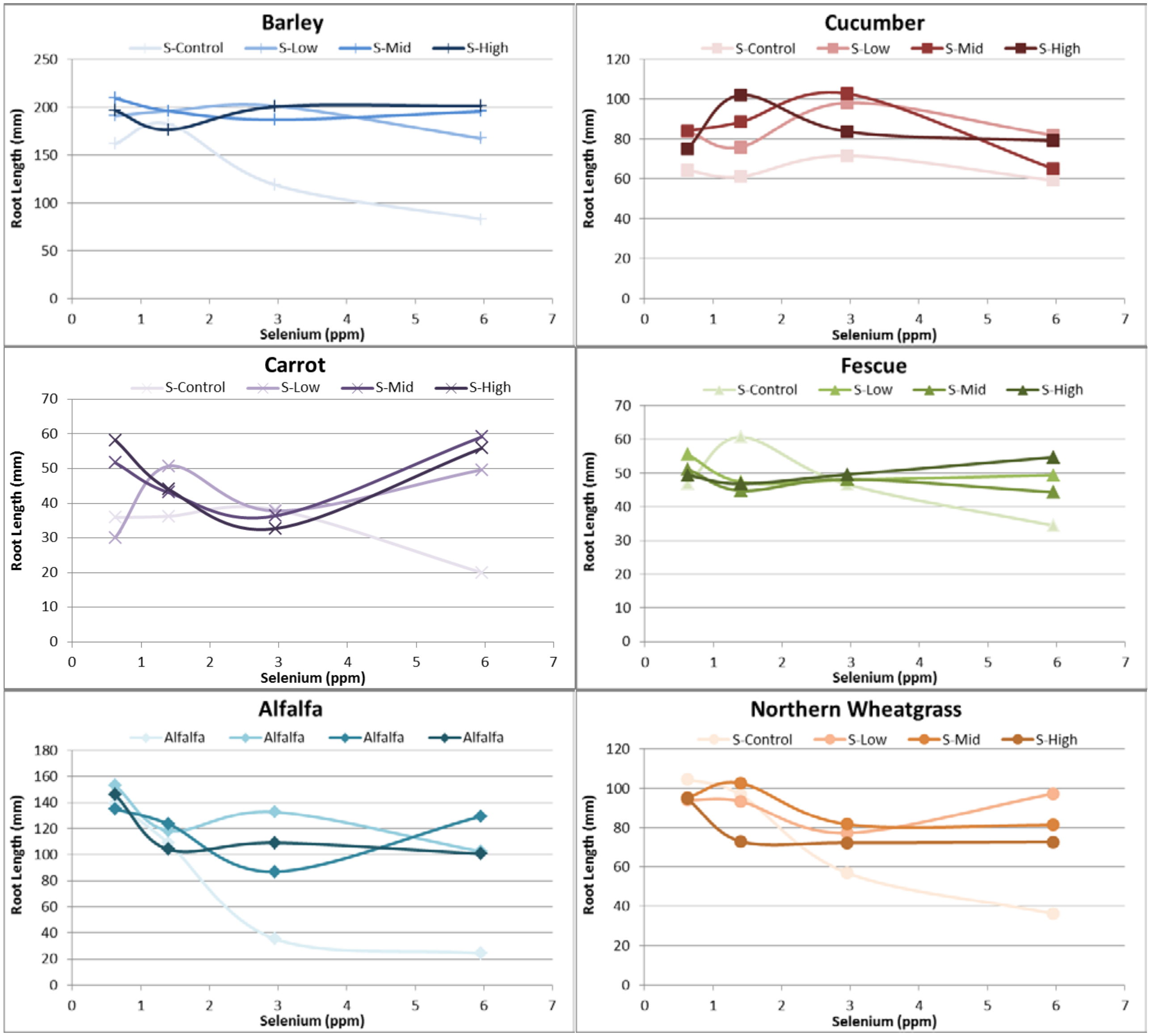

Figure 8. Plant species root length response curves for fine soils spiked with increasing levels of selenium under 4 sulfur concentrations.

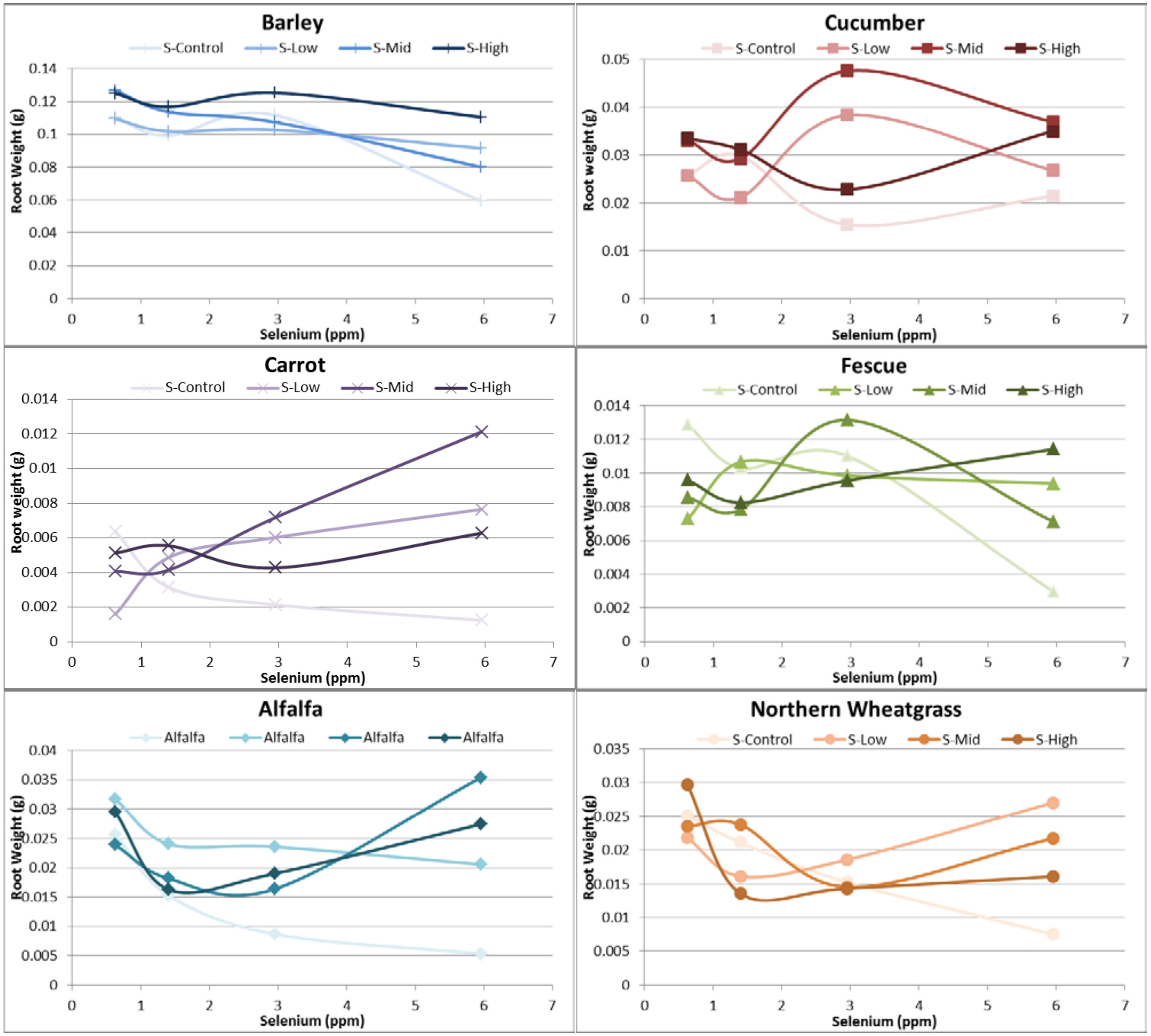

Figure 9. Plant species root weight response curves for fine soils spiked with increasing levels of selenium under 4 sulfur concentrations.

3.4.2 Aboveground biomass

The mean shoot length and weight ± standard deviation at the end of the test for the individual species and soil type are summarized in Appendix B (Figures B-7 to B-12). One-way ANOVA was used to compare shoot length and weight between the various Se and S treatments for each species and soil type, those with p-values ≤ 0.05 have treatments where the measured endpoints were significantly different (Table 13).

Table 13. One way ANOVA p-value for shoot length and shoot weight between the Se and S treatments for each species and soil type.

	Artificial Soil		Coarse Soil		Fine Soil	
	Shoot length	Shoot weight	Shoot length	Shoot weight	Shoot length	Shoot weight
Barley	0.16	0.035*	0.025*	0.0022*	0.0055*	0.0071*
Red fescue	0.00065*	3.3E-04*	0.00078*	9.2E-09*	0.00058*	0.0016*
Alfalfa	0.073	0.16	8.8E-05*	1.0E-05*	0.0099*	0.0067*
Carrot	0.014*	0.006*	0.0027*	0.0015*	0.016*	0.011*
Cucumber	0.091	0.0096*	0.013*	0.0047*	0.0052*	0.0046*
Northern wheatgrass	0.00060*	0.0107*	0.00012*	4.1E-05*	0.0022*	2.3E-08*

* Those with p values ≤ 0.05 (or with an asterisk) have mean shoot length significantly different between the Se and S treatments.

3.4.2.1 Artificial Soil

There were significant differences found in shoot length between the S and Se treatments in three of the six plant species tested in the study (red fescue, carrot and northern wheatgrass) (Table 13, Figure 10). Without any addition of S (S-Control), Se significantly reduced shoot length in carrot (Se2), fescue (Se3) and northern wheatgrass (Se3) compared to their corresponding Se0 treatment (Figure 10). The addition of S increased (not significantly) the shoot length in cucumber, fescue, carrot, alfalfa and northern wheatgrass for the Se3 treatment (Figure 10). Conversely, the addition of S decreased shoot length for barley compared to the control. The effect of S on shoot length was inconsistent in carrot, at 0.07% S (S-Low), the effect on shoot length was positive while, it was negative for S-Mid and S-High treatments. Se caused a stimulatory response (higher shoot length) in all species, in either Se1 or Se2 treatments.

There were also significant differences found in shoot weight between the S and Se treatments in all but alfalfa (Figure 11). The effect of S and Se on aboveground biomass was highly variable amongst the species, the increase in Se only significantly reduced shoot weight in northern wheatgrass. Overall, the toxicity of Se on the other species was not consistently observed through a reduction in shoot weight. All six species observed the stimulatory response (higher shoot weight) with the addition of Se, most evident at the Se1 and Se2 levels. It was noted that some S treatments did have a positive effect on shoot weight, however the antagonistic relationship between Se and S was not clear. The addition of S (regardless of concentration added) did not consistently alleviated the toxic effects of elevated Se (Se3=6ppm) in all species.

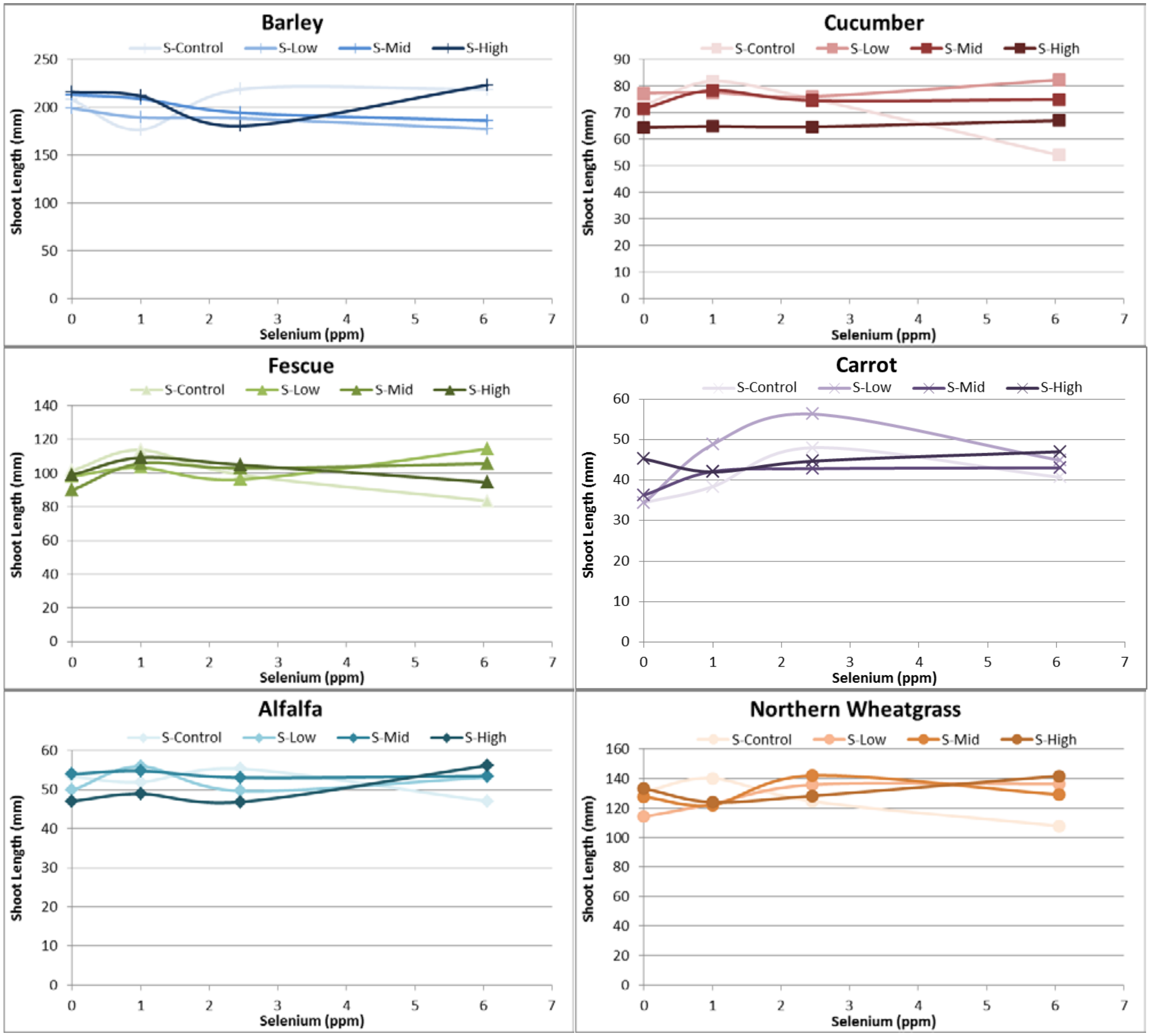

Figure 10. Plant species shoot length response curves for artificial soils spiked with increasing levels of selenium under 4 sulfur concentrations.

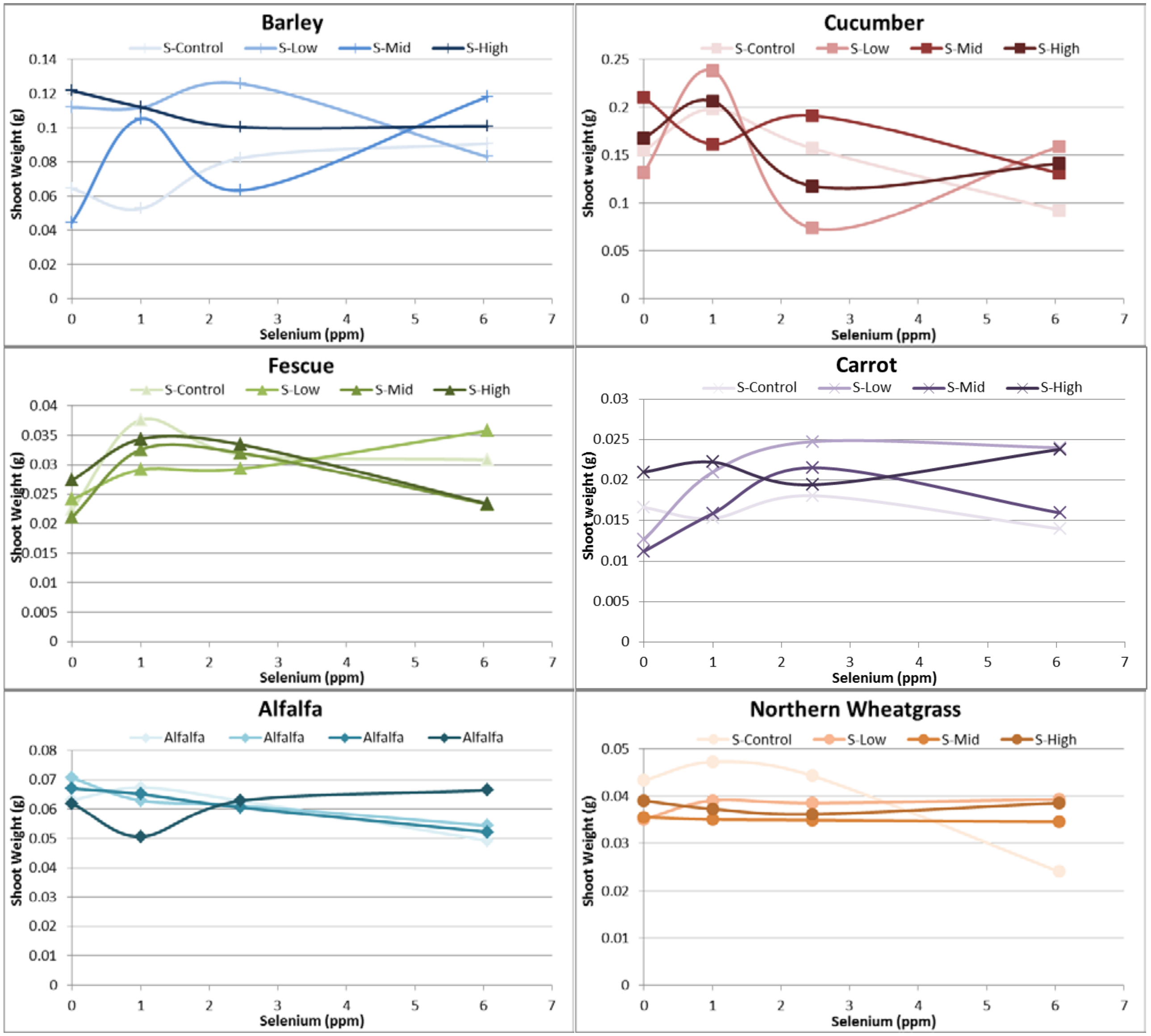

Figure 11. Plant species shoot weight response curves for artificial soils spiked with increasing levels of selenium under 4 sulfur concentrations.

3.4.2.2 Coarse Soil

The toxicity of Se was most evident in the coarse textured soil. For all species, a significant reduction in shoot length was observed in the Se3 treatment with no S added (S-Control), for some species (alfalfa and northern wheatgrass) this was also observed under the Se2 treatment (Figure 12). A few species, namely cucumber and fescue, did respond positively (through increase in shoot weight) to low levels of Se, but the hormetic effect of Se was not consistent in the coarse soil. Overall, the addition of S had a positive or neutral effect on the shoot length in all Se spiked treatments (Figure 12). The greatest increase in shoot length with the S addition was observed in grass species (barley, northern wheatgrass and red fescue). There was no consistent trend on the level of S that yielded the greatest antagonistic response with Se.

Similar to shoot length, there is a direct correlation between the decrease in shoot weight and the increase in Se concentration without any S addition (Figure 13). However, the S-High treatments (without any Se), also significantly reduced shoot weight in fescue, alfalfa and northern wheatgrass. The higher EC associated with S-High treatments may have also adversely affected the growth of those species. Therefore the antagonistic effect of S and Se was most evident at S-Low treatments for the majority of the species. For example, at Se3 (6ppm) concentration, the addition of 0.05% S (S-Low) treatment saw a 230% increase in shoot weight in cucumber, while the 0.2% S (S-High) treatment only saw a 5% increase in shoot weight compared to the S-Control treatment.

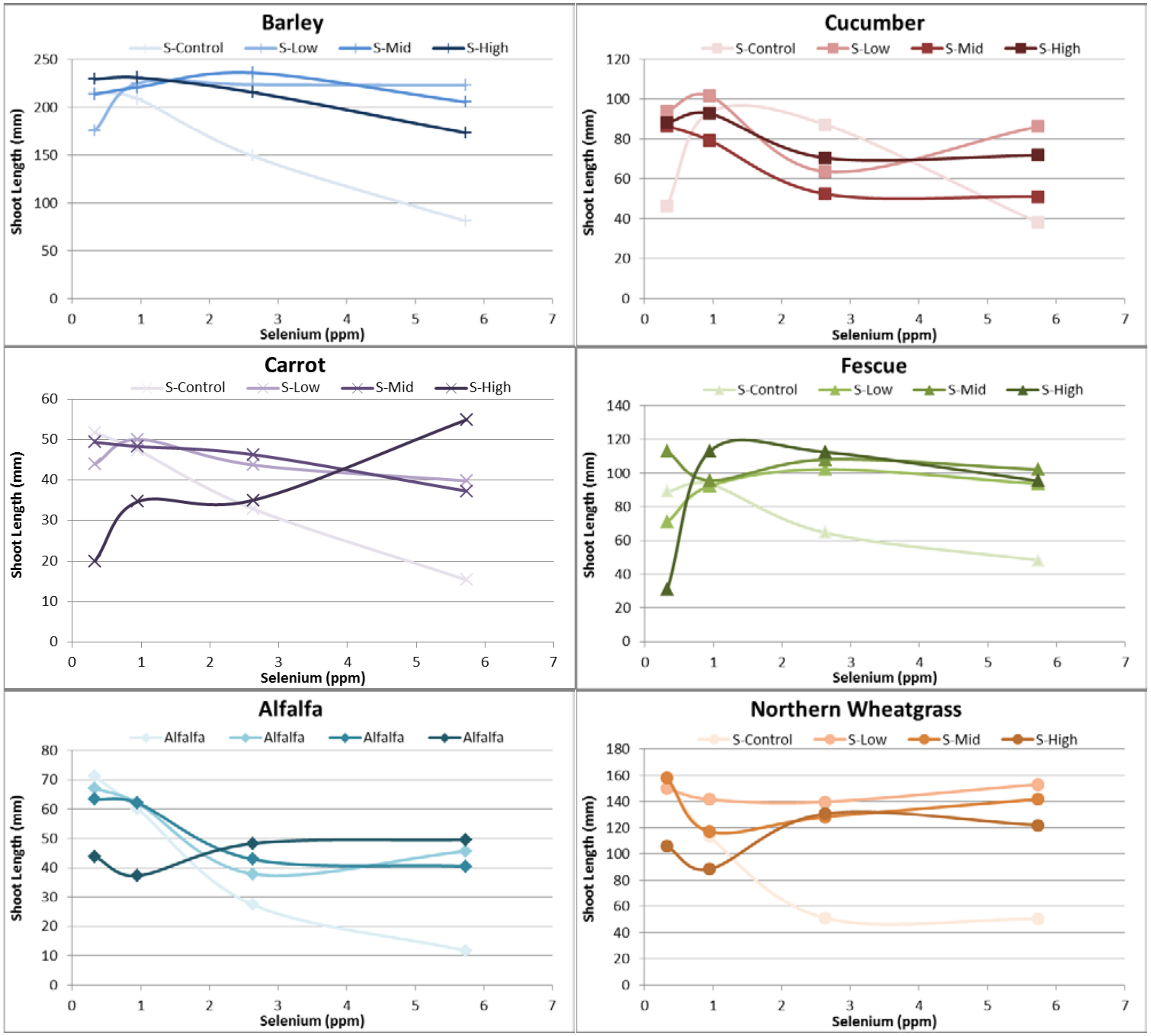

Figure 12. Plant species shoot length response curves for coarse soils spiked with increasing levels of selenium under 4 sulfur concentrations.

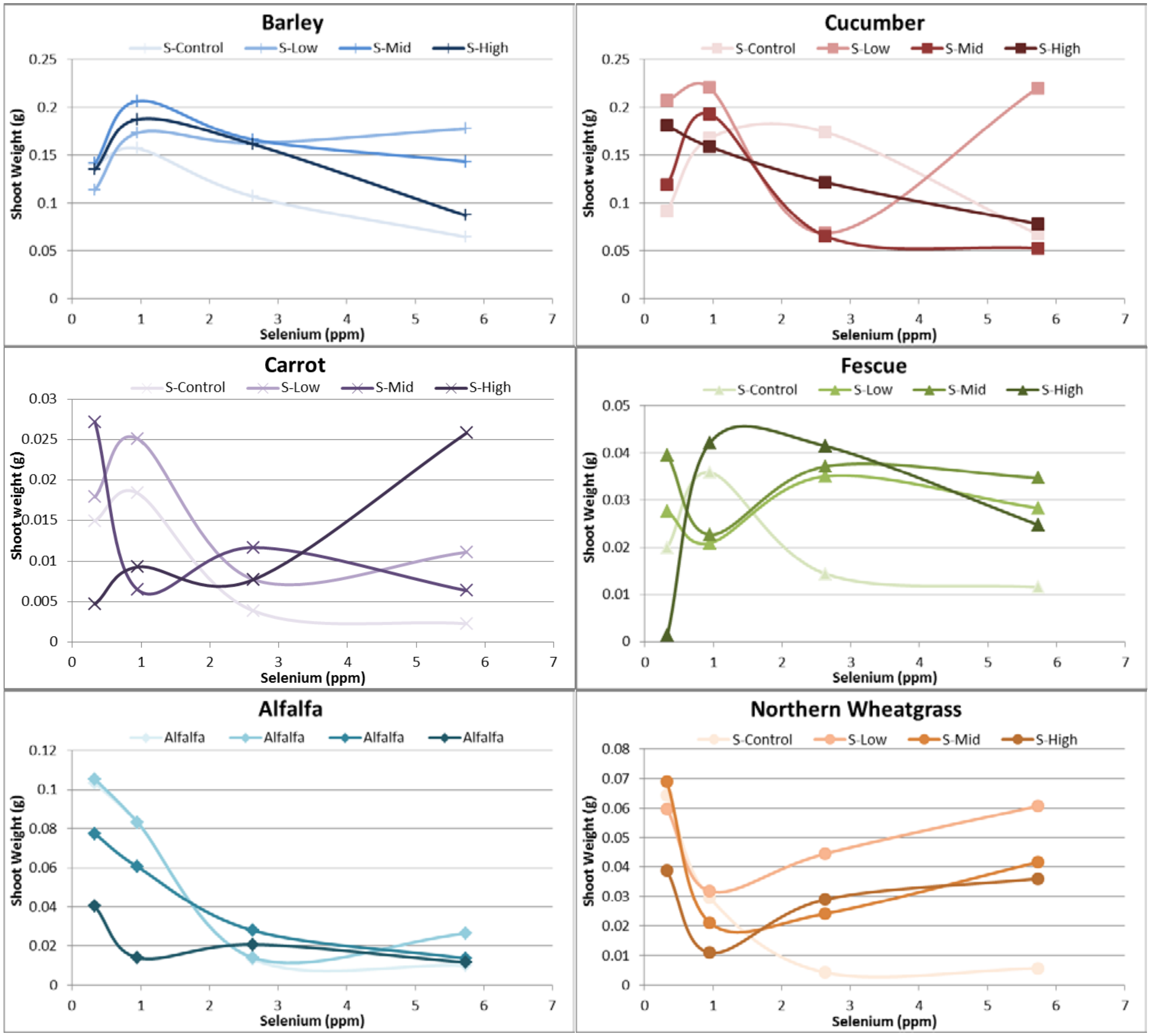

Figure 13. Plant species shoot weight response curves for coarse soils spiked with increasing levels of selenium under 4 sulfur concentrations.

3.4.2.3 Fine Soil

The toxicity of Se on all species grown in fine textured soil was expressed through a reduction in aboveground biomass; both the shoot length and weight were significantly reduced under Se3 and S-Control treatments. The addition of S (at all three levels) consistently increased (not significantly) shoot length and weight for all species at the highest Se concentration (Se3). There were a few anomalies with this data, particularly for fescue and carrot, where a reduction in shoot length and weight were observed as a result of S and not Se, however this was only evident at Se0 concentration. The antagonistic effect of Se and S was evident in all species, the three S levels yielded similar response across the species tested.

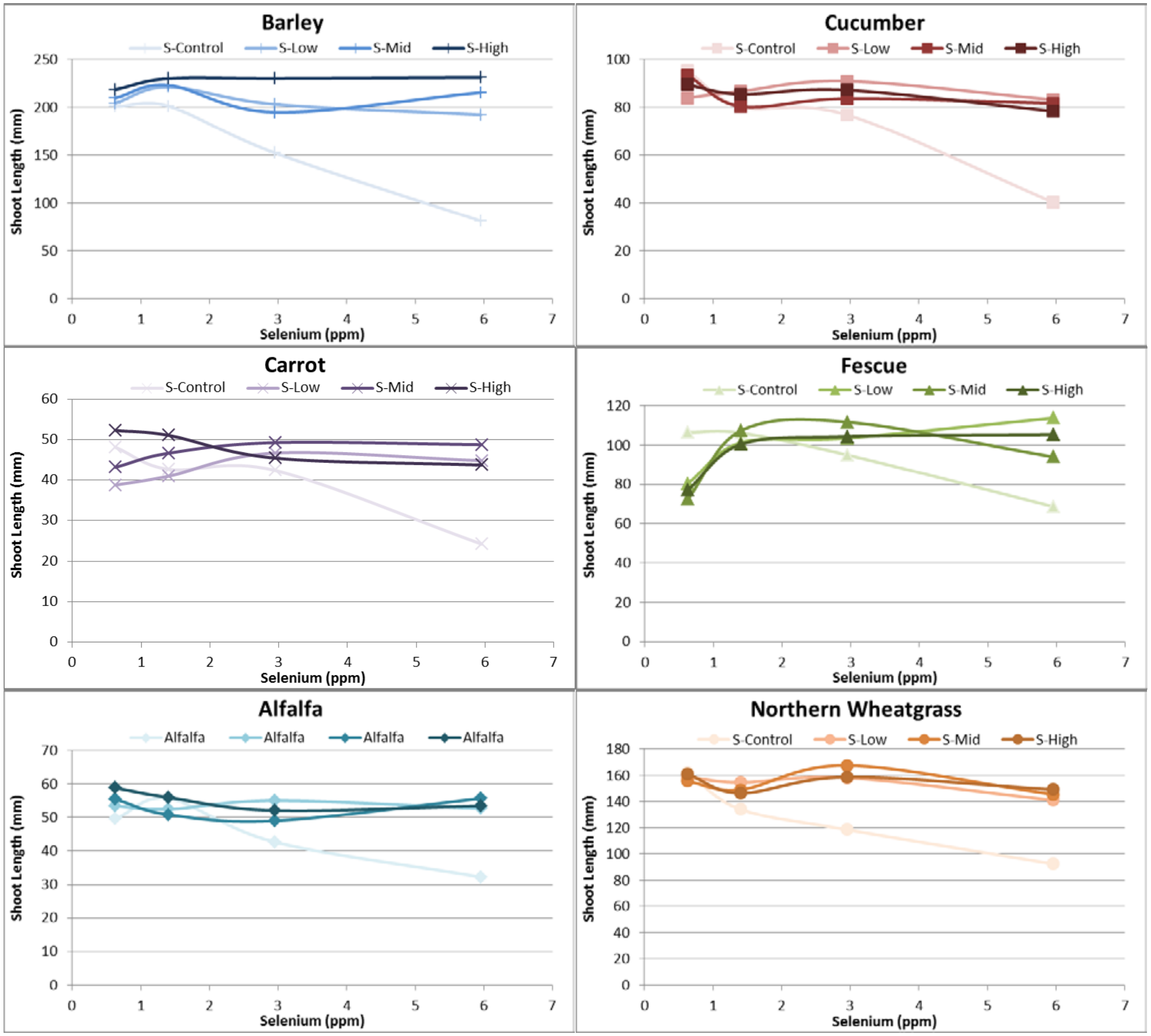

Figure 14. Plant species shoot length response curves for fine soils spiked with increasing levels of selenium under 4 sulfur concentrations.

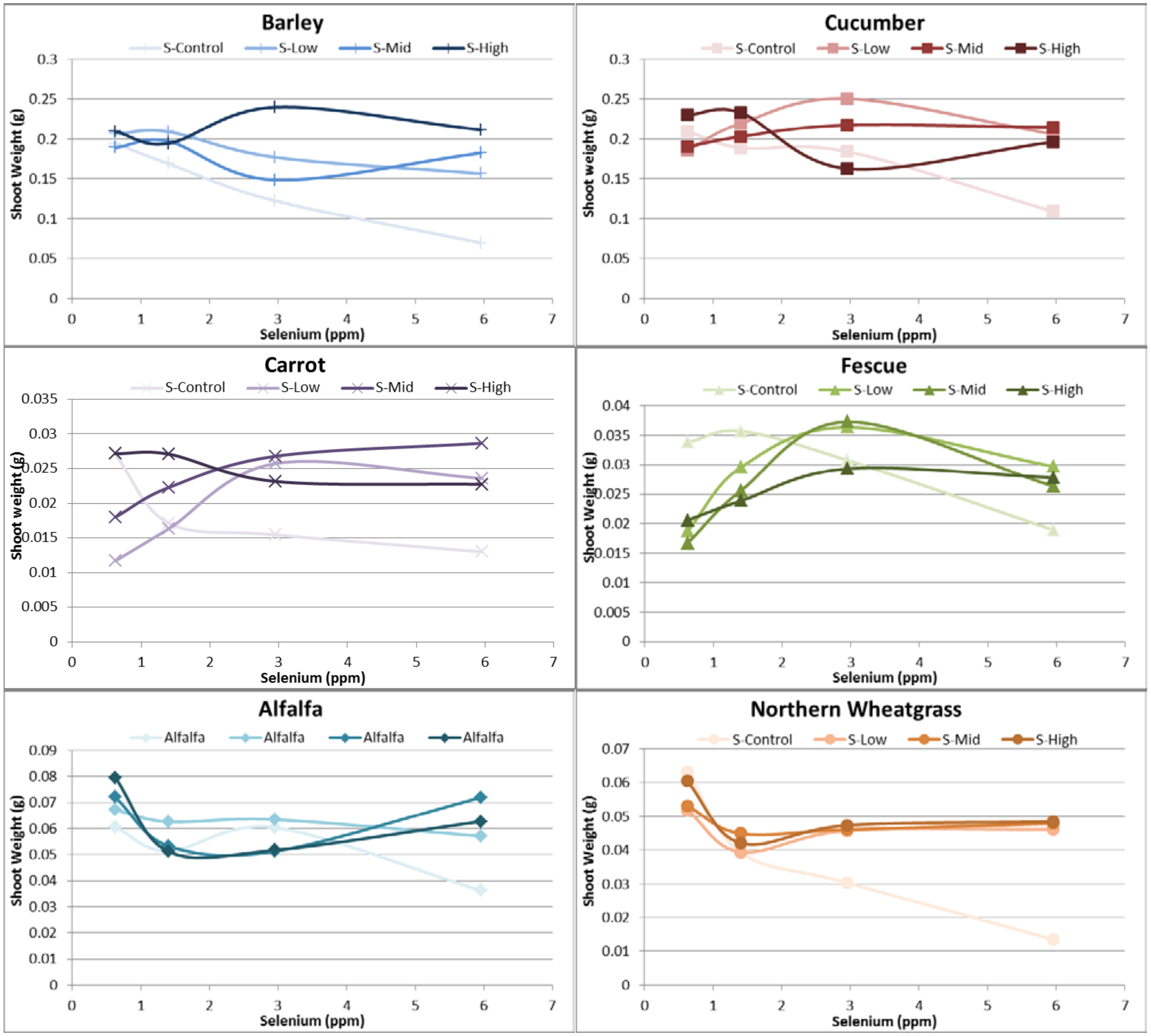

Figure 15. Plant species shoot weight response curves for fine soils spiked with increasing levels of selenium under 4 sulfur concentrations.

4.0 Discussion

4.1 Plant physiological response to Se

The toxicity effect of Se on % emergence was inconsistent amongst the six species and the three soil types. The variability was highest in the coarse soil, followed by artificial and fine soils. For the species tested, the % emergence was lowest in the coarse soil especially in the S and/or Se spiked treatments. In the coarse soil, the antagonist effect of S and Se was only observed in northern wheatgrass and carrot, however the resultant change in % emergence was not significant. Furthermore, the addition of S generally decreased % emergence in the coarse soil. It's likely that the increase in soil EC from spiking soil with S may have impacted the seed's viability especially in the coarse soil. Overall, the antagonist effect of Se and S was not consistent in % emergence for the species tested and the % emergence in the coarse soil was poor due to an increase in soil EC.

One of the morphological symptoms of Se toxicity is decreased root length (Hartikainen et al., 2001), as observed in this study in most of the Se3 treatments grown under soil with no Sulfur (S-Control). The decrease in root length as a result of Se toxicity was most prominent in the fine textured soil, as the resultant change in root length was statistically significant in all species when compared to the control (Se0). The antagonistic relationship between S and Se was observed through an increase in root length in Se3 S-High treatments. This effect was evident in the all species and soil types but the increase was only consistently significant in the fine textured soil.

As observed in this study, the reduction in root length often did correspond to reduction in root weight. Although the magnitude of the reduction in root weight was not consistent with the reduction in root length especially in the coarse textured soil. Studies have reported an increase in root width with associated decrease in root length as a result of Se toxicity (Hartikainen et al., 2001). This is not unexpected since excessive Se and S in the plant is known to enhance ethylene production; changes in root morphology, such as increased root width, is common for plants dealing with stress related to increased ethylene production (Hartikainen et al., 2001). The antagonistic relationship between S and Se was demonstrated through an increase in root weight in the Se3 and S-High treatments. Similar to root length, the most significant increase in root weight was observed in those grown in fine textured soil.

Changes to root morphology due to Se toxicity had a significant impact on shoot growth as observed in this study; there was a good correlation between the below-ground biomass data and those collected for above-ground. Se toxicity was also demonstrated through a reduction in both shoot length and weight in treatments without S. The antagonist relationship between Se and S was also observed in the above-ground biomass data, and the reduction in Se toxicity was most evident in the Se3 and S-High treatments. Overall, changes to root morphology as a result of Se toxicity likely lead to inefficient uptake in water, nutrient and phytosynthates thus impacting the growth of aboveground biomass.

Overall, the data from this study suggests toxicity of Se can be reduced by increasing S concentration (up to 0.02% S) in the artificial and fine textured soil. The antagonistic effect of Se and S occurred at lower S concentration (0.05% S) for the coarse textured soil as a result of the negative effects from higher EC and SAR due to increasing S. In most species, Se is thought to enter root cells through S transporters in the plasma membrane (Brown and Shrift 1982; White et al. 2004). However, it is unclear whether the high S concentrations in the rhizosphere reduced Se uptake by the plants through selectivity or the increase in S uptake changed the Se/S ratio within the plant tissue thus reducing the incorporation of Se into the synthesis of plant proteins. The data from this study supports the theory that the toxicity of Se was reduced by the presence of elevated S in the rhizosphere, however, the threshold for S to have the antagonistic response with elevated Se may be different depending on soil texture.

The data collected in this study supports the theory of hormetic effect of Se as discovered in previous work by Prediger et al., (2012), where low concentration of Se (Se1 = 1 ppm and/or Se2=2.5 ppm) caused a stimulatory response in plants. The hormetic effect of Se was notable in all species and soil types, and often result in higher (not always significantly) plant biomass. This study has shown that Se can be a beneficial element, and at low levels (<1 or 2 ppm), it can have a positive effect on plant growth.

4.2 Soil Texture

The emergence and growth of the plants in the coarse soil was considerably poorer in comparison to the fine and artificial soils. Due to the lack of exchange sites in the coarse textured soil, the effect of higher EC in the soil through the addition of S and Se may have inflicted stress on the plants. As mentioned previously, the EC for the coarse soil was in the Fair range according to the Alberta Tier 1 Remediation Guidelines (Alberta Environment, 2010). However, the seedlings may be more sensitive to the differences between the osmotic potential of the internal and external (soil) solution, hence resulting in less water uptake. Higher EC may not be an issue in the fine textured and artificial soils, as these soils have more exchange sites to capture the free ions in soil solution. The phytotoxicity of Se as well as the antagonistic response of Se and S was most prominent in the fine textured soil. Texture classes should be differentiated and considered separately in any future research to further elucidate the Alberta and Canadian soil Se guideline for the ecological contact pathway.

5.0 Conclusions

The results of this study demonstrated that the phytotoxicity of Se was most evident at 6 ppm Se. The antagonistic effect of S and Se was observed although not consistently in all species and the three soils used in this study. In the fine textured soil, the addition of S (at S-High level) consistently “reversed” the toxic effect of Se. Species grown in the coarse textured soil were not as responsive at the S-High level, likely due to the stress inflicted on the plants as a result of higher EC associated with the increase in S. Most species responded similarly to Se toxicity and the antagonistic effects of S and Se, but in general alfalfa and northern wheatgrass were more sensitive to Se toxicity and they also responded more positively to the addition of S.

The corresponding increase in soil EC, as a result of higher S concentration, may impose other stress on the plants should also be considered when re-evaluating guidelines, especially for the coarse textured soils. Future research into the antagonistic relationship of Se and S should consider investigating the longevity of this relationship to answer the question “is the suppression of Se toxicity by S only valid in the first growing season?”

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