Allelopathic Characteristics of *Artemisia tridentata* and *Purshia tridentata* and Implications for Invasive Species Management

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Abstract: Big sagebrush (*Artemisia tridentata*) and antelope bitterbrush (*Purshia tridentata*) are native species to the sagebrush ecosystem of northern Nevada. Both of these species exhibit allelopathic effects, whereby they produce chemicals that inhibit or prevent the growth of other plants nearby. In this experiment, extracts of sagebrush and bitterbrush are used to examine the extent and quality of this growth inhibition on Wisconsin fast plants (*Brassica rapa*). Both plants were found to severely inhibit growth to similar degrees, making them potentially viable resources for resisting exotic weed invasion.

Introduction:

Resource competition among plants is a primary determinant for distribution of a species. Nutrients, water and light are the essential limiting resources for which plants compete (Craine & Dybzinski, 2013). Competition is of particular concern when we consider the topic of invasive species management; invasive species negatively impact plant communities through competitive exclusion, niche disruption/exclusion, or even the extinction of native species, all leading to a loss of biodiversity (Mooney & Cleland, 2001). Invasive species often possess traits that allow them to thrive in new ecosystems; short generation times, niche flexibility, and the changes they instigate in the ecosystem as they spread through the landscape. These represent a few factors that allow these invaders to contribute to a positive feedback loop of invasive success as they overrun and crowd out native species (Mooney & Cleland, 2001). Plants in general compete for resources through a variety of mechanisms such as greater root growth, faster increase in biomass, and increased height. These mechanisms may help the plant compete for water, access to light, and soil nutrient availability. Some species of plants, such as black walnut (*Juglans nigra)*, spotted knapweed (*Centaurea maculosa*) and garlic mustard (*Alliaria petiolata*), even release chemicals into the soil to harm or inhibit the growth of nearby neighbors, which is known as allelopathy (Koochecki, et al. 2013).

In the Great Basin desert and sagebrush steppes, resources are scarce. Intense droughts are frequent, resulting in very dry conditions during hot summers and freezing winters, making water a particularly critical resource that plants must compete for (Nichols, 1989). Well-established, mature shrubs often have deeper root systems, permitting them to avoid some of the drought stress that afflicts their smaller counterparts (Donovan, 1994). Invasive species such as cheatgrass (*Bromus tectorum*), hereafter referred to as *B. tectorum*, often out-compete young native plants for water and nitrogen by proliferating more dramatically, and through earlier seasonal germination and growth compared to many of the native species they compete with (Pellant, 1996). This creates a positive feedback loop that can eventually result in an invasive species monoculture, completely excluding native species from large areas (Monaco, 2003). As a result, preventing or inhibiting the germination of invaders is an attractive option for biological control.

Allelopathy is a chemical-mediated competitive mechanism between plants, whereby plant-synthesized metabolites are disseminated to the surrounding area by means of leaching, exudates, or decomposing plant material (Meiners et al., 2012). Further, allelopathic tendencies of plants have been shown to correlate to environments with limited resources such as the Great Basin (Meiners et al., 2012; De Souza, 2010). These traits have been shown to affect nutrient dynamics among communities, species diversity and richness, and soil composition. This in turn may affect other plants by altering their metabolism, life stages, respiration, and photosynthesis (Ambika, 2013; Bogatek, 2007). Allelopathy, as indicated by Overholdt et al. (2012) in their study of aqueous allelopathic extracts on invasive species growth, can prove an important biological filter to invasion, and may, with due management, mitigate effects of, or even prevent, exotic plant invasions. Given the current status of the *B. tectorum* invasion in the Great Basin, we believe this to be an important avenue of research.

For the purpose of this study, we considered the allelopathic characteristics of big sagebrush (*Artemisia tridentata)* and antelope bitterbrush (*Purshia tridentata*), hereafter referred to as *A. tridentata* and *P. tridentata*, on the growth of surrounding plants, with an eye to the implications of these effects on invasive species control. Root length, plant height, and germination data was collected in order to examine the effect of compounds found in *A. tridentata* and *P. tridentata* on the relative health of a test plant species. We predicted that allelopathic tendencies of *A. tridentata* and *P. tridentata* inhibit growth of grasses and forbs to a greater extent than non-allelopathic shrubs. Plants in a controlled environment exposed to compounds from *A. tridentata* and *P. tridentata* bark and foliage infused in water were expected to exhibit lower levels of growth than those in the control group, who received only water. We hypothesized that inhibition of plant growth should vary between the two plant extracts being used.

Materials and Methods:

To determine the effects of chemical compounds found in *A. tridentata* and *P. tridentata* on plant growth, three groups (big sagebrush group, antelope bitterbrush group, and control group) of 40 Wisconsin fast plants (*Brassica rapa*)*,* hereafter referred to as *B. rapa*, seeds were placed in three Styrofoam celled seed trays, and were administered water that had been exposed to *A. tridentata* and *P. tridentata*, respectively. *B. rapa* was used instead of *B. tectorum* in order to control for potential adaptations in the local *B. tectorum* populations, and to provide us with a novel interaction model. The control plants received distilled water. Plant materials were harvested from several mature, healthy plants found at the foothills of Peavine Peak, Reno, Nevada (39.33730 N, -119.50033 W) on November 18, 2013. Harvested plant matter consisted primarily of mature leafy foliage with a small (<10%) amount of bark, seeds, and flowers. While allelopathy is often a root to root interaction, above ground material was used in order to determine if leaf litter was accounting for the relatively large, clear areas surrounding the plants in field observations. Harvested plant matter was dried at 25 degrees Celsius for 24 hours, and then ground to a fine powder, yielding 149.9g of ground *P. tridentata* material and 149.7g of ground *A. tridentata* material. Ground *A. tridentata* was added to an Erlenmeyer flask containing 1750ml of distilled water and allowed to steep with a stir bar for 24 hours. By continually agitating the solution and maintaining a room temperature for this extended period of time, it was hoped that all water-soluble chemicals would be extracted without the potential breakdown that might occur when boiling or simmering them. This process was then repeated with the ground *P. tridentata* material, and both flasks were strained to remove solid plant matter from the remaining usable liquid. The resulting infusion was then further diluted with water at a 1:1 ratio.

On November 21, 2013, seeds were singularly placed in a Styrofoam cell containing potting soil, covered in soil, and sprinkled with small amounts of treated or untreated water depending on the group, after which 400ml of each infusion was added to the pan containing the seed trays. Trays were then placed under grow lights for the duration of the experiment. Four days later, this process was repeated, again with 400ml of liquid administered to each group. Plants were treated with a further 800ml per tray (400ml treated water from flasks, 400ml distilled water, mixed/800ml distilled water for control group) three days later. To prevent undue evaporation of remaining liquid in the pan, exposed portions of the pan were covered, with only the tray of seedlings receiving direct light. Plants were harvested on December 2, 2013, for a total of twelve days of growth, and whereupon they were lightly brushed to remove dirt from the roots and weighed. Root/aboveground growth was also measured, and is depicted in Table 1. Data was then analyzed with R software.

Results :

Using ANOVA statistical analyses in R, we found significant correlations between treatments and the majority of measured statistics.

Overall plant mass was drastically reduced by both treatments (ANOVA F value 215.3, P value < .001, see figure 1) as was plant height (ANOVA F value 259.9, P value < .001, see figure 2) and root length (F value 49.78, P value <.001, see figure 3).

Germination rates did not differ significantly between the control and the *P. tridentata* treatment (X-Squared = .127, df = 1, p-value = .361); however, the *A. tridentata* treatment did significantly impact germination rates (X-Squared=6.56, df= 1, p-value = .005204, see figure 4).

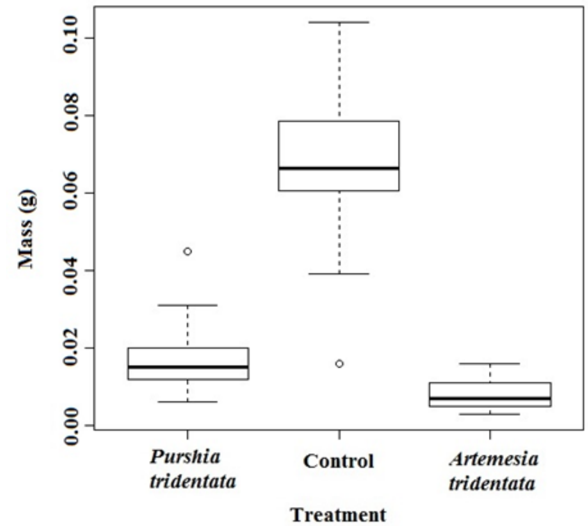

Figure 1. Boxplot demonstrating the significant effects on overall wet plant mass by *A. tridentata* and *P. tridentata* plant matter infusions. (ANOVA F value 215.3, P value < .001)

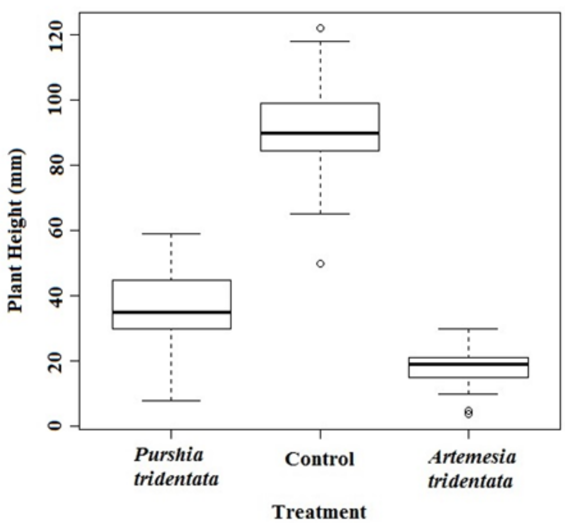

Figure 2. Boxplot demonstrating the significant impact on overall plant height by *A. tridentata* and *P. tridentata* plant matter infusions. (ANOVA F value 259.9, P value < .001)

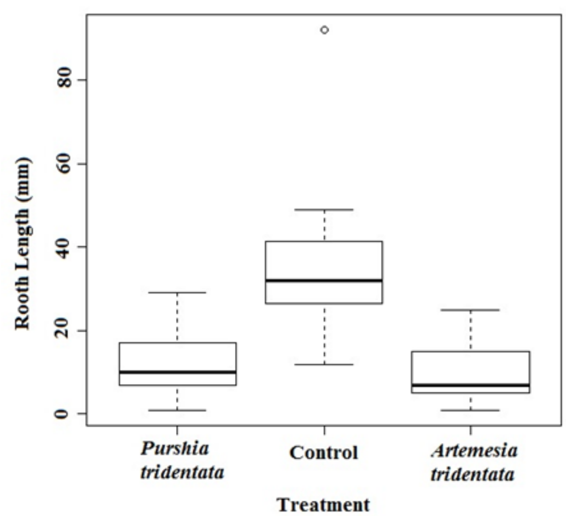

Figure 3. (above) Boxplot demonstrating the significant impact on root length of *A. tridentata* and *P. tridentata* plant matter infusions. (F value 49.78, P value <.001)

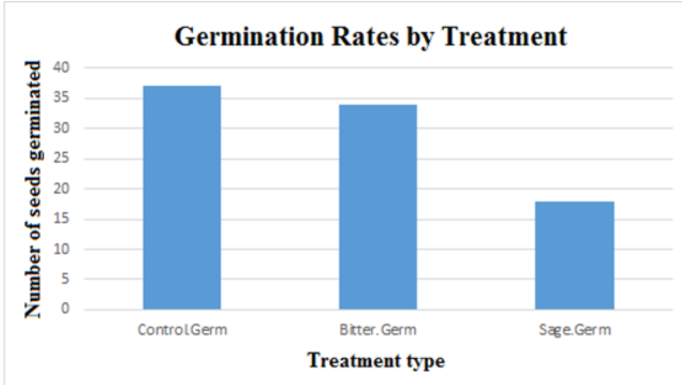

Figure 4. (left) Bar graph demonstrating the insignificant difference in germination rates between the control and *P. tridentata* plant matter infusion (X-Squared = .127, df = 1, p-value = .361) as well as the significant difference between the germination rates of the control and *A. tridentata* treatments. (X-Squared=6.56, df= 1, p-value = .005204).

Table 1: Raw Data Measured

Control			Bitterbrush			Sagebrush		
Plant Height (mm)	Root Length (mm)	Total Mass (grams)	Plant Height (mm)	Root Length (mm)	Total Mass (grams)	Plant Height (mm)	Root Length (mm)	Total Mass (grams)
86	32	0.076	30	20	0.006	20	15	0.01
88	43	0.054	33	17	0.008	21	7	0.008
113	26	0.072	59	20	0.031	30	25	0.016
84	15	0.061	45	15	0.024	30	11	0.011
89	32	0.079	45	15	0.014	28	12	0.013
90	39	0.073	58	7	0.02	10	2	0.003
88	23	0.066	49	17	0.019	28	20	0.016
96	37	0.072	56	29	0.029	19	15	0.011
95	40	0.061	25	20	0.013	15	16	0.005
100	44	0.078	53	7	0.045	5	1	0.004
85	92	0.066	40	15	0.012	10	4	0.005
82	25	0.063	45	6	0.021	17	5	0.004
111	43	0.098	35	5	0.019	15	7	0.007
92	27	0.06	45	16	0.02	4	5	0.003
100	49	0.083	38	20	0.022	20	6	0.007
100	31	0.0855	35	15	0.013	21	10	0.007
80	22	0.104	35	10	0.013	15	5	0.006
118	33	0.094	30	11	0.013			
95	12	0.061	30	7	0.017			
65	30	0.047	31	6	0.017			
122	43	0.093	26	7	0.006			
102	43	0.064	41	10	0.017			
104	45	0.081	52	6	0.019			
96	48	0.071	35	17	0.009			
98	32	0.078	32	10	0.012			
72	27	0.039	8	1	0.007			
90	35	0.068	33	7	0.012			
85	32	0.059	10	1	0.006			
97	30	0.086	45	20	0.026			
90	14	0.066	40	15	0.012			
50	21	0.016	25	7	0.015			
82	27	0.053	33	10	0.019			
68	30	0.058	30	7	0.01			
80	32	0.058						
85	27	0.062						
92	23	0.067						

Table 1. Table of raw plant data for each treatment, detailing individual plant measurements for plant height, root length, and total wet mass.

Discussion:

We hypothesized that both *A. tridentata* and *P. tridentata* would exhibit allelopathic effects on plants growing in soil that contained their secondary compounds, and we further predicted that one would serve as a more effective deterrent to competing vegetation than the other. Observational data in the field showed a lower incidence of *B. tectorum* in and around *A. tridentata* populations (unpublished data), which suggested that the *A. tridentata* would be the more detrimental of the two and this was confirmed by our laboratory experiment, most importantly where germination was concerned. We conclude from our results that both *A. tridentata* and *P. tridentata* exhibit allelopathic effects, and that they are fairly similar in magnitude, barring germination rate where *A. tridentata* had a significantly higher impact (see figure 4). We suggest that both *A. tridentata* and *P. tridentata* merit further study as possible invasive species control mechanisms, and that investigation into the mechanisms of their allelopathic affects would also be worthwhile.

Current methods of controlling invasive species in the Great Basin, and specifically *B. tectorum*, include a range of mechanical and chemical methods including timed herbicide applications, prescribed burns, applied grazing, and hand pulling, which have all been employed with limited success (Vollmer, 2008). However, all of these methods present additional challenges and costs. Herbicide quantities must be carefully measured and applied to avoid potential damage to native plants such as mountain mahogany (*Cercocarpus*), *A. tridentata* and *P. tridentata*, all of which contribute to the maintenance of a healthy ecosystem (Vollmer, 2008). Prescribed burns carry manageable hazards, but must be repeated year to year, and require additional monitoring and evaluation of biomass before being carried out (Bunting, 1987). Hand pulling is tedious and costly, and applied grazing requires monitoring and analysis in much the same way that prescribed burning does (McFayden, 1998).

A biological control option is attractive as a "fire and forget" tactic that can be applied once, and that will then maintain itself over time (McFayden, 1998). Biological control species are generally natural predators of the invasive species targeted for control, such as ladybugs released to control aphids (McFayden, 1998). Native species may also act as biological control agents if they can outcompete invaders and thus exclude them or otherwise hinder their rate of invasion (McFayden, 1998). A native species that could successfully exclude *B. tectorum* and allow the Great Basin ecosystem to eventually recover from the invasion is an attractive concept; however, a native species would require augmentation, either via manual planting or some other method of dispersal, in order to effectively compete with *B. tectorum* across its broad range. One potential application of this research could be the use of a treatment similar to what was prepared in the laboratory setting in conjunction with planting in disturbed areas of the landscape to potentially hinder the germination of *B. tectorum*. If the active allelopathic compounds could be identified and cheaply synthesized, it could prove an effective addition to the tools currently available to land management personnel.

Further study is required, however, especially if dense *A. tridentata* and *P. tridentata* planting is to be used as a control mechanism to prevent the spread of *B. tectorum* and other invasive species. This study examined the effects of a concentration of compounds, and how it compares to exposure rates in the field is uncertain. A series of laboratory and field experiments is recommended as a long-term effort to determine the viability *A. tridentata* and *P. tridentata* as large scale control mechanisms.

Future plans for research include repetitions of this study with a range of allelopathic compound concentrations. Further, we would like to expand the study to include other Great Basin shrubs and grasses that might also exhibit allelopathy to examine permutations of allelopathic compound combinations. These additional studies would help control for potential flaws in our initial experiment with regards to dosage concentration. These studies should further include the use of a control subject such as the fast growing plants used in this study, as well as known invasive species such as *B. tectorum*, in order to note any differences in the effectiveness of the compounds or resistances that the invasive plants might possess. We also recommend drying experimental plants and taking wet and dry mass measurements in future studies in order to account for water weight and to detect possible water uptake inhibition as a mechanism of allelopathy, as the mechanisms of many of these chemicals are little understood.

It should be noted that the amount of water used in this experiment far exceeds what *A. tridentata* and *P. tridentata* materials are generally exposed to in the Great Basin. Future research should consider the inclusion of *A. tridentata* and *P. tridentata* material that is dry or slightly moistened within the soil, or even soil taken from beneath the plants themselves to more closely replicate natural conditions. Further experiments might be designed to discover the mechanisms by which *A. tridentata* and *P. tridentata* work to both inhibit germination and reduce growth of competing plants, perhaps allowing for eventual artificial replication.

This research would ideally conclude in a long-term field study involving the complete clearing of several plots of *B. tectorum* habitat, after which they would be used to test the effects of the new treatment as well as traditional methods of control such as herbicides, grazing, and combinations of other previously used methods. These experimental manipulations would benefit greatly by being carried out over the course of several years, in order to account for variations in rainfall and overall weather conditions from year to year.

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