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The Impact of Interactions Among Native Grassland Species: A Study of Interactions Between Two
Invasive Species (*Bromus tectorum* and *Setaria faberi*) and Two Native Species (*Helianthus
annuus* and *Rudbeckia hirta*)

An Undergraduate Thesis Proposal

By

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Abstract

Allelopathy is a description of an interaction between two plant species, usually negative, that impacts their growth or germination. There has been ample data published regarding the impact of allelopathic species in agricultural settings, but little attention has been given to the potential interactions between invasive and native species. Two culprits that have employed allelopathy to great effect in agricultural settings are cheatgrass (*Bromus tectorum*) and giant foxtail (*Setaria faberi*). This study posits that allelopathic interactions, similar to those found in agriculture, are occurring between native and invasive species on the Great Plains.

A set of greenhouse trials was established to determine the effects of interplanting of native and invasive species (simulating the annual fight for germination and resources in Spring). The invasive species biomass was harvested after 6 weeks for the second set of trials, set to simulate native species germinating under last season's growth of invasive tissue. This scenario is common in the grasslands of Nebraska, where germination often occurs under the dead foliage of last season's growth.

In examining the interactions between the two invasive species, described above, and two native species, sunflower (*Helianthus annuus*) and black eyed susan (*Rudbeckia hirta*), it was found that there were small differences between the control and experimental populations. Vigor and speed of development was negatively impacted in both *Rudbeckia* and sunflower, likely due to interspecies competition. Both invasive species showed a deleterious effect on native plant growth compared to the control. There was no impact on germination or growth in the trials with dead invasive biomass spread over the surface of the soil.

Introduction

Throughout humanity's migrations across our planet we have always had plant species in tow. From the dawn of hunter-gatherer migrations, there is archeological evidence that various species have accompanied our travels (Novak, 2001). Most of these newcomers are unsuccessful in establishing a foothold in a new climate. However, there is the odd species that finds success in its new environment. For most of these lucky few, the original colonist only sows a few successive generations before perishing and vanishing from its temporary home. There are a very small number of species that manage to avoid the guillotine of a non-native climate. These handful of pioneering species are often a cause of great concern.

When a non-native species beats the evolutionary odds and manages to establish a long-standing, breeding population in its new environment we christen them "naturalized". This naturalized species often succeeds by "imitating" the growth patterns of native species. These naturalized species have a further rarer class of species within their ranks- the "invasive". An invasive species is characterized by aggressive expansion into a non-native habitat. Furthermore, an invasive species is so fecund, aggressive, and dominant in its new habitat that it is prone to causing profound ecological damage (Vitousek, 1996).

While many of these invasive plant species are dominant in high disturbance ecosystems (such as farmland or urban areas), it is useful to understand that even our most pristine parks and refuges are impacted. It is estimated that between 5% and 25% of all vascular plant species present in in the United States National Parks System are non-native (Vitousek, 1996). This frightening statistic can be thought of as the canary in the coalmine - signaling that these invasive species have permeated even our most treasured "pristine" landscapes. Cheatgrass (*Bromus*

tectorum) is a prime example of an invasive that continues to impact ranchers and National Parks alike as it spreads across the Great Plains and Western U.S. (see figure 1). Armed with the knowledge that invasive plant species don't stop their march towards ecological domination at our backyard or pasture fence, we must act.

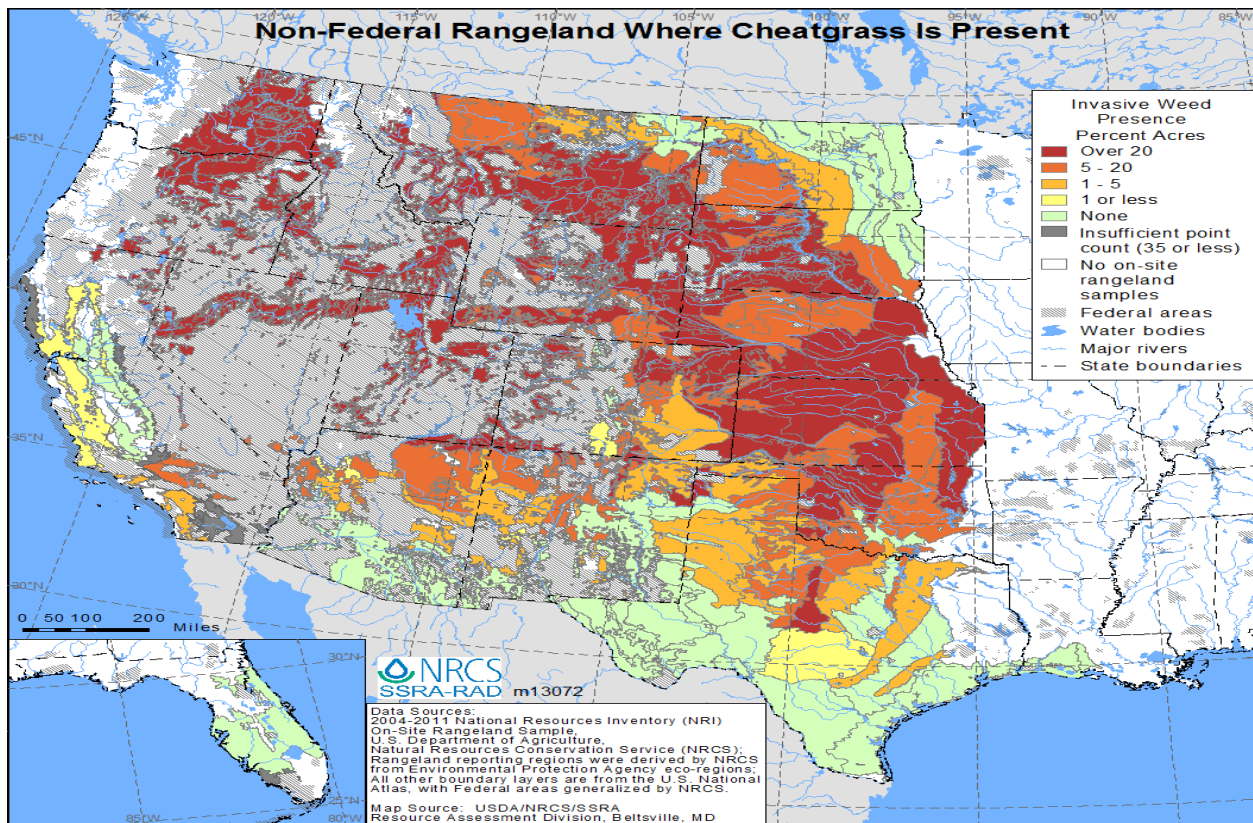


Figure 1. Distribution of Cheatgrass, one of the fastest spreading invasive species (USDA, 2014)

To act effectively, one must understand the tools of one's enemy. One of the most potent tools at many invasive species' disposal is allelopathy. The term allelopathy stems from the Greek allilon & pathy to mean "mutual harm" or "suffering" (Willis, 2007). It is, broadly speaking, an adaptation wherein one plant produces chemicals that inhibit the growth, reproduction, germination or survival of another plant (Willis, 2007). It is possible that this tool

is employed by many species that have become invasive in across the Great Plains. Two culprits that have employed this tool to great effect are cheatgrass (*Bromus tectorum*) and giant foxtail (*Setaria faberi*) (Rice, 2012; Bell, 1972). These species arrived onto the Great Plains from different lineages and evolutionary backgrounds. However, they both have successfully established themselves as aggressive competitors in our prairies, pastures, and croplands. When these species are found, they are often present in patches with remarkably low species diversity (Stachon, 1980). This total suppression of other species is the result of chemical warfare- reducing the germination and growth of all competition (Willis, 2007).

However, some testing has been done in Europe, concluding that there is no allelopathic germination suppression of native European species by invasives (De Fabbro, 2014). The effects of invasive allelopathy on native grassland species of the Great Plains remains unknown. I hypothesize that the same allelopathic traits that cause reduction in crop germination and growth are also responsible for reduced germination and growth in species found in native prairie ecosystems. To test this hypothesis, I am going to take a selection of two native species, sunflower (*Helianthus annuus*) and black eyed susan (*Rudbeckia hirta*) (both present across the Great Plains), and observe their interactions with the invasive species mentioned above.

An interesting additional interaction that may factor into my results is the allelopathy of sunflowers themselves (Azania, 2003). Another intriguing trait possessed by our natives - black eyed susan have been shown to have high rates of successful establishment in areas dominated by invasive species, such as roadside ditches (Johnston, 2014).

Materials & Methods

There will be two main trials. The first experiment will be a trial in which the sunflower seeds and black eyed susan seeds will be interplanted with the cheatgrass and foxtail seeds. In the first trial, there will be 10 replications of each mix (each invasive paired with a native). Seeds will be buried beneath ½ inch of potting soil. Measurements will be taken after 6 weeks of growth regarding seedling vigor (height and health) and germination rate (% seeds successfully germinated).

The second trial will involve invasive tissues (fresh leaves and roots) that will be spread over the surface of soil and planted with native seeds (10 replications for each native invasive pairing). The study will be observing data for vigor (plant height and health) and germination rate (% seeds germinated). The leaf and root tissue will be obtained by harvesting greenhouse grown specimens of invasive species.

Standardized greenhouse potting soil will be used, sourced from Beautiful Land Products in Iowa and no fertilizers or herbicides will be present for either of these trials. All seeds have been cold stratified for 8 weeks prior to germination. Plastic cells will be used to hold the media, measuring 2” across. Regular watering will occur using municipal tap water from the City of Lincoln. Fluorescent grow lights mounted on wire racks will be used to provide the greenhouse conditions for the experiment. Both native plant’s seeds were procured from Stock Seed Farm and the invasive seeds were procured from the UNL Weed Studies Department. The entire experiment will be conducted at 25C.

The goal of these methods is to determine the role of allelopathy in common invasive-native interactions in Nebraska’s Prairies. The study is attempting to replicate the co-germination of natives and invasives - a common fight for survival in Spring. The native seeds germinating under invasive tissue seeks to imitate the conditions that might occur after a mowing or grazing.

For future research, a field survey and several field trials would be a good next step but are beyond the scope of this project. These experiments merely seek to imitate natural interactions. We cannot know the true applicability until these results are replicated in the wild. It may also be interesting to consider moisture levels and soil type when evaluating these allelopathic interactions in the future. There may be some advantages and disadvantages to clay or sandy soils that must be taken into account when discussing these interactions. In addition, there is a vast precipitation gradient across the state and Great Plains as a whole. There is likely a good deal of difference between how ecosystems react to allelopathic invasives in the arid West or the humid East.

Results

The experiment showed that there was not a significant impact to the germination rate of *Rudbeckia* in both interplantings of cheatgrass and foxtail (see figure 2). *Rudbeckia* experienced 40% germination loss in both invasive plantings compared to the 100% germination rate found in the controls. However, this is not likely a result of allelopathy, but could be a result of interspecies competition or natural variation in germination. *Rudbeckia* plantings in controls germinated in, on average, 6 days. Cheatgrass and foxtail germinated in all trials in which they were planted.

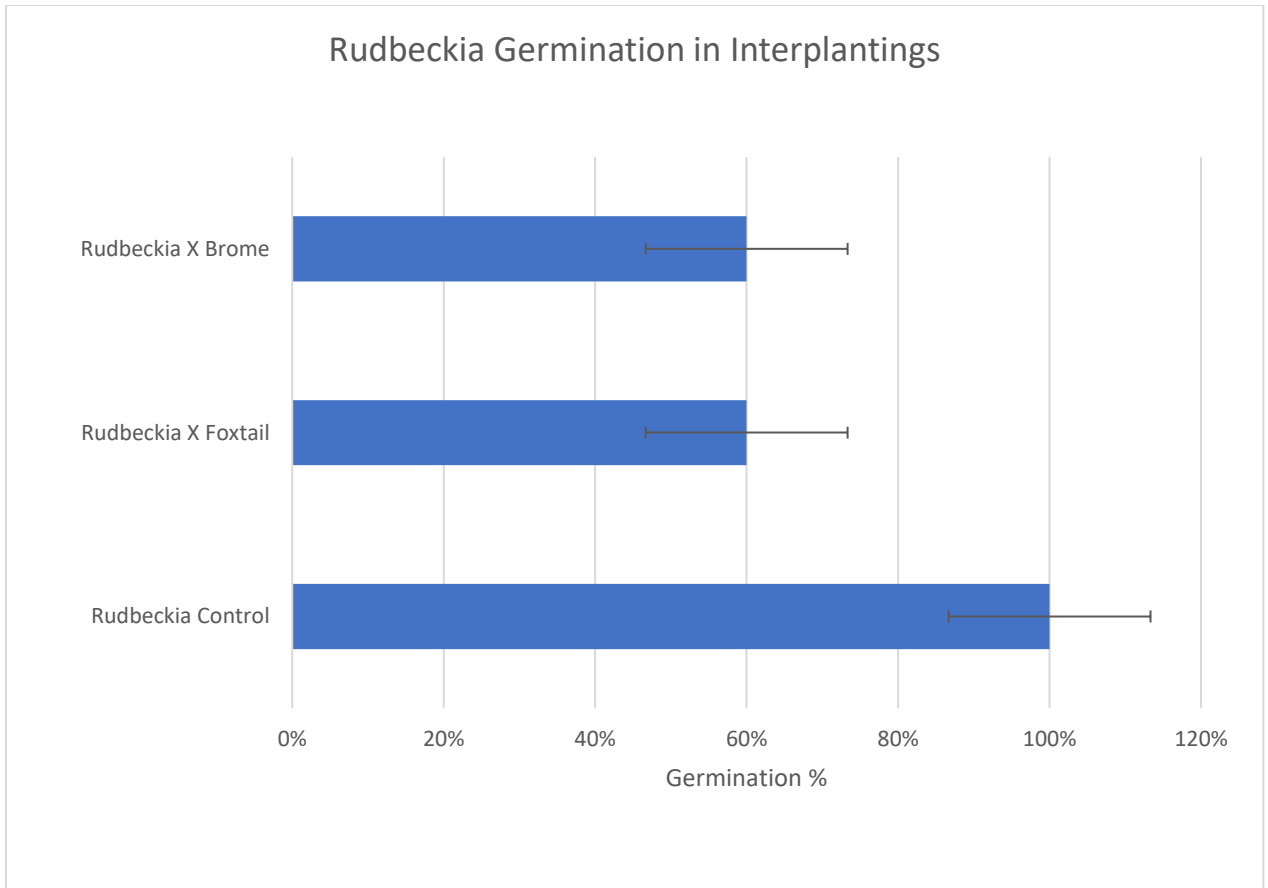


Figure 2

Sunflowers in the control and in the invasive plantings showed inconsistent rates of germination, as could be expected with native seed. There was not any interaction regarding the germination of sunflowers compared to the control. However, the data regarding vigour seem to suggest interspecies competition had a small effect on sunflower height.

	Native Germination	Invasive Germination	Mean Native Height (inch)	Mean Invasive Height (inch)
Sunflower Control	40%	0	3.03	0
Sunflower x Foxtail	30%	70%	2.5	1.18
Sunflower x Brome	60%	100%	2.66	2.98

Table 1

Both sunflowers and *Rudbeckia* showed slight decreases in vigour when interplanted with the invasive species as measured at 6 weeks growth (see figures 3, 4, 5, & 6). There seemed to be a slight correlation between the two trials in that interplantings compared to the control. This is not likely due to allelopathy but is likely due to interspecies competition.

	Native Germination	Invasive Germination	Mean Native leaf count
Rudbeckia Control	100%	0	1.77
Rudbeckia X Foxtail	60%	100%	1.45
Rudbeckia X Brome	60%	100%	1.54

Table 2

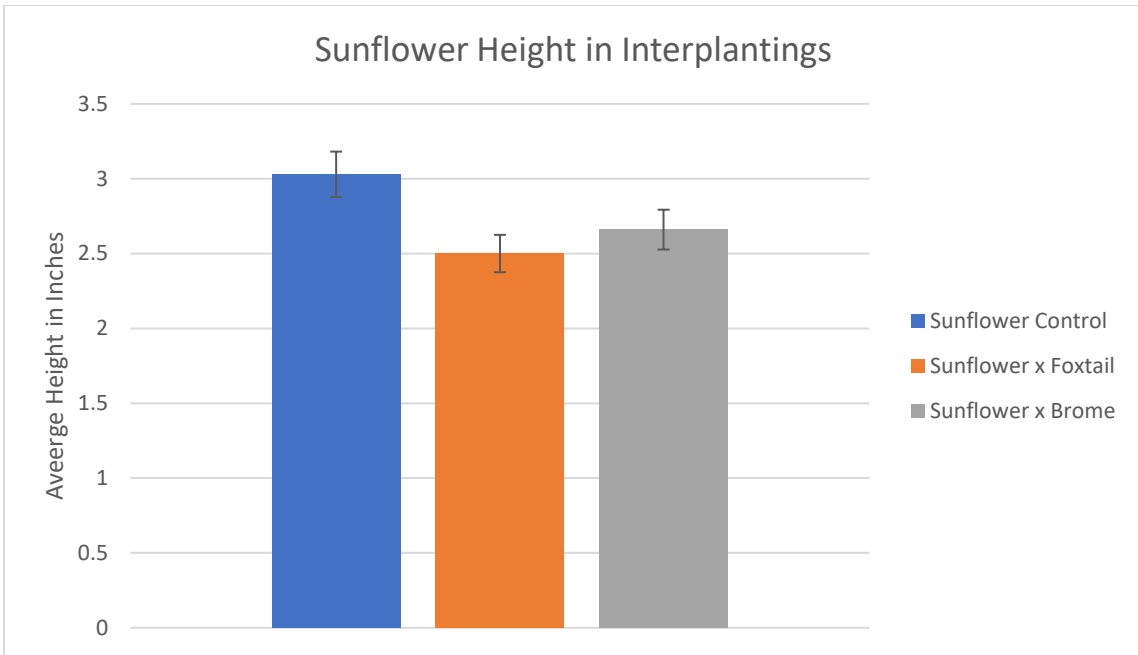


Figure 5

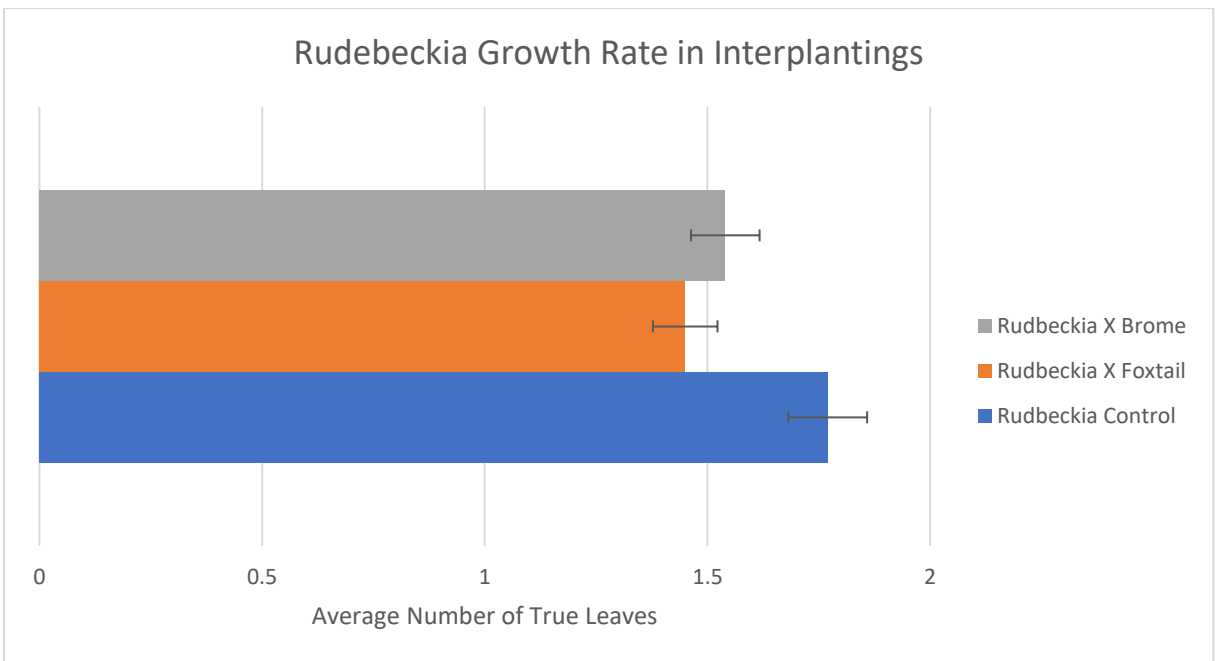


Figure 6

There was no interaction when the dead plant matter of cheatgrass and foxtail was placed over sunflower and *Rudbeckia* seeds (see figure 5 & 6). The weights of the biomass applied at

the time of planting are listed in table 3. Germination was not slowed nor reduced by the application of the invasive tissue and plant vigor was not impacted. Germination was ~4 days for the sunflowers and ~6 for the Rudbeckia. There was 100% germination in all trials.

Planting	Native Germination Time (days)	Invasive Biomass (g)
Sunflower Control	4	0
Sun x Foxtail	4	0.03
Sun x Brome	4	0.15

Table 3

	Native Germination Time (days)	Invasive Biomass (g)
Rudbeckia Control	6	0
Rudbeck. X Foxtail	6	0.05
Rudbeck. X Brome	6	0.12

Table 4

Discussion

The objective of this research was to determine if there was a similar allelopathic interaction between invasive and native plant species as has been documented in much of the literature cited below. The results that were recorded seem to highlight a few major findings:

1. There appears to be no significant reduction in germination in *Rudbeckia* when grown in conjunction with either giant foxtail or cheatgrass.

This statement is supported by the data present in figures 3 and 4. A 40% reduction in germination is within the typical variation expected for a native species. This seems to suggest that there is an active mechanism utilized by giant foxtail and cheatgrass independently, perhaps competition for nutrients, water, and light.

2. There appears to be a slight decrease in the vigor of native species when interplanted with cheatgrass and giant foxtail when compared to controls.

The data collected from the interplantings appeared to show some correlation between the impact of the invasives on the two natives being studied. It is likely that such a set of interactions can be traced back to the competition between invasive species and the natives, and not allelopathy. Both species seemed to cause the native specimens to perform only slightly worse than the control.

3. No evidence of allelopathy was found in either cheatgrass or giant foxtail when applied to the surface of the soil before germination.

There is a great deal of evidence in the literature for foliar allelopathy, such as is found in Winter Rye (Achrya, 2017). However, such a result was not obtained for the species studied. It appears as though suppression of germination and growth was only a factor when there was a live specimen. Dead foliar and root matter on the surface of the soil had no impact on the germination time or growth rate of sunflower or black eyed susan.

There appears to be no support for the hypothesis that the same allelopathic interactions that are occurring in agricultural settings are occurring in native grasslands.

Summary & Conclusions

Context in Europe

The root of these questions regarding allelopathy and weeds establishment outside of their native ranges is discussed in a study by Del Fabbro titled : “Allelopathic effects of three plant invaders on germination of native species: a field study” (2014). This study takes place in Europe and examines the “Novel Weapons Hypothesis” at the root of the allelopathic interactions between invasive and native species.

Being a European study, it measures the impact of allelopathic compounds produced by two American species and one Asian species. The seeds are sown outdoors in small test plots. A mix of natives is sown in beds with mixes of invasives and the germination is recorded over the course of 9 weeks.

Contrary to the Novel Weapons Hypothesis, it seemed as though germination was not repressed by the invasive species. This study provides some evidence that allelopathy is not as impactful as other research, including mine, has suggested. The author offers up some commentary that hypothesizes that native plants allelopathic interactions may have been present in the “control” plot. This would imply that both natives and invasives are utilizing allelopathy to the same effect. Therefore, the author concludes, invasives which use allelopathy are no more capable of suppressing germination than natives. Allelopathy is but one tool in an invasive

species toolbox. Field studies such as this are hopefully in my future. I would be highly interested in replicating this design with a format centered around North American interactions.

Further interesting comparisons between this paper and previous literature can be drawn in the history of *Veratrum album L* or False Hellebore. This is a poisonous weed native to Asia that has been naturalized in European mountain grasslands. This weed is of interest because, according to genetic data collected by Treier (2011), it seems to have colonized Europe in several waves. These waves seem to coincide with the glaciations that have occurred over the past few millennia. These glaciations appear to eliminate the populations of False Hellebore, only to have them recolonize the area when the glaciers recede and humans reenter the area.

This is another demonstration of humanities impact on our environment. We are quick to designate undesirable species as weeds, but we don't often stop to think about how they established themselves. There is evidence that False Hellebore has been used medicinally as a topical analgesic (Teier, 2011). This could be a medicinal herb that was brought along with human colonists after each glacial maximum. However, through our modern lens, it has no purpose and should be eliminated. Perhaps there is a lesson to be learned here that can extend to a species has been studied above- cheatgrass.

Context in North America

It is thought that Cheatgrass was first introduced to the United States from Europe due to the depletion of America's native pastures. (Novak, 2011) European livestock were accustomed to eating European grasses, so when the few palatable Native American grasses were depleted in the first European settlements on the East Coast, European forage grasses were brought across the Atlantic. The intent was to increase the palatability of American pasture for European

livestock. In this, they were successful- but at a cost. Cheatgrass Grass is highly palatable for cattle for the period of its life cycle. However, it becomes dangerous to eat when setting seed (Novak, 2011). Cheatgrass has now spread over the Western U.S. in vast swaths. Its allelopathic traits no doubt suppressing the natives it encounters along the way. It is a story repeated a thousand times throughout our history as a species; a plant is useful until it jumps the fence and escapes captivity- only then do we vilify it and call it a weed. Cheatgrass has been thoroughly vilified in the last few decades, as has False Hellebore. However, it is a good practice in empathy to remember the friendly origins of these weeds. Like a peppermint plant taking over a garden, many of our greatest allies in the plant kingdom are also our most dangerous bedfellows.

What has only been briefly mentioned in the study, but should be elaborated upon, is the allelopathic properties of the Sunflower (*Helianthus annuus L.*). The sunflower is native to North America, along with all other members of the *Helianthus* genus. This species makes for an interesting focus of the study as it involves the relative of a set of common weeds imported from the Americas, rather than imported to. Sunflowers are grown as a food crop around the world. The largest producers of Sunflowers are Russia, Ukraine, and Argentina (Azania, 2003).

However, Sunflowers are also an important crop across the tropics. The success of an introduced temperate species in the tropics is helped in large part by the genus's thermo and photo neutral growth patterns(Azania, 2003). This allows Sunflowers to be successfully grown in any region with adequate soil, warmth, and precipitation. These criteria are easy to satisfy as most members of the *Helianthus* genus are tolerant of mild frosts, extreme heat, arid conditions, and many soil types (Azania, 2003).

Issues with this useful genus begin to arise when examining their interactions with other plant life. Sunflowers have been shown to produce a medley of allelopathic compounds

throughout their leaves, stems, and root exudates (Azania, 2003). The later of which is most impactful to agricultural and conservation settings. Root exudates, as well as the “green manure” or dead plant matter, of sunflowers has been shown to inhibit the germination and growth of a wide swath of crops and pioneer species. Root exudates are particularly impactful in tropical regions where multiple rotations of sunflower can cause a potent build-up of these compounds in soils (Azania, 2003).

Further studies could include the examination of other members of the *Helianthus* genus and their interactions with native species in their introduced ranges. Such an examination may prove useful in identifying strategies for control of these potent allelopathic traits. This literature holds particular import for this study as Sunflower’s interactions with invasives may be influenced by its own allelopathic traits. It is important to understand, in the context of this study, how complex and multifaceted these allelopathic interactions can be.

To examine the real-world applicability of these results, I would like to reference the control of invasive species in Georgia’s roadside ditches. While this may seem like a trivial pursuit, it is highly important to catalogue and control species in roadside ditches. Roads, being vectors for human travel, are also a primary vector for plant’s travel. The frequent disturbances present in the human dominated ecosystem that is a roadside ditch are also key in assisting invasive and potentially invasive species propagation (Johnston, 2014).

There is a direct link between the density of invasive species in Georgian forests and proximity to a roadway (Johnston, 2014). The human dimension of weed transport and establishment is rarely so clear and actionable. There have been several instances in which researchers have identified allelopathic interactions in roadside ditches. These allelopathic interactions lead to decreased species diversity and increased invasive stands (Johnston, 2014).

This is highly relevant to the research presented above as cheatgrass, giant foxtail, black eyed susan, and annual sunflower are all likely to be interacting in ditches across Nebraska at this very moment. Understanding that these species move along human routes of transport can help us to determine the best control strategies for the situation at hand.

Examining the Population Dynamics of Weeds

The relative contribution of natural landscapes and human-mediated factors on the connectivity of a noxious invasive weed examines (Alvarado-Serrano, 2019) examines the genetic diversity of a problematic weed and its surprising interconnectivity over a large range of the Eastern U.S.. *Ipomoea purpurea* or the Common Morning Glory is a introduced species hailing from Mexico and Central America. It is well known in American gardens for its rapid growth and daily display of its flowers every morning. However, it is also one of the most recent problematic weeds introduced to the American Midwest. It is prone to smothering crops with its vines and is often resistant to herbicide.

When examining the movement of invasive species, and their subsequent impact on the surrounding ecosystem, there is a surprising degree of homogeneity (Alvarado-Serrano, 2019). For example, research has been conducted observing genetic markers of Morning Glory across roughly a dozen sites in the Midwest. These sites were then compared to the genetic drift from a control, sourced from a native specimen in Mexico. Surprisingly, there was very little genetic difference in the supposedly isolated sites (Alvarado-Serrano, 2019). This seemed to correlate directly with population density of the region being examined, with more populous areas having a greater degree of homogeneity. This is likely due almost entirely to human impact and propagation. Morning glories have heavy seeds that do not distribute over even short distances and their seeds are poisonous. This precludes any natural distribution and shows what a crucial

role humans play in the distribution of invasive species. Such information can likely be applied to other weed species, which could explain how advantageous traits such as herbicide resistance or allelopathy spread so quickly through distant regions (Alvarado-Serrano, 2019).

If this study were to be conducted under ideal conditions, it would involve much larger sample sizes and the integration of field trials. The data presented in this study was interesting, but not without its flaws. The lack of germination presents a confounding variable. It would have been very intriguing to determine if there was indeed an interaction between Canada thistle and our native species, as was described in previous literature (Stachon, 1980). Furthermore, there should be a field survey conducted to determine the stands of the species in question which currently exist in prairies and grasslands across Nebraska.

Cousens' *Dynamics of Weed Populations* (1995) examines the very foundation of what constitutes a weed. From the roots of agriculture in the Middle East to our modern struggles with herbicide resistant weeds, Cousens paints a picture of population dynamics on a grand evolutionary scale. This publication gives the creation of what we call weeds a human focus by zeroing in on the path these plants have taken. Many have originated from warm climates where short life cycles after disturbance were advantageous. Their place in our fields and ecosystems are often a result of centuries of travel and unique impact that agriculture has had on the Earth's landscapes.

Historic Context

What we dismissively call weeds are the complex product of human migration and agriculture. Weeds introduction into new regions closely follow the path of new human migration. In Australia, where sheep herding exploded in the last century, it was found that one

of the most significant vectors for the introduction of new invasive species was the gastrointestinal tract of sheep (Cousens, 1995). Weed seeds could be transported for weeks before being deposited in a new pasture. Such a mode of distribution is also found in cereal agriculture, where a study in Utah found that 36% of grain in the state contained significant amounts of weed seed (Cousens, 1995). Weed seeds are often found on the mud of a traveler's shoe, the food that they eat, and even in the stitches of their clothing. Wherever humans and their livestock have migrated, they have changed the surrounding ecology by their very footsteps.

Inevitably, we see the natural world through a human lens. The designation of certain plant species as weeds is the product of that lens. Our commodification of naturally occurring plant species has allowed species such as wheat to spread from Anatolia to the furthest corners of the globe and become virtually unrecognizable compared to its wild cultivars. Such a process has occurred to hundreds of crop species, but perhaps thousands of introduced species.

Each species that we designate as a weed has a story. For example, *Bromus tectorum* or cheatgrass is an annual grass native to Western Europe. It has followed the footsteps of Europeans wherever they have gone- from Australia to Argentina and from the Western U.S. to Western China (Novac, 2012).

Furthermore, giant foxtail is native to Northern Japan. Its journey can be traced down the Silk Road where it spread to Europe. From there, European colonization of the America's has brought it past the coasts and Westward - to the heart of the Great Plains(USDA, 2014). Finding a habitat similar to its home, Giant foxtail has made good use of its grassland lineage to take root across Nebraska. Giant foxtail has nearly circled the world over the course of centuries, and its story is not rare. The tools it employs, such as allelopathy, likely hold a great deal of importance in the story of how it successfully reproduced in new ranges for hundreds of years.

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