

**Effects of Dalmatian toadflax on grass biomass and forb biodiversity in
Western Montana grasslands**

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Abstract

Invasive species are known to displace resident flora through competition, which can lead to a decrease in biodiversity. This is a concern even on wildlife refuges such as the National Bison Range, where Dalmatian toadflax (*Linaria dalmatica*) is particularly aggressive. Although there is abundant research on the eradication of toadflax, previous work has failed to address the effects of Dalmatian toadflax on grassland species. This study compared grass biomass and forb biodiversity between paired plots with and without high densities of Dalmatian toadflax and found strong evidence that grass biomass is reduced in the presence of high densities of toadflax. This study indicates that current eradication methods have not been entirely effective at eliminating the negative effects of toadflax. This has implications for management of grazing on the range, as a reduction of bison grazing in areas with high density of toadflax may assist eradication efforts.

Keywords: Dalmatian toadflax, grasslands, invasive species, grass biomass, biodiversity, National Bison Range

Introduction

Grasslands in North America are currently at risk for ecosystem destruction from agricultural and commercial development, invasive species, grazing management, and fire suppression (Wick 2015). These changes threaten ecosystem biodiversity, compromising the services that it provides to humans and wildlife (Myers 1996). Invasive species, in particular, are known to displace indigenous flora by outcompeting them for resources due to uniquely competitive characteristics in a new environment (Allendorf & Lundquist 2003). It is now thought that invasive species are a major component of global environmental change as they promote species extinction, which decreases native species diversity (Vitousek et. al., 1996). This is of concern for grasslands in the United States, as the majority of pre-settlement grasslands have been converted to other land uses and conversion is still occurring (Connor et. al. 2001).

Montana is home to five types of grassland ecosystems, though only a small portion of this land is protected (State of Montana 2017). In the Western grasslands of Montana, aggressive invasive species such as cheatgrass, spotted knapweed, and leafy spurge are a widespread issue (Pearson et. al. 2016). Affected grasslands include those on the National Bison Range in Charlo, Montana. The National Bison Range is one of the last intact publicly-owned intermountain native grasslands in the U.S., therefore making it a valuable area for conservation (USFWS 2017). One of the most prominent invasives, Dalmatian toadflax (*Linaria dalmatica*), is abundant on the National Bison Range in Montana and is known to thrive in disturbed areas (Sing et. al 2016, Jacobs & Sing 2006).

Dalmatian toadflax was introduced as an ornamental from Eastern Europe in the early 1900s and has since spread in the U.S., including all but nine counties in Montana (Alex 1962, Jacobs & Sing 2006). It is a noxious weed in the Plantain family and has yellow snapdragon-like flowers and heart-shaped, waxy leaves. Dalmatian toadflax can grow to 1-4 ft tall and is capable of profuse seed production - a large plant can produce up to 500,000 seeds (Sing et. al. 2016, Robocker, 1970). Underneath the ground's surface, Dalmatian toadflax spreads in patches via vegetative regeneration from buds on root crowns and lateral roots (Jacobs & Sing 2006). This can result in patches of high-density toadflax stems next to lower density areas due to the lateral spreading of daughter stems of the same individual. A taproot of a toadflax plant can extend 1.2-3 m deep underground, while the roots of most prairie grasses only occupy the top 1.2-1.5 m of soil (Sing et. al. 2016, Weaver 1958). Due to this, the plant is especially competitive with winter annuals and shallow-rooted perennials, such as grasses, and is thought to outcompete other species because of its deep root structure that assists water uptake during drought (Robocker 1974, Coupland et. al. 1962).

Since most grasses present on the grasslands of Montana are shallow-rooted in comparison, there is a concern that Dalmatian toadflax is competitively displacing grass and forb species. In addition, it has been noted to hybridize with yellow toadflax (*Linaria vulgaris*), another invasive species present in Montana and on the range (Ward et. al. 2009). Many methods have been widely employed in attempt to eradicate Dalmatian toadflax, including herbicides, biological controls, burning, tilling, and grazing (Sing et. al. 2016). The National Bison Range has been employing the toadflax Weevil (*Mecinus janthinus*) since 1992 as a moderately successful biocontrol and has sprayed pesticides intermittently most commonly in fall in recent years (Zarate 2011, 2016). However, employed strategies are not completely effective due to the hardiness of the plant and high weevil mortality, thus it continues to spread in abundance on the National Bison Range (Sing et. al. 2008).

Though there is abundant literature on the biology of Dalmatian toadflax in general and recommendations on how to eradicate, specific information on the extent of how the plant affects native Montana flora composition is lacking. The denoted “invasiveness” of a species, often based upon rate of spread, may not accurately predict its impact (Ricciardi & Cohen 2007). Therefore, additional research on the immediate and local effects of toadflax are needed in addition to its colonization rates. It is likely that Dalmatian toadflax is competitively displacing native grassland forbs and grasses, causing a decline in species diversity. This study compares grass biomass and forb diversity in grassland areas of the National Bison Range with high densities of Dalmatian toadflax stems with adjacent areas of low density.

Since invasive species have the potential to displace native species and degrade ecosystems, I hypothesize that the presence of Dalmatian toadflax in high densities will have a negative effect on the growth of grasses and forb biodiversity. Therefore, I expect to see lower

average grass biomass and lower forb diversity in areas with high densities of Dalmatian toadflax stems in comparison with the areas with low density.

Methods

Study Area

This study was conducted from late June to early August on grassland ecosystems of the National Bison Range, which are characterized by bunchgrasses and native forbs. Selection of sites was done carefully to ensure slope incline, slope face, topography, and dominant vegetation were identical. Plots were within three meters of each other to ensure that abiotic factors do not differ. Each site also had geographical separation by rivers or hills to avoid pseudoreplication. Two locations were selected for sites, with four sites on North Boundary Road and three in Pauline (A).

At each site, there were paired 3x3 m plots, one with a high density of toadflax stems (treatment) and one with a low density of toadflax comparatively (control). Out of the total 14 plots at 7 sites, half of the plots had a low density of toadflax and half had a high density of toadflax. Low-density plots were characterized as having 20% or less of the number of toadflax stems in the high-density plot. Out of a total of 144 0.25 m x 0.25 m subplots in each plot, 10 subplots were randomly selected for a total of 140 subplots utilized for biodiversity counts and grass collection. Toadflax stems were counted in each subplot sampled, and four additional subplots in each plot were chosen randomly for toadflax counts only to improve accuracy.

Data Collection

In each subplot, all grasses from this year's growth were collected, dried, and weighed. All species collected were rooted to ensure that litter was not collected, but roots were trimmed

for uniformity. Grass samples were placed in paper bags and dried in a dry oven at 100°C for 24 hours before weighing.

To measure competition of Dalmatian toadflax with forbs and grasses, the same ten random subplots in each plot were used. Within each plot, number and types of forbs were counted and species and counts of grasses were recorded. If a subplot could not be sampled (e.g. excessive trampling, large holes), a new random subplot was generated.

Statistical Analysis

All statistical analyses were completed using R (v3.5.1 GUI 1.70 El Capitan build) and RStudio (v1.1.456). To determine if grass biomass differences are statistically significant, I performed a paired t-test on my grass biomass averages for the plots with high densities of toadflax against those with low densities. There were seven plot averages for those with high and low toadflax densities. A paired t-test was also used to compare Shannon diversity index values. Lastly, I performed a regression between the counts of grasses and forbs at each site, between areas of high and low-density. A square root transformation was used to normalize my grass and forb count data.

Results

Average grass biomass was significantly greater in plots with low densities of toadflax. (Fig. 1, 3.791 $\pm$ 0.744 with vs. 5.098 $\pm$ 0.946 without, $t_{0.05(2),6} = -2.6294$, $p = 0.039$).

Forb biodiversity, meanwhile, was not significantly different between treatments. (Fig. 2, 1.927 $\pm$.307 with vs. 1.675 $\pm$.264 without, $t_{0.05(2),6} = 1.268$, $p = 0.252$).

However, the linear relationship between forb and grass counts (Fig. 3) was found to be significant ($y = -0.4392x + 4.4004$, $r^2 = .344$, $p = 0.045$).

Discussion

This study found significant evidence that grass biomass is lower in the presence of higher densities of toadflax on the National Bison Range. This indicates that eradication efforts should continue and management strategies should take grass health into priority consideration. It was also found that there is a significant negative linear relationship between grass and forb counts, likely indicating that they are competing with each other for limited resources. It was hypothesized that high-density toadflax presence would decrease forb biodiversity. However, there was no significant difference found between plots with high and low densities of toadflax.

Grass Biomass

The difference between average grass subplot biomass in plots with high and low concentrations of Dalmatian toadflax was statistically significant. This is likely due to competition between the shallow-rooted grasses and the toadflax. Due to their deep taproots and extending lateral roots, toadflax is likely better at consolidating water and nutrients from the soil (Robocker 1974, Coupland et. al. 1962). Previous research has found that toadflax decreases biomass of some forbs and grasses; for example, one study observed a 62% average decrease in dry weight biomass of grass and other forbs in toadflax infested vs. toadflax-free plots (Robocker 1974). Due to this, my hypothesis seems to be the most likely explanation for the significant differences in biomass. Though grazing has been proven to be effective for reducing toadflax density, such as with cows and sheep, a bison's diet is composed of less than 10% forbs (Plumb & Todd 1993). Given that grasses are competing with toadflax, high grazing in areas with toadflax with bison could exacerbate the current problem. Therefore, reduction of bison grazing in areas with high density of toadflax may assist eradication efforts.

Though unlikely, there is one possible source of error from the discovery that yellow salsify (*Tragopogon dubius*) seedlings looked extremely similar to a bunchgrass that I was recording. Upon realization that it was not a bunch grass and that the plot only had this grass and not the true bunchgrass, I returned and collected via random subplots nine samples of the plant and averaged their weight. Given that this weight was adjusted and that this species was small and uncommon as a seedling, it is not likely that it impacted my final results.

Forb and Grass Counts

Following the finding of significant biomass differences for the grasses, I plotted the counts of grasses against the counts of forbs and performed a regression (Fig. 2). This was also statistically significant, which may indicate a competitive relationship between the forbs and grasses on my plots. The dominance of grasses indicates a reduction of forbs and vice versa. Strong competition for resources such as light, water, space, and nutrients between grasses and forbs is present on prairies (Weaver 1958). In the plots where grasses performed better, which were those without the Dalmatian toadflax, forbs did not perform as well in number. Similar to the conclusion drawn above, if a competitive relationship exists, allowing bison and other species to graze in areas of high concern may assist in the growth of toadflax and forbs over grasses.

Biodiversity of Forbs

Though there was not a significant difference between the biodiversity values for forb species in the plots with and without toadflax, the mean biodiversity index was higher for the plots with toadflax (Fig. 3). This could indicate that there is a present, but smaller difference in forb biodiversity that I was unable to find statistically significant with my sample size. Even so, this was not predicted by my hypothesis. The biodiversity of forbs may not be as affected as

hypothesized by the presence of toadflax. The toadflax might not act as a competitor to the other forbs, but may be primarily competing with the grasses instead. A third explanation may be the benefit of smaller forbs from pollinators that toadflax may attract with its showy flowers. During this work, I observed large numbers of pollinators (bees particularly) visiting the toadflax flowers. Smaller forbs may benefit from an influx of pollinators in areas with a lot of toadflax which could negate the effects of competition, leading to a situation where the toadflax would benefit forb biodiversity.

In addition, though overall biodiversity differences were not found to be significant, toadflax may inhibit the success of a few select species. This was found with Pennyroyal (*Mentha pulegium*), which is a threatened species. After researchers prescribed fires, toadflax was found to have a long-term competitive advantage that is maintained by the higher plant density and a seed bank established after a fire (Dodge et. al., 2008). Future research is needed to assess the effects of a high density of toadflax on forb diversity in general and on select species of concern.

Considerations

During this study, it was noted that bison and deer tended to migrate frequently near the plot areas. It is possible that some plots may have been grazed or stepped on during these migrations, which could affect my final forb and grass counts as well as grass biomass. To control for this, those plots that were clearly trampled were not chosen for the study and new subplots were selected if grasses and forbs were flattened. It was assumed that grazing was comparable between two plots at a given site since they were in close proximity to each other.

Another form of disturbance by animals is waste excretions by bison, deer, and elk. Observed on the range have been large piles of bison excrement especially, which would cause

an influx of nitrogen to a localized area encouraging grass and forb growth. Although no large piles of bison excrement were noted at my plots, there is the potential that in the months or years prior certain spots had the influx of nitrogen. However, this was assumed to be standard across both plots at a certain site as the excrement does decompose quickly and both plots are likely to have the same input given their proximity.

Conclusion and Future Recommendations

This study primarily indicates that the presence of high densities of Dalmatian toadflax impacts grass biomass negatively on the National Bison Range. This study suggests that eradication efforts have not been entirely successful at eliminating Dalmatian toadflax in the area. The significant negative relationship found between counts of grasses and forbs may indicate a competitive relationship and have implications for management of grazing on the range. Although the hypothesis that biodiversity of forbs is altered in the presence of high densities of toadflax is not supported here, future studies with a greater number of sites could definitively determine whether there is a significant difference.

To improve study design, average biomass of toadflax could also be collected in addition to counts of the species. An environmental risk assessment paper focusing on Dalmatian toadflax states that, to keep biomass reductions of cool-season grasses under 25%, they established a risk threshold of 1,163 kg/ha dry weight of Dalmatian toadflax (Sing & Peterson 2011). Future investigations could record biomass found on the National Bison Range and compare those values to this calculated threshold.

In conclusion, the results of this study provides evidence that grasses on the range are negatively affected by the presence of Dalmatian toadflax. Invasive species pose a threat to the remaining intact grasslands, including the National Bison Range. Without investigating and

addressing the issues that they pose to native flora, we will see these natural areas degrade over time. As grasslands provide us, and wildlife, with extensive ecosystem services, priority must be set on conserving these valuable lands.

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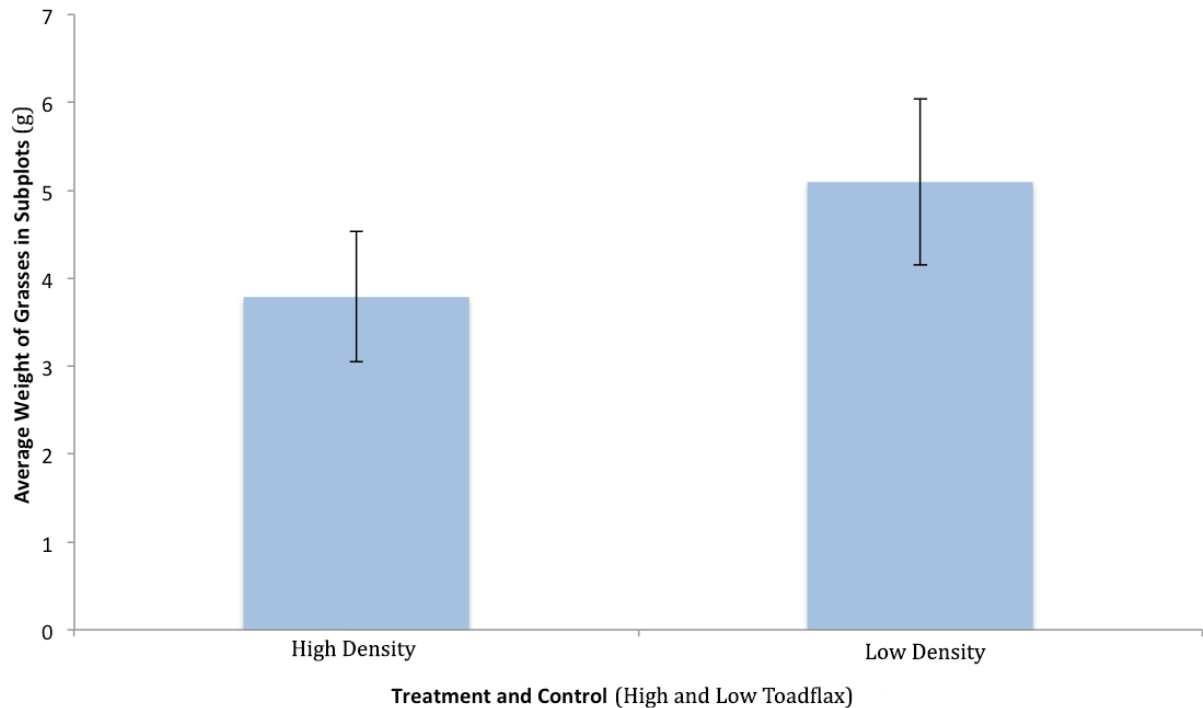


Figure 1. Average Grass Biomass with High and Low Densities of Toadflax. This displays the difference in means between the average grass biomass at plots with high and low densities of toadflax. It is statistically significant with a p -value of 0.039.

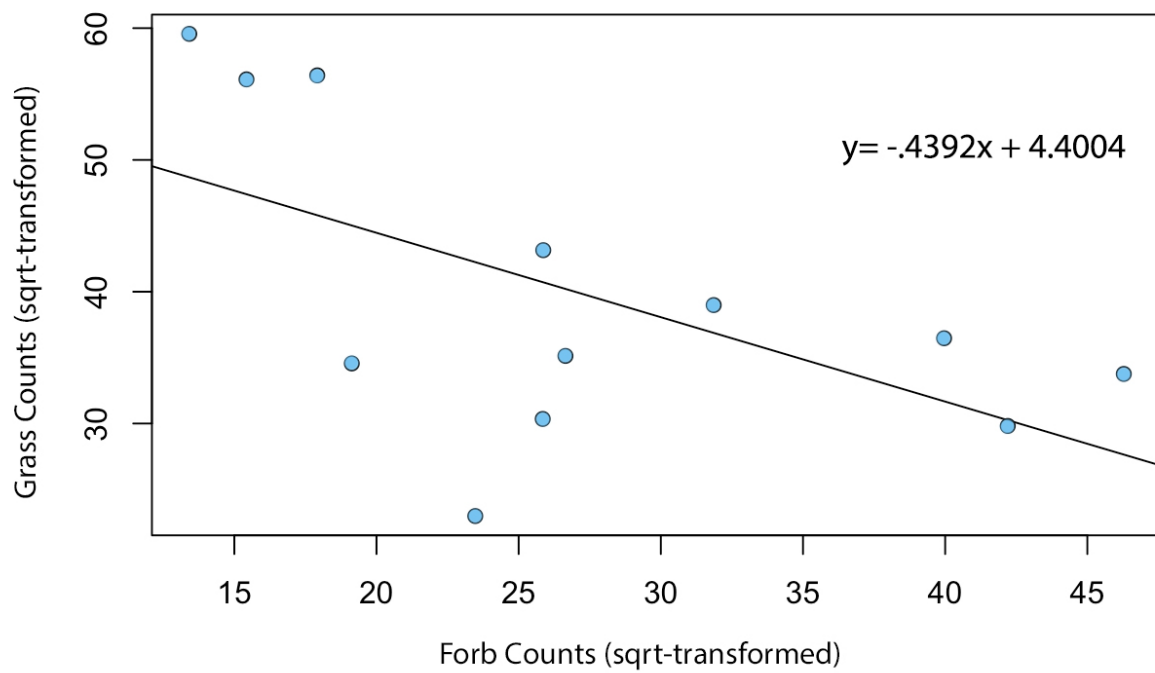


Figure 2. A Linear Relationship Between Forb and Grass Counts. This shows the predictive relationship between counts of grasses and forbs. The relationship was statistically significant with a p -value of 0.045.

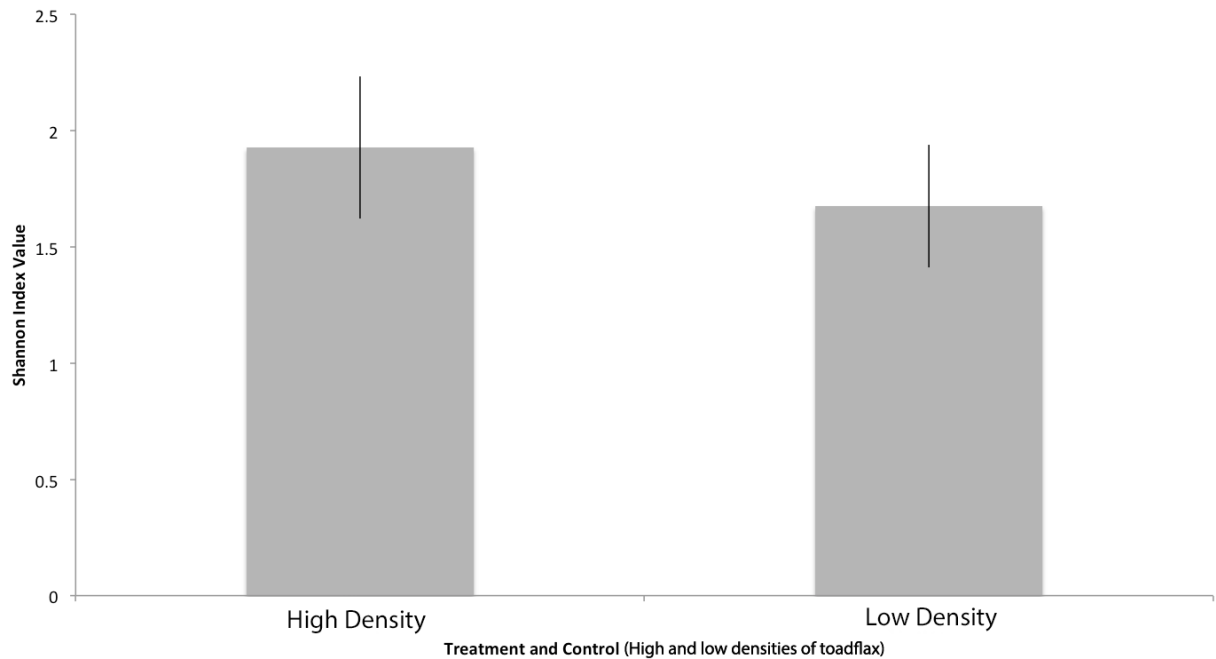


Figure 3. Shannon Diversity Index Values for Control and Treatment Plots. This bar chart displays the average biodiversity calculation for forbs for the control and treatment plot with error bars. Though the treatment (high density of toadflax) had a higher mean, the results of the paired t-test were not significant, with a p -value of 0.252.

Appendix



(A) **Site Map on the National Bison Range.** Selected plots were along service roads, with three plots in Pauline and four on North Boundary Road.