Allelopathic Effects of Yarrow and its Uses in Rangeland Conservation

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ABSTRACT

California rangelands have a long history of mismanagement that has resulted in the conversion of historically rich fields of forbs and perennial grasses to homogeneous prairies of exotic invasive European annual grasses. Rangelands are estimated to hold almost a third of the world's soil carbon and have the ability to store much more carbon when composed of perennials rather than annuals. The study of allelopathy, or the interaction between plants mediated by secondary metabolites, could provide an eco-friendly control technique for exotic invasive grasses. To test if yarrow could be used to manage invasive species in California rangelands I tested multiple application techniques on the seeds of *Stipa pulchra,* a native perennial grass, and *Avena fatua*, an exotic annual grass. I tracked the germination and growth rates of the grasses with two different yarrow applications: planted with yarrow seeds and planted with a layer of yarrow leaf litter mulch. The *Stipa pulchra* populations showed little resistance to the allelopathic effects of the *A. millefolium*, 58% of the control group germinated within two weeks of planting compared to 25% of the population planted with a layer of yarrow leaf litter. The growth rates of the *S. pulchra* populations were not significantly affected. The *A. fatua* grew vigorously despite any of the treatments and even thrived where the purple needle grass experienced declines in growth. Based on the results yarrow holds little potential for large scale applications in California rangelands.

KEYWORDS

Secondary Metabolites, Germination Rates, Growth Rates, Perennial Grasses, Annual Grasses

INTRODUCTION

The mismanagement of grasslands in California for the past century has converted once diverse fields of forbs and perennial grasses to large swaths of exotic invasive European annual grasses. This transition is thought to have begun in the 1600s during the Spanish Mission period, due to seeds carried in goods being transported into the New World (Amme and Pitschel 1989). A total of 1,152 non-native plants were brought in, of which 94 are now considered invasive plants by the California Invasive Plant Council (Barbour et al. 2007). Many of these are extremely aggressive invaders, due to their evolution in a Mediterranean climate (Barbour et al. 2007, MacDougall et al. 2009). Managing these weeds cost the U.S. an average of $2 billion annually making them more costly than any other pest. Forage quality, for both wildlife and livestock, animal and plant diversity, and soil water stores are all negatively impacted by these exotic invasives (DiTomaso 2000).

Rangelands, which include grasslands, are estimated to hold almost a third of the world's soil carbon (Schuman et al. 2002). The mismanagement of these substantial carbon sinks is significantly decreasing their potential climate change mitigation (Schuman et al. 2002, Wilkes et al. 2009). Increasing perennials is one of the main ways to increase soil organic matter (SOM) which in turn increase carbon sequestration, erosion control, beneficial microbial communities and water retention (Wilkes et al. 2009). There are currently two main avenues for restoration of native perennials in grasslands: fire and grazing. Low intensity prescribed fires can effectively reduce the cover of invasive grasses for several years after application (Marty et al. 2015). Moderate to intensive grazing practices can effectively increase habitat for native vertebrates, increase native plant cover and decrease exotic plant cover (Marty 2005, Barry et al. 2007). Both burns and grazing are effective at removing invasive plant cover and freeing up space for natives to recolonize and thrive but the longevity of the results vary greatly and require continued active management of the invasive species (Marty et al. 2015).

Application of allelopathic plants or allelochemicals provides a possible avenue of long term, cost effective, and passive control (Macias et al. 2017). Allelopathy is the study of the interaction between plants mediated by secondary metabolites. Secondary metabolites can be a diverse array of chemicals produced by plants to defend against competition, herbivory, viruses and diseases (Wink 2010). For example for weed management in organic ecosystems, allelochemicals are effective in the stunting of plant growth (Macias et al. 2017). Multiple studies of the allelopathic properties of *Achillea millefolium*, or western yarrow, a forb native to California grasslands, have somewhat promising results. A study using aqueous extracts of yarrow to test its inhibitory effects had a negative correlation between increasing extract concentration and growth/germination rates of plantain, or *Plantago major* (Behzadi et al. 2016). Another study using extracts had mixed results with the millefolium extract showing decreasing germination rates without changing growth rates (Magdalena et al. 2022). Although these studies mostly demonstrate that extracts of yarrow do impact the growth and germination of other plants it's still unclear if these properties extend to natural conditions when yarrow is grown alongside both native and exotic grasses.

This proof of allelopathic properties leaves the question: Can the allelopathic qualities of *A. millefolium* help manage exotic invasive grasses in California grasslands? Answering this question will require breaking it down into multiple parts. First I aim to identify how yarrow affects the two grass species of interest, those being wild oats (*Avena fatua)*, an exotic annual, and purple needle grass (*Stipa pulchra)*, a native perennial. Will there be a marked decrease in germination rates and growth rates as demonstrated in past experiments as in Behzadi et al. 2016 and Magdalena et al. 2022? And, how will native and exotic grasses be differently inhibited by yarrow? Because *S. pulchra*, the native grass, coevolved with yarrow I expect it to have an immunity to the secondary metabolites that would inhibit growth whereas *A. fatua* will have no defenses (Inderjit et al. 2011). Yarrow is also native to the regions in Europe that wild oats are native to but yarrow creates different arrays of allelochemicals based on environmental factors (Apel et al. 2021). Thus, despite being the same species, the allelopathic effects of Californian yarrow and European yarrow may be completely different. Finally an effective, low cost, and easily scaled up application technique must be found. Although it's expected that the most effective application is planting with pre-established yarrow this isn't feasible at large scales. I am hoping to find significant effects with planting yarrow seeds or leaf litter as mulch.

METHODS

Species of interest

To study the differences of the allelopathic effects on native vs non-native grasses I used the species: *A. millefolium* (yarrow), *A. fatua* (wild oats), and *S. pulchra* (purple needle grass). Yarrow is a native perennial forb/flower that blooms from April to September. It can grow up to 1 meter tall and prefers sunny, frequently disturbed areas. It can tolerate fairly severe drought conditions (Jepson 2012). Wild oats and purple needle grass are both grass species. *Stipa pulchra* is a native perennial bunchgrass that was widespread throughout California pre-European contact. It can grow up to 1.3 meters tall and can tolerate serpentine soils and very little water. *A. fatua* is an annual exotic grass that originated in the Mediterranean. It is an aggressive seeder, blooming March through June (Jepson 2012).

Study Sites

I conducted this research at the Oxford Tact Facility (OT), a student run farm owned by UC Berkeley, and at the Berkeley greenhouse. OT, located in North Berkeley, has clay soils and is irrigated through drip tape. I was allotted a 10 meter long bed with pre-established yarrow plants in it. It was previously an herb row managed by the student garden but had been neglected for several seasons. I prepped the beds by removing all plants other than the yarrow. The plants grown inside in the greenhouse were grown in small 4 x 6 grid planters, each cell being 5 x 7.5 cm, using the potting soil provided by the facility, “super soil”. The temperature and the lights were left unregulated. I did not add any fertilizer in the watering of the plants with the goal of mimicking more natural conditions and to avoid any confounding factors.

Controls

All of the control trays were set up inside the greenhouse. I used 24 replicates, one full tray, for both grass species. I used seeds collected within the past two years to ensure viability. Each cell received one seed, planted 1/4 inch deep with the awn pointed up. Each tray received

500 ml of water once a week. To measure growth I used a ruler depressed to soil level. The longest leaf was pulled, taught and measured at its tip. Measurements were taken once a week for six weeks after seeds had been given 2 weeks to germinate.

Seed treatment

The first application technique was planting the grass seeds alongside yarrow seeds. This treatment was also conducted inside the greenhouse. There were 24 replicates of each species for this application. The grass seeds were planted in the same manner as the control. One yarrow seed was added to each cell planted 1/8 inch deep next to the grass seeds. They received the same watering and measuring regimes as the control groups.

Leaf litter treatment

The final populations grown inside the greenhouse were those with the yarrow leaf litter treatment. I gathered leaf litter from the plants in the field, selecting a few leaves from each plant. The leaf litter was used as a mulch for the final two trays of 24 replicates. The grass seeds were planted as in the control and then topped with a thin layer of yarrow leaves. The leaves were removed only to count germination rates and were reapplied after. They received the same watering and measuring regimes as the control groups.

Field Experiment

I conducted one experiment outside on my plot at the Oxford Tract Facility. Because there were pre established yarrow plants I wanted to see how the seeds would respond to being planted in close proximity to them. I prepped the beds by removing all weeds within a 2 foot radius of the yarrow plants. I planted 30 of each grass species seeds within 30 cm of yarrow plants. There was one section of just purple needle grass, one of just wild oats and one with both seeds. I didn’t water these seeds as there were plenty of heavy rains following planting. This treatment was unsuccessful due to the failure to think of the large seed bank in the plot I was using. When the seeds I planted began to germinate, so did the hundreds of other wild oats seeds already in the soil. This made measuring both germination and growth rates almost impossible and resulted in the termination of this field experiment.

Statistical analysis

I performed a series of analysis of variance statistical tests to determine differences between the control, seed and leaf litter treatments. The data was split up to compare species to test the treatments. All tests were run as one-way ANOVAs with a confidence level of 0.05. All tests were conducted using R statistical software version 4.3.2 (R Core Team, 2021 and the package R commander. I ran one ANOVA for each species for growth rates with the treatment groups (control, seed and leaf litter) as the explanatory variables and height as the response variable. For the germination tests the response variable was the percentage of seeds germinated within the two week period.

RESULTS

Germination

The germination rates for the *S. pulchra* populations decreased in percentage germinated with the yarrow application techniques with the control having a germination rate of 58%, the seed treatment with 42% and the leaf litter with 25%. This stepwise decrease can be clearly seen in the *S. pulchra* germination graph with most of the seeds germinated between days 7 and 12, with rates flattening at day 13 (Fig. 1a). With a one-way ANOVA test I found statistically significant differences (F = 7.1489, num df = 2, denom df = 23.015, p-value = 0.003848). The *A. fatua* populations showed varying effects on germination with the yarrow applications (Fig, 1b). The control had a germination rate of 75%, as did the leaf litter treatment. The seed treatment had a 100% germination success rate. The majority of the seeds germinated before the 10th day, with rates plateauing at day 13 (Fig. 1a). The ANOVA test returned non-significant results (F = 2.1486, num df = 2, denom df = 25.222, p-value = 0.1375).

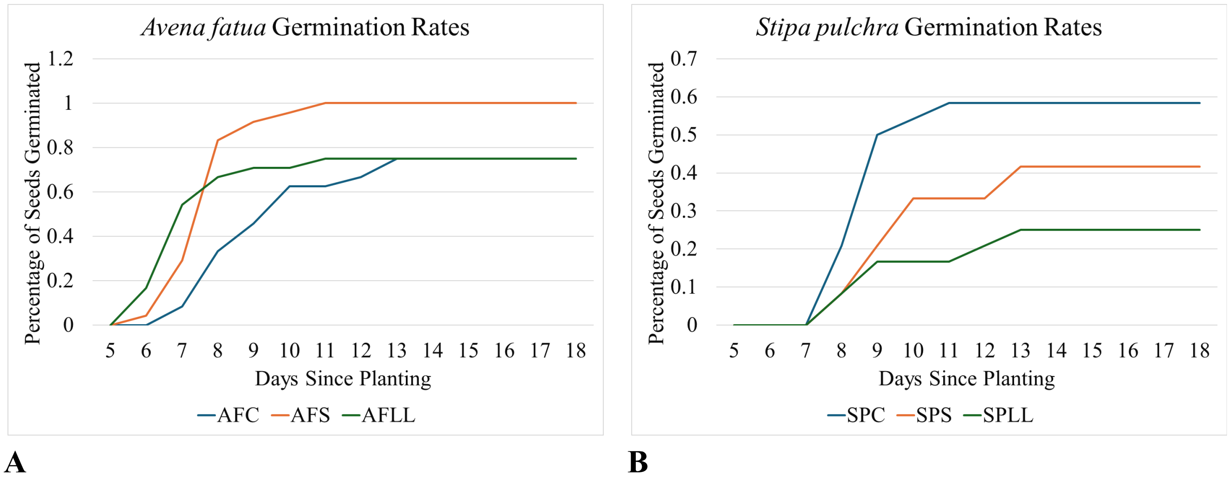

Figure 1. The percentage of seeds germinated by day of all the *S. pulchra* populations (A). The percentage of seeds germinated by day for all the *A. fatua* populations (B). C-control, S-seed, LL-leaf litter

Growth

Although the growth rates of the *S. pulchra* populations with the yarrow treatments appear be slower than the control, seen in figure 2A, the ANOVA returned non-significant results (F = 1.5376, num df = 2.000, denom df = 92.901, p-value = 0.2203). Perhaps repeating the experiment with more replicates would yield more conclusive data or running the experiment for a longer duration as we can see the rates of growth showing more differences in the final week (Fig. 2a). The *A .fatua* control group was revealed to have the lowest growth rate out of all the *A. fatua* populations. There was a positive correlation between yarrow applications and growth with the wild oats, especially with the leaf litter mulch, in figure 2B. The ANOVA test of the exotic grass growth rates showed significant differences in growth rates between treatments (F = 12.872, num df = 2.00, denom df = 224.05, p-value = 0.000005108).

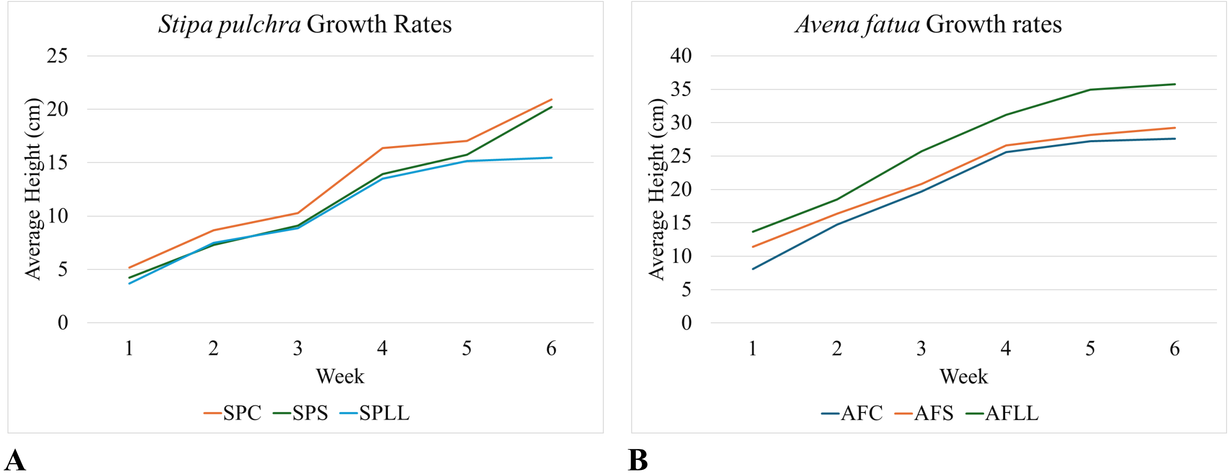

Figure 2. The growth rates of all (A) *S. pulchra* populations and (B) *A. fatua* populations measured by average height of grass by treatment measured once a week. C-control, S-seed, LL-leaf litter

DISCUSSION

The *S. pulchra* populations demonstrated surpessed germination and growth rates from the yarrow and did not respond to potential resistance to the allelochemicals of *A. millefolium*. The data illustrated an unexpected relationship between the yarrow applications and the growth *A. fatua* (Figure 2). The wild oats grew vigorously despite any of the treatments and even thrived where the purple needle grass experienced significant declines in growth, with the leaf litter treatment exhibiting growth by 30% higher than that of the control. The germination of *A. fatua,* however, was not negatively impacted by any of the yarrow applications.

Effects on *Stipa pulchra*

The native grass species, *S. pulchra*, revealed a significant reduction in germination rates with both *A. millefolium* treatment techniques (Figure 1). Total germination within the control group was 58% within two weeks of planting compared to 42% of the population planted with yarrow seeds and 25% of the population planted with a layer of yarrow leaf litter. The higher efficacy of the applied leaf litter is consistent with typical allelopathic principles of suppression of germination.

Many allelopathic plants contain the majority of their relevant secondary metabolites in their leaves. Yarrow specifically contains all of its allelochemicals in its leaves with most extending throughout the entirety of the plant (Farasati Far et al. 2023). The allelochemicals that affect germination rates are phenolic acids, tannic acids and flavonoids. These decrease germination respectively through disruptions of membrane structure and functions, cytotoxic effects and metabolic interferences (Einhellig 1994). Yarrow contains multiple different phenolic acids and the flavonoid kaempferol (Apel et al. 2021, Farasati Far et al. 2023). By watering through the layer of leaf litter the water soluble compounds are expected to leech from the leaves and percolate into the soil. Although flavonoids are not particularly water soluble, phenolic acids are highly soluble. The high levels of phenolic acid in the leaf litter likely accounts for the stunted germination rates in comparison to those present when grown with germinating yarrow.

The growth rates of the *S. pulchra* populations didn't respond to the yarrow treatments with a significant decrease. When planted with *A. millefolium* seeds the difference in growth rates is negligible but with the leaf litter there was still a visible drop in rates (Figure 2). The relevant allelochemicals that affect growth act in a multitude of ways, these being phenolic acids, alkaloids and monoterpenes. Yarrow contains multiple different phenolic acids, including caffeic acid, a particularly effective allelochemical, four different monoterpenes and at least one alkaloid (Einhellig 1994, Farasati Far et al. 2023). Phenolic, specifically caffeic, acids and many alkaloids interrupt plants' natural deterioration of Indole-3-acetic acid (IAA), a powerful growth regulator that decreases growth rates when concentrations are high (Einhellig 1994, Bunsangiam et al. 2021). Caffeic acid decreases plants uptake of nutrients from the soil including nitrogen, phosphorus and potassium. Reduction in nutrient uptake has a direct correlation with amounts of chlorophyll produced and subsequently on dry weight (Alsaadawi et al. 1986, Farasati Far et al. 2023). Monoterpenes have also been shown to stunt root growth (Einhellig 1994, Farasati Far et al. 2023). The combined effects of these allelochemicals supports the explanation for the decrease in growth rates for the treatments with yarrow.

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Effects on *Avena fatua*

The effects on the yarrow treatments on germination rates of the *A. fatua* populations were inconclusive. Planting with yarrow seeds led to the highest germination rate in all treatments of 100% followed by the leaf litter and control groups with a rate of 75% and these results were statistically non-significant. Wild oats are more resistant to allelochemicals in terms of germination rates compared to other invasive grass species (Jabran et al. 2010). There was no impact on germination rates by the allelochemicals previously listed for the *S. pulchra* populations: phenolic acids, tannic acids and flavonoids (Einhellig 1994).

The growth rates showed the opposite effects from the *A. millefolium* treatments compared to the *S. pulchra* populations (Figure 2B). Despite the fact that phenolic compounds and other allelopathic plants such as mulberry decrease the growth rates of wild oats. The groups planted with a yarrow leaf litter mulch grew at the fastest rate (Almaghrabi 2012, Bajwa et al. 2017). The lack of effects from the treatments is consistent with studies where *A. fatua* has exhibited resistance to multiple different types of herbicides (Adamczewski et al. 2013). Only certain allelochemicals affect the pathways relevant to decrease in wild oats growth. The phenolic compounds used in Almaghrabi's (2012) study: salicylic acid, ferulic acid, hydroxybenzoic acid and hydroxyphenyl acetic acid, are all phenolic acids but none are present in yarrow. Another possible reason for a lack of allelopathic inhibition of growth could be the lack of water solubility in many non-phenolic acid allelochemicals, preventing percolation into the soil and interaction with the seedling. An alcohol or acetone based extract could better access chemicals that may have a stronger inhibitory effect. As for the increase of growth rates with the application of yarrow leaf litter this may be due to the water retaining properties of mulch. WIth a layer of leaf litter there is higher water retention and therefore the wild oats won't be as water limited as other treatment groups.

Broader implications

Based on the findings from this study, *A. millefolium* is not an ideal candidate for biological control of exotic species in California rangelands. Although there are clear changes in plant growth and development from yarrow applications, they do not affect non-native plants more so than their desired native counterparts. A generalized broadcast treatment would not keep invasives out after their removal with prescribed fire or grazing as intended as there was no effect on their germination. It would effectively keep the native plants out which would be counter intuitive. Instead of the native grass having built up a resistance to the allelochemicals I observed the allelochemicals being specialized to stunt native growth. Using an allelopathic plant from Europe may yield the opposite results, being co-evolved to European annual grasses they could potentially affect them more so than California perennials. Improving perennial richness in rangelands can greatly improve our soil carbon storage but further research is needed to find an effective approach.

Limitations

Future research includes looking into different application methods, specifically how to ensure as many allelochemicals as possible reach the soil in future experiments. This work may include non-water based extracts as many allelochemicals are not water soluble. Many studies on allelochemicals use ethanol or acetone based extracts to isolate certain chemicals. It would also be beneficial to test the effects of yarrow on non-grass exotic invasives such as *Oxalis pes-capse,* a member of the wood sorrel family that has invaded many central california coastal agricultural sites. Looking into more realistic field experiments or repeating the field experiment in this study could also yield beneficial data on environmental factors working with allelopathic effects in limiting growth of exotic invasives such as drought and nutrient deficits.

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