Bioavailability of Allelochemicals in Soil

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14. ABSTRACT

The successful spread of the weeds Acroptilon repens (Russian knapweed, RK) and Centaurea maculosa (Spotted knapweed, SK) has been attributed to the exudation of allelopathic chemicals by their roots. RK releases 7,8-benzoflavone and SK releases (+/-)-catechin. Understanding the conditions resulting in optimum sorption of these allelochemicals could provide a means to arrest the spread of the weeds that exude these allelochemicals. Our objective was to determine which soil constituents and conditions provide maximum sorption of the allelochemicals (+/-)-catechin and 7,8-benzoflavone and determine if the optimum soil sorption conditions diminish the phytotoxicity of these allelochemicals. We found that 1) sorption of (+/-)-catechin and 7,8-benzoflavone onto soil with or without carbon was 100%. This rendered (+/-) - catechin and 7,8-benzoflavone unavailable, and 2) carbon significantly inhibited the growth of RK while promoting the growth of bluebunch wheatgrass (BBWG). Thus, application of carbon to RK infested sites may limit its spread on military bases.

15. SUBJECT TERMS
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List of Acronyms

AC	**activated carbon**
ALSAC	**Alkali sacaton**
BBWG	**Bluebunch wheatgrass**
IF	**Idaho Fescue**
RK	**Russian Knapweed**
SERDP	**Strategic Environmental Research and Development Program**
SK	**Spotted Knapweed**

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I would like to thank Leanna Reynolds and Melanie Ballard for their work in setting-up and conducting the laboratory and greenhouse studies. Also, Thanks is extended to Dr. Dale Gardner at the USDA Poisonous Plant Laboratory for conducting the allelochemical sample analysis, Dr. David Hole for his help with the statistical analysis, and the Utah Agriculture Experiment Station for providing the greenhouse space.

We are grateful to the Department of Defense's Strategic Environmental Research and Development Program (SERDP) for providing the funding for this project which enabled us to undertake this project. Appreciation for technical assistance is extended to Mr. Bradley Smith, Executive Director and Drs. Robert Holst and John Hall, Sustainable Infrastructure Program Managers, former and present, and to the HydroGeoLogic, Inc., staff for their administrative assistance.

1. Executive Summary

The spread of invasive weed species such as, *Acroptilon repens* [Russian knapweed (RK)] and *Centaurea maculosa* [(Spotted knapweed (SK)] are wreaking havoc upon native plant ecosystems in the western U.S. at tremendous environmental and economic costs. Their successful spread has been attributed to the exudation of allelopathic chemicals by their roots into surrounding soil. RK releases 7,8-benzoflavone and SK releases (+/-)-catechin. These chemicals are phytotoxic to native plant species. The toxicity of these allelochemicals is a function of their bioavailability in the soil solution which is regulated by soil sorption (retention) processes. Very little is known about the sorption of these allelochemicals in soil. Understanding the conditions resulting in optimum sorption – or minimal bioavailability – of these allelochemicals could provide a means to arrest the spread of the weeds that exude these allelochemicals.

The objective of this project was to determine which soil constituents and conditions (pH and organic matter content) provide maximum **sorption** of the allelochemicals (+/-)-catechin and 7,8-benzoflavone and determine through **bioassays** if the optimum soil sorption conditions could diminish the phytotoxicity of these allelochemicals to native species.

Sorption studies included sorption edges (amount sorbed vs. pH) and isotherms (amount sorbed vs. equilibrium concentration) for (+/-)-catechin sorption onto soil constituent surfaces, and due to the low aqueous solubility of 7,8-benzoflavone, its desorption from soil with water or methanol was investigated. Bioassays included hydroponic studies – a species screening study with RK and studies testing the phytotoxicity of 7,8-benzoflavone and (+/-)-catechin – and greenhouse column studies examining the effect of activated carbon and soil texture on RK, SK and native plant species establishment.

We found that **1)** sorption of (+/-)-catechin and 7,8-benzoflavone onto ferrihydrate and soil with or without AC was 100%. This rendered (+/-) - catechin and 7,8-benzoflavone unavailable. Thus, it is unlikely that (+/-)-catechin and 7,8-benzoflavone were the allelochemicals responsible for the successful spread of RK and SK' and **2)** AC significantly inhibited the growth of RK while promoting the growth of Bluebunch Wheatgrass (BBWG). Thus, application of AC to RK infested sites and restoration with BBWG may limit the spread of RK.

The information gleaned from this study can be used to develop a management strategy to mitigate the spread of RK. By knowing the soil conditions that are susceptible to the spread of RK and through the combined use of weed and soil maps, those areas can be identified and targeted and treated with AC. This would enhance the growth of native species while suppressing the growth of RK. Ultimately, this low cost, low maintenance management strategy would provide an environmentally sound means of controlling invasive weeds, minimizing their disturbance of natural landscapes, and increasing the vegetative cover of native plant species on military bases.

2. Objective

The project objectives were to 1) determine the soil constituents and conditions that provide maximum sorption of the allelochemicals (+/-)-catechin and 7,8-benzoflavone in soil and 2) determine through bioassays if the optimum soil sorption conditions could diminish the phytotoxicity of these allelochemicals to native species.

The work in this proposal was relevant to the SERDP Statement of Need (SON) for Invasive Species Control because it involves basic research that potentially provides an environmentally sound means of controlling invasive weeds, minimizing their disturbance of natural landscapes and increasing the vegetative cover of native plant species. This addressed the SON specifying that SERDP will "entertain proposals in habitat enhancement, particularly through control of exotic pests and promotion of natural species, to minimize disturbance of natural landscapes and increase vegetative cover, thereby controlling a growing threat to environmental security while improving training conditions." In particular, it targeted the spread of Russian (*Acroptilon repens*) and spotted knapweed (*Centaurea maculosa*), two invasive weed species that are wreaking havoc upon native plant ecosystems in the western US at tremendous environmental and economic costs.

3. Background

Russian knapweed (*Acroptilon repens*) and spotted knapweed (*Centaurea maculosa*) are considered some of the most devastating invasive weeds – environmentally and economically – in the western regions of the United States. The successful spread of these weeds has been attributed to the exudation of allelopathic chemicals by their roots into surrounding soil. These chemicals are phytotoxic to native plant species. The flavones, (+/-)-catechin and 7,8 -benzoflavone, exuded by SK and RK, respectively, have recently been identified as the allelochemicals that may be responsible for their spread with reported phytotoxic activities in the range of 50 to 250 $\mu g\ ml^{-1}$ (Bais et al., 2002; Stermitz et al., 2003).

A relationship between soil properties (mostly soil texture) and the spread of the knapweed species has been noted (Goslee et at., 2001; Grant et al., 2003; Hierro and Callaway, 2003). Yet, very little is known about the sorption of (+/-)-catechin and 7,8-benzoflavone in soil. We surmise that the phytotoxic activity of these allelochemicals in soil is, in part, regulated by their sorption (or retention) by soil solid phases, where an increase in sorption will result in a decrease in the amount of bioavailable (amount in soil solution) allelochemical and hence, a decrease in phytotoxic activity (Figure 1).

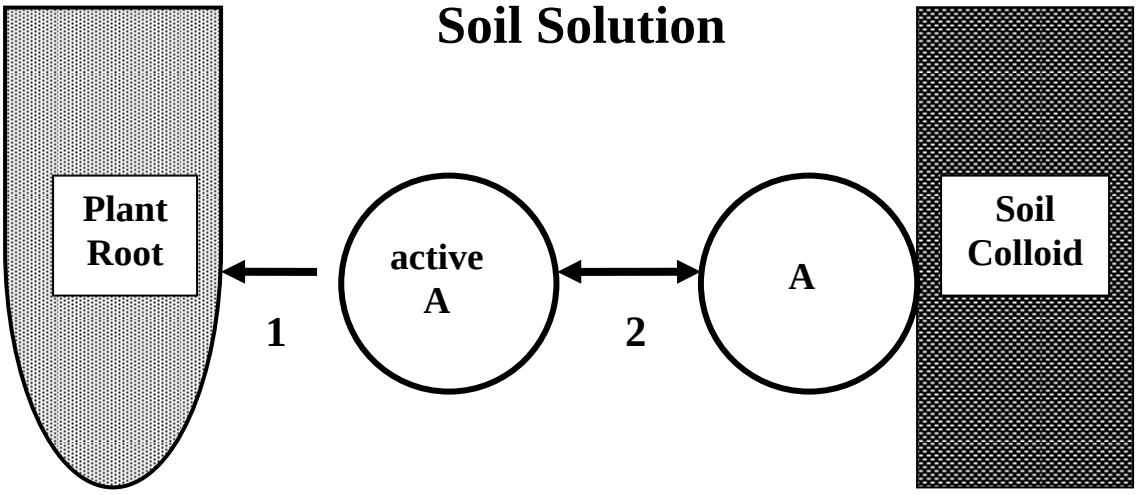

Figure 1. Phytotoxicity of an allelochemical (A) as a function of its bioavailability (1), which is regulated by its sorption to soil colloids (2).

Sorption is generally defined as the transfer of matter from the solution phase to the solid phase and encompasses the processes of adsorption, precipitation and partitioning. For organic chemicals, such as (+/-)-catechin and 7,8-benzoflavone, adsorption and partitioning phenomenon will be most important. Here, we use adsorption to mean the net accumulation of matter in a two dimensional molecular arrangement at a solid/aqueous interface. Partitioning occurs in

response to solute-solvent rather than solute surface interactions and involves the "partitioning" of the chemical into the three dimensional network of soil organic matter in response to the compound's increased solubility in the organic phase relative to soil water. The polarity of an organic molecule is an important factor determining the degree to which it is sorbed by soils (Pepper et al. 1996). Nonpolar organics will partition into soil organic matter phases, whereas, polar organic compounds will behave similar to inorganic ions and sorb to charged soil surface sites. In either case, the soil organic fraction would act as a strong sorbant. If the organic matter content of soils is low, which is often the case for semi-arid grassland soils, then it is likely that sorption of an organic chemical will also be low and it will be readily bioavailable. Kulmatiski and Beard (2006) reported that the addition of activated carbon decreased cover of diffuse Knapweed (*Centaurea diffusa*) in the field, which they attributed to activated carbon's ability to sequester organic alleochemicals.

When considering adsorption phenomenon, it is the soil colloidal fraction (soil particles with diameters less than 1 μm) that is responsible for regulating the bioavailability of the adsorbate (chemical that accumulates on the soil surface). Important soil adsorbents (colloidal solids on which material accumulates) are oxides of iron, aluminum and manganese; silicate clays, calcium carbonate, and soil organic matter (humus). We observed, at a study site near Dinosaur National Monument in Utah, that, Russian knapweed did not invade areas dominated by soils rich in iron oxides (Morris et al., 2004). Perhaps, (+/-)-catechin adsorbed onto iron oxide surfaces and was rendered inactive, thus negating Russian knapweed's competitive edge over native species at the site. The degree of adsorption onto soil colloidal surface is dependent on soil pH (Petrie et al., 2002; Sparks, 2003). Generally, soils from semi-arid regions in the Western U.S. contain abundant levels of calcium carbonate, which buffers soil pH in the alkaline range from about 7.5 to 8.5 (Doner and Grossl, 2002). We surmise that the solubility and adsorption behavior of (-)-catechin and 7,8-benzoflavone under alkaline conditions may enhance their bioavailability and contribute to the successful spread of Russian and spotted knapweed in the West. Interestingly, Grant et al. (2003) observed that Russian knapweed infestation was greater in clayey soils than sandier soils in semi-arid grasslands of Colorado. They attribute this to the adsorption and accumulation of volatile allelochemicals to biologically active levels in clayey soil.

Recent studies question the importance of (+/-)-catechin as the allelochemical responsible for the successful spread of SK (Blair et al., 2006; Perry et al., 2007). Perry et al. (2007) report that detection of (+/-)-catechin in soils associated with SK was infrequent and, when detected, the levels were lower then previously reported. Similarly, Blair et al. (2006) report that (+/-)-catechin measured in soils associated with long-term infested SK sites was either undetected or detected at low levels (three orders of magnitude less than levels reported earlier and responsible for growth inhibition in sensitive native species), and that soil moisture appears to be an important factor regulating (+)-catechin soil levels. The information regarding the sorption behavior of (-)-catechin and 7,8-benzoflavone in soil is scant – especially the conditions and soil constituents responsible for optimal sorption. If these can be identified then it may be possible to mitigate the spread of RK and SK by diminishing the bioavailability of exuded allelochemicals to native plant species.

4. Materials and Methods

Soil phase sorbents used for sorption studies and greenhouse column study included: ferrihydrite, a high surface area amorphous iron oxide commonly found in soils; and soil media consisting of sand containing either 5% or 20% (w/w) Kidman fine sandy loam (65 % sand, 25 % silt, and 10% clay) with or without activated carbon. The granular activated carbon was purchased from Sigma Chemcial Co. (St. Louis, MO) and had a 4 -14 mesh particle size. The activated carbon was added so that it comprised 4% of the total weight of the soil medium. The ferrihydrite was prepared and characterized using procedures detailed in Grafe et al. (2002). The (+/-) -catechin and 7,8 benzoflavone used in the sorption and hydroponic toxicity studies were also purchased from Sigma Chemical Co. (St. Louis, MO). Analyses of (+/-)-catechin and 7, 8 benzoflavone in solution samples were conducted at the USDA Poisonous Plant Laboratory in Logan, Utah using gas chromatography. This involved compound separation by gradient elution on a 5µm, reverse phase octadecyl column (250mm x 4.6mm) and detection at 210nm. The gradient elution consisted of a water-methanol mixture. External calibrations were performed with standards for the accurate quantification of analytes.

4.1 Sorption Batch Experiments

4.1.1 (+/-) – Catechin

(+/-) – Catechin sorption experiments included both sorption isotherm (amount sorbed vs. equilibrium concentration of sorbate) and sorption edge (amount sorbed vs. pH) experiments. Sorption edges with ferrihydrite were conducted in a flat bottomed 500 mL glass beaker under ambient conditions at a suspension concentration of 0.25 g L^{-1} with a background electrolyte concentration of 0.05 M $NaNO_3$. One hundred mg L^{-1} (+/-)-catechin was added to the suspension and the pH of the suspension was adjusted to 10.0 with 1 M NaOH. A pH titration was performed on the suspension by adding 1M HNO_3, and once pH reached equilibrium at 8.0, 7.0, 5.0, and 3.0, a 10 mL suspension sample was collected and transferred to a 50 mL polypropylene centrifuge tube. The control consisted of the same titration without ferrihydrite. The 10 mL suspension samples were shaken on a mechanical shaker at low speed for 3 hours after which samples were centrifuged (4000 rpm for 10 minutes). The supernatant was collected and delivered to the USDA Poisonous Plant Lab for (+/-)-catechin analysis by gas chromatography (GC).

Sorption isotherms were performed on the soil medium with and without activated carbon. This required weighing 1 gram of soil medium into 50 mL polypropylene centrifuge tubes. Then 10 mL solutions of 0, 10, 25, 50, and 100 mg L^{-1} (+/-)-catechin were added to each tube. Each treatment was run in duplicate. Controls contained no soil medium. Tubes were shaken on a mechanical shaker at low speed for 3 hours, centrifuged, and supernatant collected and analyzed as mentioned above. The amount sorbed was the difference between the initial sorbate concentration and that measured in the equilibrium solution. More detailed information regarding the experimental set-up can be found in Grossl et al. (1997) and Grafe et al. (2002).

4.1.2 7,8-Benzoflavone

Due to the low aqueous solubility of 7,8 benzoflavone, sorption isotherms within the relevant active concentration range reported by Stermitz et al. (2003) could not be performed. Instead desorption experiments were conducted where 7,8-benzoflavone was extracted with either water or methanol from the soil medium containing 20% (w/w) Kidman fine sandy loam, with or without activated carbon. This extraction procedure was a modified version of that reported by Alford et al. (2007). Specifically, 2500 μg of 7,8-benzoflavone in methanol was added to 10 grams of soil medium to make a slurry. The methanol was allowed to volatilize from the slurry which was contained in 40 mL scintillation vials. Twenty-five mL of either distilled-deionized water or methanol was added to each vial which was sealed and shaken on a mechanical shaker at low speed for 1.5 hours. Soils were then separated from extracting solutions via centrifugation (4000 rpm for 10 minutes). Solution extracts were delivered to the USDA Poisonous Plant Lab for 7,8-benzoflavone analysis by GC.

4.2 Bioassays

Seeds used in the bioassays came from the following sources: Russian knapweed, bluebunch wheatgrass, and alkali sacaton seeds were collected at the field near Dinosaur National Monument. The spotted knapweed came from the Center for Invasive Plant Management at Montana State University – they were collected locally there. The Idaho fescue came from the USDA, ARS, WRPIS, Washington State University, Regional Plant Introduction Station – they were collected in Idaho.

All bioassay data were statistically analyzed using analysis of variance (ANOVA) procedure and the level of significance was calculated from the F values of ANOVA (SAS, Inc. Cary, NC). Means were compared with a least significant difference (LSD) test.

4.2.1 Hydroponic Screening Study

This study was designed to determine if Russian knapweed (RK) growing together (sharing the same nutrient solution containers) with bluebunch wheat grass (*Pseudoroegneria spicata*) (BBWG) and alkali sacaton (*Sporobolus airodes*) (ALSAC) inhibited their growth. Plant seeds were germinated in germination trays. Upon seedling establishment, seedlings were transplanted to 50 L solution culture tubs. The nutrient solution recipe is listed in Mackowiak et al. (2005). All treatments received the same the same nutrient solution. The only treatment differences were the following plant combinations:

RK control
BBWG control
ALSAC control
RK vs. BBWG
RK vs. ALSAC
BBWG vs. ALSAC

Eight plants were grown in each tub. Controls consisted of 8 plants of the same species, while combined treatments consisted of 4 plants of both species. Within each tub, plants were spaced equidistant from one another and in tubs containing mixed species they were planted in an alternate pattern (Figure 2).

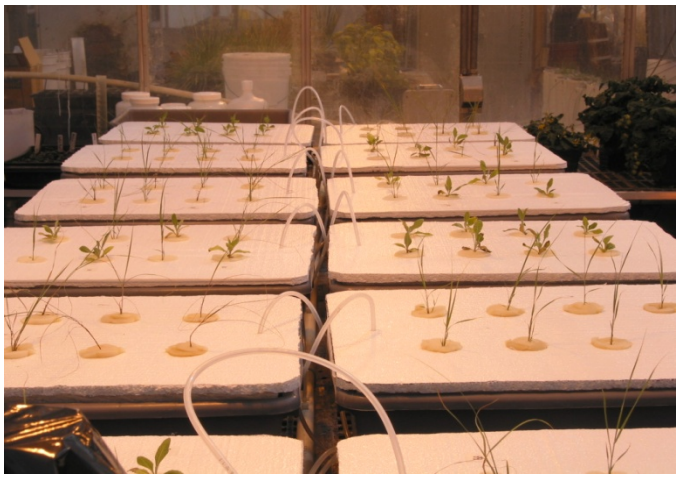

Figure 2. Hydroponic screening system after seedling transplant.

The nutrient solution level was checked daily with a portable glass manometer bearing a mark showing the liquid level setting. Refill solution was added whenever the solution level dropped below the manometer mark. The nutrient solution in each tub was vigorously mixed and aerated using polyvinyl chloride manifolds fed from an in-house air supply. Nutrient solution pH (5.5 ± 0.1) was measured in the greenhouse unit each day. Greenhouse lighting was supplemented with four 400-watt high-pressure sodium lamps to provide an average daily irradiance of approximately 26 mol m^{-2} d^{-1}. Sixty days after seedling transplant, plants were harvested and root and shoot dry weights were measured.

4.2.2 Hydroponic Phytotoxicity Studies

4.2.2.1 (+/-)-Catechin

Spotted knapweed and Idaho Fescue seeds were germinated in germination trays. Seedlings were transplanted into 1 L foil lined glass vessels containing aerated nutrient solutions. The nutrient solution recipe was the same used in the Hydroponic Screening study. The (+/-)-catechin treatments were added approximately 3 to 4 weeks after seedling transplant to ensure that plants were established and healthy. Treatments consisted of 0, 25, 50, and 100 mg L^{-1} one-time doses of (+/-)-catechin. Each treatment was replicated five times resulting in a total of 40 hydroponic vessels. Nutrient solutions were monitored every two days, refill solutions were added accordingly and pH was adjusted to 5.5. Plants were grown for about 60 days after treatment addition, they were then harvested and root and shoot dry weights were measured.

4.2.2.2 7,8-Benzoflavone

The same system was used for the 7,8-benzoflavone phytotoxicity study, except that RK and BBWG were the indicator plants. Also, there were only two 7,8-benzoflavone treatments: zero 7,8-benzoflavone (control) or 200 mg L^{-1} 7,8-benzoflavone added. These treatments were replicated five times. Plants were grown for about 60 days after treatment addition, they were then harvested and root and shoot dry weights were measured.

4.2.3 Greenhouse Column Studies

Soil Columns were constructed from PVC pipe with an inner diameter of 10cm and length of 30 cm. Columns were packed with soil media to a bulk density of 1.4 g cm^{-3}. Columns were planted with RK and BBWG where each column either contained two RK plants, two BBWG plants, or one RK and one BBWG plant. Thus, there were a total of 12 treatments consisting of following treatment combinations:

1) Carbon : Activated Carbon versus No Activated Carbon (Control)
2) Plants: RK alone, BBWG alone, RK vs. BBWG
3) Soil Texture: 5% versus 20% Kidman fine sandy loam

The exact same experimental set-up was used for SK and IF studies. Each treatment was replicated 5 times resulting in 60 columns. Columns were arranged in a completely randomized design. RK and BBWG seeds were germinated in germination trays and upon establishment seedlings were transplanted into the soil columns. A partition consisting of a brass sheet was placed between plants to separate shoots from one another (Figure 3).

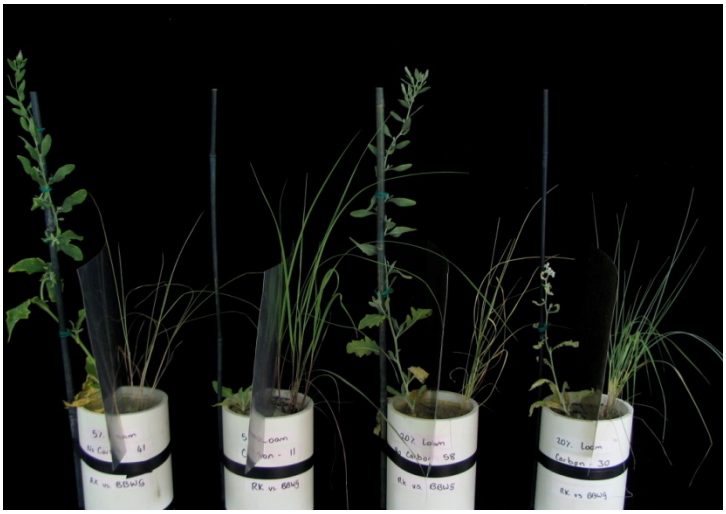

Figure 3. Greenhouse columns with RK and BBWG growing together. Note partition separating RK and BBWG shoots.

All columns were given a blanket application of 0.5 g 20-10-20 fertilizer and 0.6 g Scotts micromax micronutrient fertilizer. Columns were watered nearly every other day to maintain

soil water content at 15% (w/w). Plants were grown for 60 days after seedling establishment. At the end of the study, above and below ground plant biomass was collected and fresh and dry weights (dried at 80 C for 24 hours) measured. To collect below ground biomass, the soil plugs were removed from the columns and the roots were separated from soil. Dried and milled shoot and root tissue was digested using a wet digestion with nitric acid and hydrogen peroxide, and digests were analyzed for nutrient elements and trace metals via ICP emission spectrometry. Fresh root mass was digitally imaged and root lengths were measured. Also, rhizosphere (soil adhering to plant roots) and bulk soils where submitted to the Utah State University Analytical Laboratory and analyzed for total and bioavailable (DTPA extract) nutrient and trace metals.

5. Results and Accomplishments

5.1 Sorption Batch Experiments

5.1.1 (+/-) – Catechin

Sorption of 100 ppm (+/-)-catechin onto ferrihydrate from pH 3.5 to 9.0 was 100% - none was detected in equilibrium solutions (Figure 4). In samples with without sorbents, equilibrium solution concentrations of (+/-)-catechin decreased with increasing pH when pH values were greater than 7.0. We believe that the decrease was due to the formation of polymerization products, however, we were not able to determine the identity of these products.

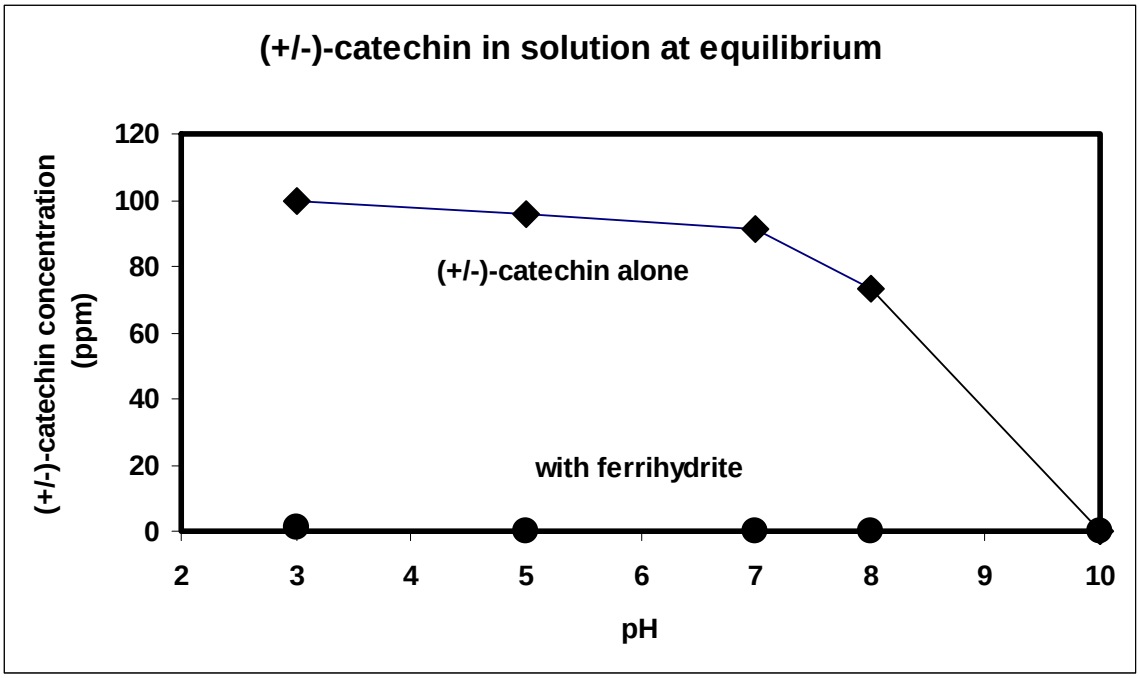

Figure 4. (+/-)-catechin sorption on ferrihydrite and with no solid added as a function of pH.

Sorption isotherms where initial (+/-)-catechin concentrations ranged from 10 to 100 ppm, also revealed 100% sorption of (+/-)-catechin on the soil medium with and without activated carbon (Table 1). It should be noted that even the medium with 20% Kidman soil had a relatively course texture, most natural soils contain more silt and clay. Hence they would have finer textures and greater surface area for sorption.

Table 1. Sorption Isotherm for (+/-)-catechin on a soil medium consisting of 20% (w/w) Kidman fine sandy loam + sand with and without activated carbon (AC).

Soil without AC		
Initial ppm	Equilibrium ppm	% Sorbed
0	0	100
10	0	100
25	0	100
100	0	100
Soil with AC		
Initial ppm	Equilibrium ppm	% Sorbed
0	0	100
10	0	100
25	0	100
00	0	100

5.1.2 7,8-Benzoflavone

Because of the low aqueous solubility (0.9 mg L^{-1}) of 7,8-benzoflavone, we were not able to conduct sorption edges and isotherms for this compound – especially within the active phytotoxic range. Instead, we investigated its desorption from the soil medium with and without activated carbon using either water or methanol as extracting agents. We were not able to extract any 7,8-benzoflavone from the soil both with and without AC using water as an extracting agent. We were able to extract all of the 7,8-benzoflavone from the soil without AC using a methanol extraction. However, in soil with AC, even methanol could only extract 0.1 % of the total sorbed 7,8-benzoflavone (Table 2).

Table 2. Desorption of 7,8-benzoflavone in either water or methanol from soil medium consisting of 20% (w/w) Kidman fine sandy loam + sand with and without activated carbon (AC) – spiked with 2500 mg 7,8-benzoflavone.

Soil without AC		
Extracts	***Expected ppm***	***Measured ppm***
Water	100	0
Methanol	100	100
Soil with AC		
Extracts	***Expected ppm***	***Measured ppm***
Water	100	0
Methanol	100	0.1

The limited aqueous solubility and near 100 % soil sorption of (+)-catechin and 7,8-benzoflavone would limit their soil solution activity and hence their phytotoxicity. This raises questions regarding their significance as allelopathic agents responsible for the spread of RK and SK.

5.2 Bioassays

5.2.1 Hydroponic Screening Study

This screening study was designed to determine if compounds released by RK roots could inhibit the growth of the native species bluebunch wheatgrass (BBWG) and alkali sacaton (ALSAC). We chose these two natives because they were the most common ones found at local field sites with RK and are frequently used in restoration of disturbed soils in the West. Plus, the literature was lacking regarding native plant species affected by possible RK allelopathy. No screening was performed for SK, since Idaho fescue (IF) is reportedly vulnerable to (+/-)-catechin released by SK (Blair et al., 2006). Thus, IF was used as a target species in SK studies.

BBWG above ground biomass production decreased by 80% when grown with RK compared to when BBWG was grown alone in tubs (Figure 5). This suggests that BBWG was sensitive to an unidentified phytotoxin released by RK. ALSAC biomass production was not significantly different when grown alone or with RK – ALSAC exhibited no phytotoxic response. Based on these results, BBWG was chosen as the target native species in RK studies.

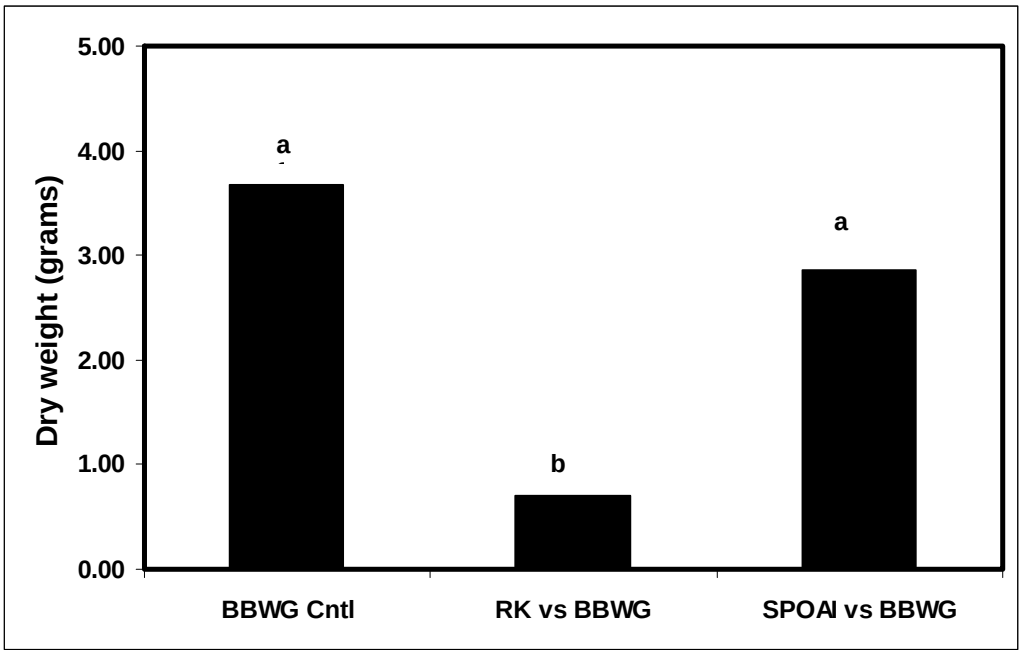

Figure 5. Shoot dry weights (grams) for BBWG when grown alone compared to when grown with BBWG and ALSAC (SPOAI). Same letters above bars within a given treatment indicate that treatment values were not significantly different at the 5% level of probability.

5.2.2 Hydroponic Phytotoxicity Studies

No significant decline in RK and BBWG biomass production was observed when treated with 200 ppm 7,8-benzoflavone. The benzoflavone was added in solid form and did not dissolve during the course of the study. Essentially plants were exposed to a continuous dose of about 0.9 mg L^{-1} (aqueous solubility) of 7,8-benzoflavone, which should be close to the maximum level present in the soil solution when sorption is not considered. If soil sorption is considered, solution levels would be lower yet. Thus, 7,8-benzoflavone is not likely responsible for the successful spread of RK.

No significant decline in SK and IF biomass production was observed when they were treated with 10, 50, and 100 ppm (+/-)-catechin. The catechin was added in solid form to the hydroponic vessels but dissolved within 24 hours. Even at this dose level, plants did not exhibit any sensitivity to (+/-)-catechin. As reported above, sorption processes would drop (+/-)-catechin levels in the soil solution effectively to zero, and as was the case for 7,8-benzoflavone, (+/-) – catechin was not likely responsible for the successful spread of SK.

5.2.3 Greenhouse Column Studies

The greenhouse column studies were designed to test our hypothesis that the presence of AC – by acting as a sorbent for allelochemicals – should promote BBWG growth in columns where

both BBWG and RK were grown together while inhibiting the growth of RK. Likewise, AC should promote IF growth in columns where both IF and SK were together while inhibiting the growth of SK. Contrary to our hypothesis, RK shoot and root growth was significantly inhibited in all cases where AC was applied (Figure 6 and 7). This occurred in treatments were RK was growing alone or with BBWG. In contrast, BBWG shoot and root growth was promoted by AC (Figures 6 and 7). This suggests that AC may not only be acting as a sink for any allelochemicals produced by RK but AC is negatively impacting an unknown factor required for the growth of RK, while AC is positively impacting the same factor and consequently promoting the growth of BBWG.

Analysis of the elemental nutrient content of plant tissue, and rhizosphere and bulk soils suggests that AC is limiting – by acting as a sink – essential micro nutrient metals (iron, zinc, manganese, and copper) required by RK. Perhaps, RK requires more of these essential metals than BBWG. Plus, the mechanism used by BBWG to scavenge these metals is enhanced by AC. Other than AC, soil texture only affected BBWG growth, where BBWG shoot biomass was significantly higher in the 20% soil (2 g) vs. 5% soil treatment (1.3 g).

SK and IF did not demonstrate the same response to AC as RK and BBWG (Figures 8, 9, and 10). The only significant response to AC was SK root weights (Figure 9). SK also exhibited an increase in shoot and root biomass production when plants were grown in the 20% soil vs. the 5% soil medium (Figures 10 and 11). IF was not influenced by either AC or soil texture. Difference in RK/BBWG response compared to SK/IF response to AC and soil texture may be due to differences in the plants' water use efficiencies and overall differences in their growth environments. SK and IF may have greater water requirements than RK and BBWG. Hence the 15 % (w/w) watering schedule may have been too low for SK and IF and limited their overall growth. Possibly, AC and 20% soil medium had a greater water holding capacity, thus enhancing SK growth. IF, with a lower water use efficiency, was under water stress no matter what the soil treatment. Conceivably, if the soil water levels were increased and SK and IF were not subjected to water stress, we may have observed a response to AC with SK and IF, similar to the one that we observed for RK and BBWG.

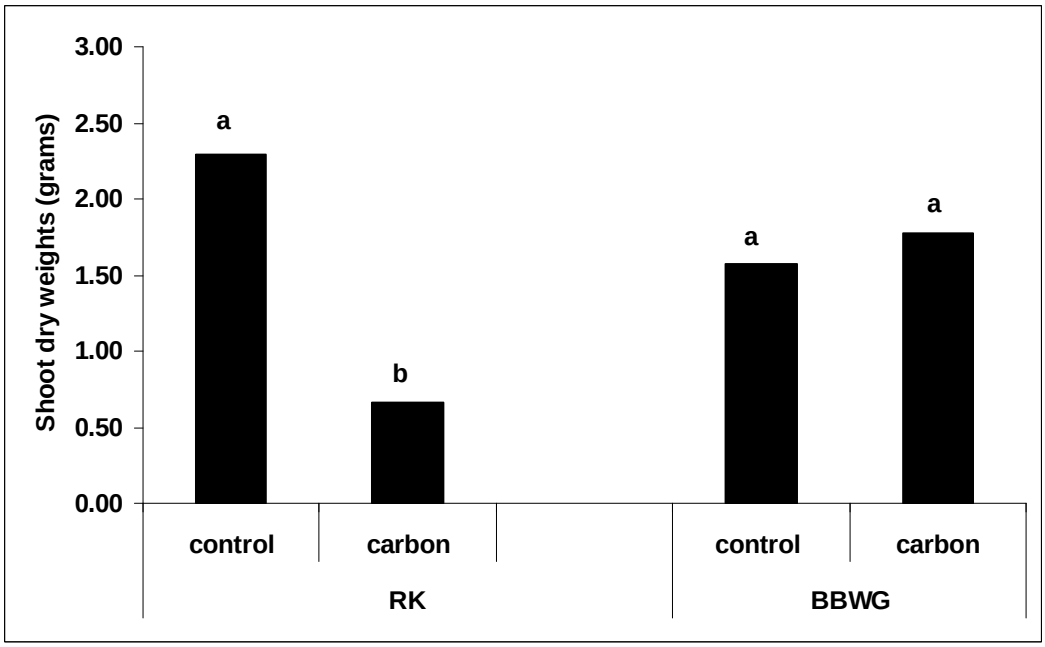

Figure 6. RK and BBWG dry matter production in soil columns with and without AC. Same letters above bars within a given treatment indicate that treatment values were not significantly different at the 5% level of probability.

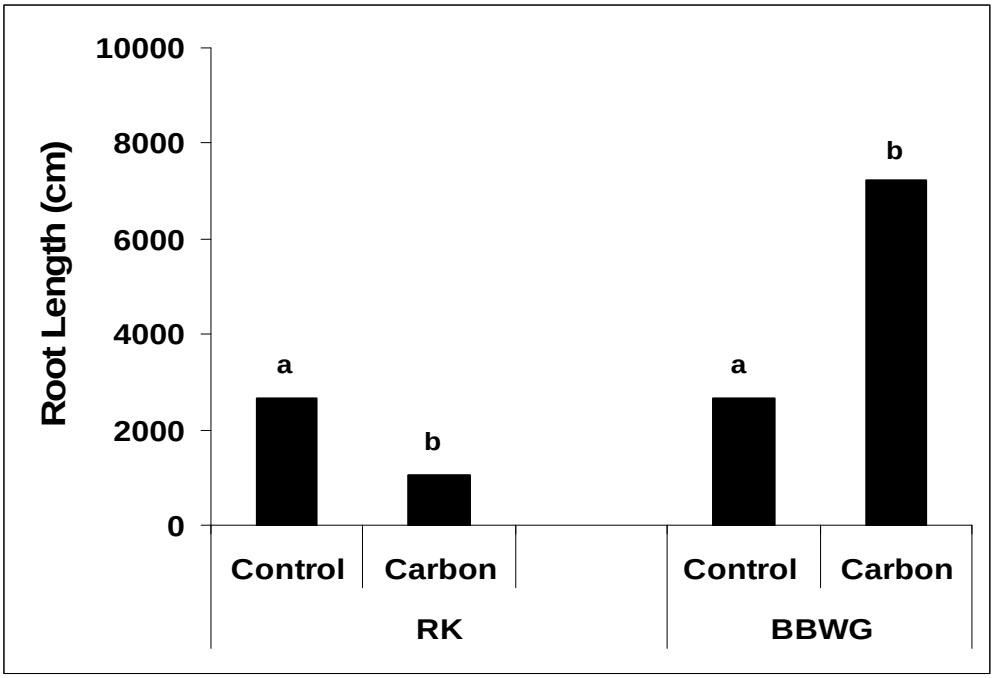

Figure 7. RK and BBWG root lengths in soil columns with and without AC. Same letters above bars within a given treatment indicate that treatment values were not significantly different at the 5% level of probability.

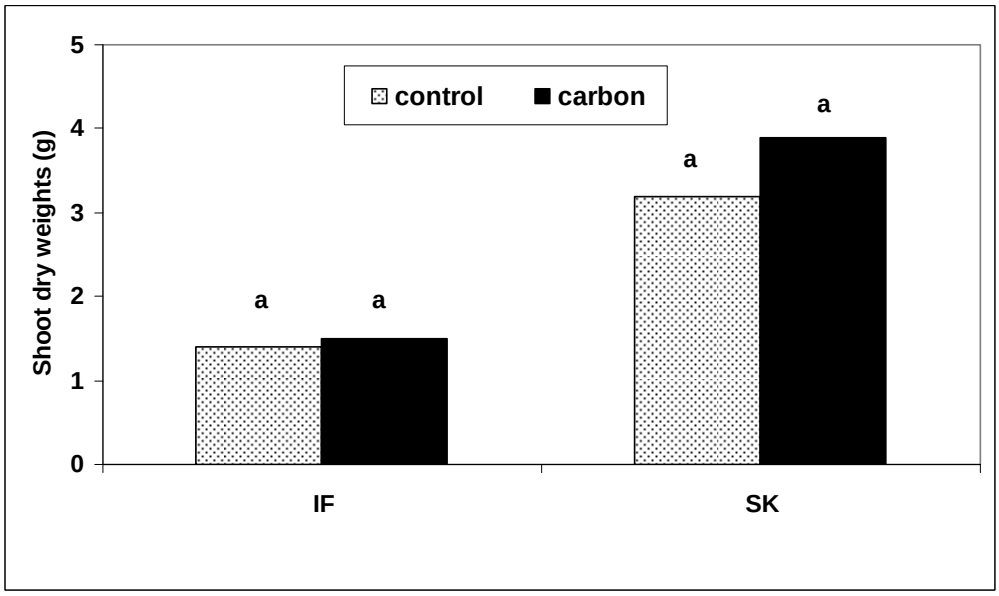

Figure 8. SK and IF dry matter production in soil columns with and without AC. Same letters above bars within a given treatment indicate that treatment values were not significantly different at the 5% level of probability.

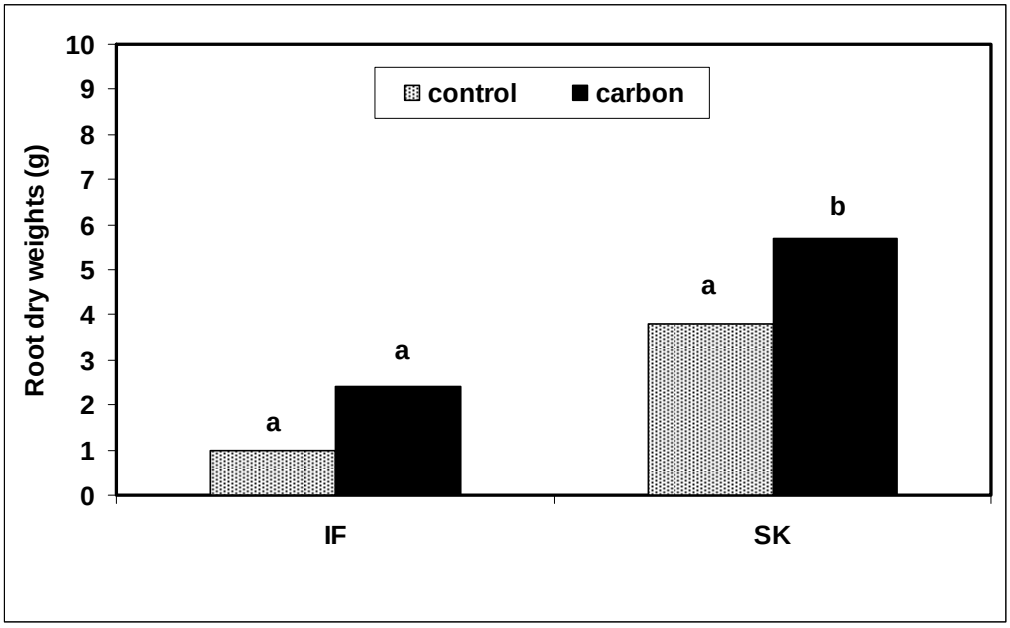

Figure 9. SK and IF root lengths in soil columns with and without AC. Same letters above bars within a given treatment indicate that treatment values were not significantly different at the 5% level of probability.

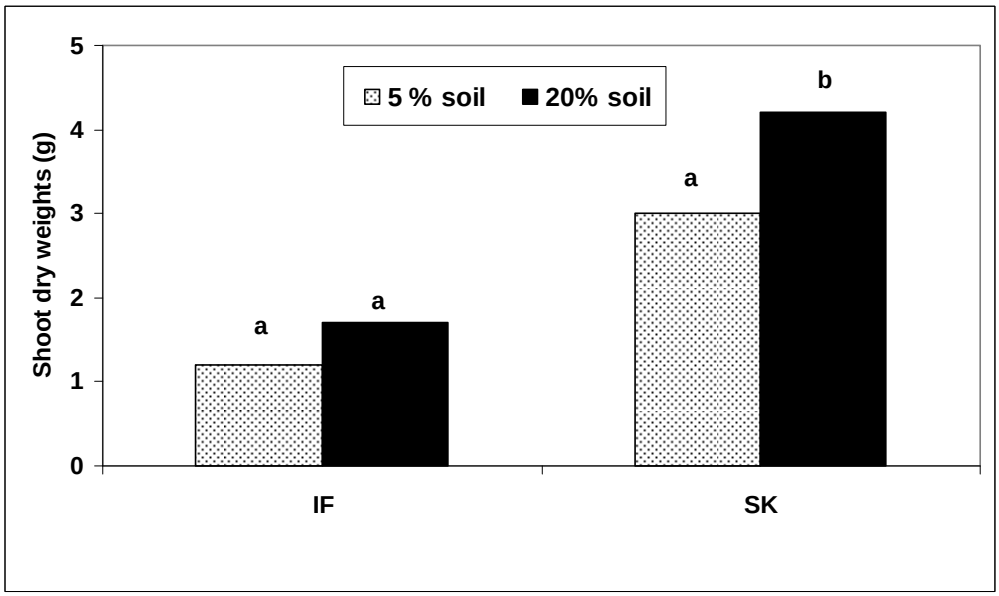

Figure 10. SK and IF dry matter production in soil columns with either 5% or 20% soil media. Same letters above bars within a given treatment indicate that treatment values were not significantly different at the 5% level of probability.

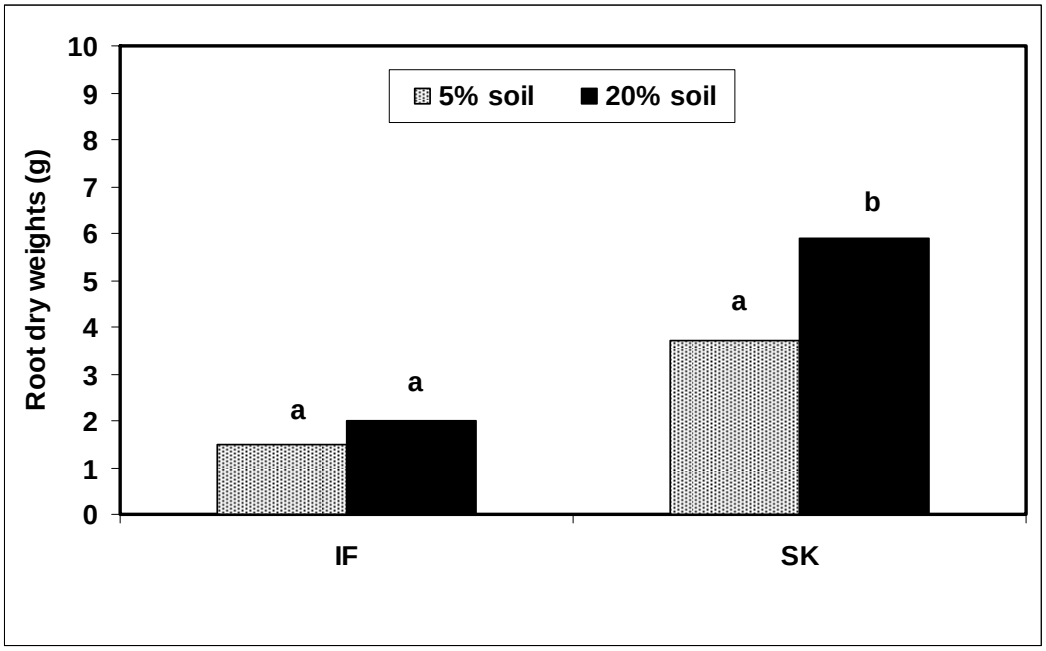

Figure 11. SK and IF dry matter production in soil columns with either 5% or 20% soil media. Same letters above bars within a given treatment indicate that treatment values were not significantly different at the 5% level of probability.

6. Conclusions

What we can conclude from this study is that **1)** sorption of (+/-)-catechin and 7,8-benzoflavone onto ferrihydrate and soil with or without AC was 100%. This rendered (+/-)-catechin and 7,8-benzoflavone unavailable and well below cited phytotoxic levels. Thus, it is unlikely that (+/-)-catechin and 7,8-benzoflavone were allelochemicals responsible for the successful spread of RK and SK. **2)** AC significantly inhibited the growth of RK while promoting the growth of BBWG. Thus, application of AC to RK infested sites and restoration with BBWG may limit the successful establishment of RK.

The information gleaned from this study can be used to develop a management strategy to mitigate the spread of RK. By knowing the soil conditions that are susceptible to the spread of RK, those areas can be selectively targeted and treated with AC. This would enhance the growth of native species while suppressing the growth of RK. For example, we superimposed a weed map plotting RK infestations at the Morris Ranch in Dinosaur National Monument over a soil map containing simple texture designations obtained from the National Resources Conservation Service (NRCS). Indeed, RK infestations were limited to the finer textured soils (Figure 12), which was consistent with our observations and those reported by Grant et al. (2003). The weed maps together with soil maps can serve as a guide to predict areas that may be susceptible to allelopathic spread of weeds. These areas can then be treated with AC and seeded with BBWG to restore lands impacted by RK. This is a low cost, low maintenance management strategy that provides an environmentally sound means of controlling invasive weeds, minimizing their disturbance of natural landscapes, and increasing the vegetative cover of native plant species. It may be especially useful for weed control on military bases where land areas are vast and only limited areas need to be treated. It may even provide a means to dispose of nontoxic organic wastes (i.e. composts and biosolids) that might behave similarly to AC.

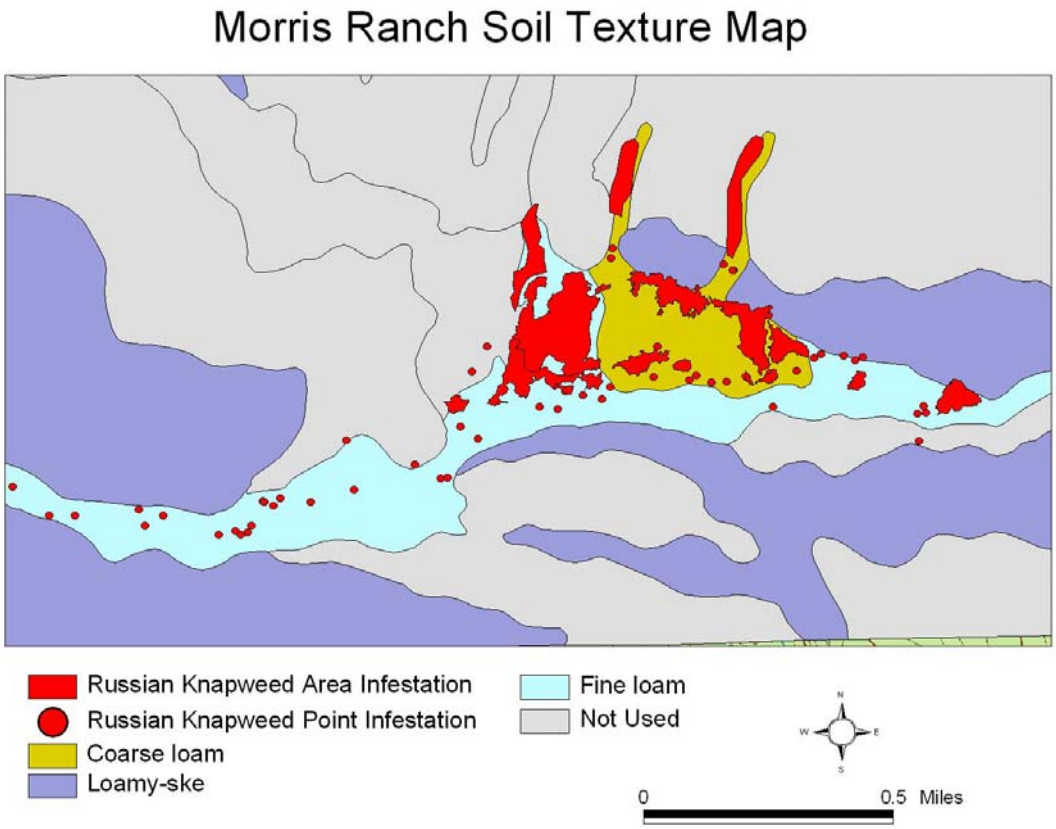

Figure 12. Russian knapweed weed map superimposed over a soil texture map. Map site is the Morris Ranch in Dinosaur National Monument.

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