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Assessing Collateral Damage: Interaction among native thistles and the introduced biological control agent Rhinocyllus conicus in the Colorado Front Range

Zach Amir
University of Colorado, Boulder, zaam4146@colorado.edu

Assessing Collateral Damage: Interactions among native thistles and the introduced biological control agent, *Rhinocyllus conicus* in the Colorado Front Range

By:
Zachary Amir
Department of Ecology and Evolutionary Biology, University of Colorado at Boulder

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Thesis Advisor:
Dr. Time Seastedt
Department of Ecology and Evolutionary Biology, University of Colorado at Boulder

Defense Committee:
Dr. Tim Seastedt, Department of Ecology and Evolutionary Biology
Dr. Pieter Johnston, Department of Ecology and Evolutionary Biology
Dr. Gregory Carey, Department of Psychology and Neuroscience

Abstract

Invasive species are reducing native biodiversity in many distinct regions across the globe. While biological control agents have been effectively utilized to manage invasive plant species and preserve existing biodiversity, not all biological control agents are successful. The invasive thistle seed head weevil (*Rhinocyllus conicus*) was originally introduced to America as a biological control agent to manage musk thistle (*Carduus nutans*), but it has been found to attack several species of native thistle. This study examined the movements of the invasive thistle seed head weevil away from musk thistle into two different species of native thistle in the Colorado Front Range: wavyleaf thistle (*Cirsium undulatum*) and fringed thistle (*Cirsium centaureae*). Specifically, the proximity of invasive musk thistle to wavyleaf thistle was observed to see if it has any influence on thistle seed head weevil attack upon wavyleaf thistle. Additionally, fringed thistle was observed in three different habitat types in the Colorado Front Range (i.e. meadow, riparian, and tree line habitat) to see if the invasive thistle seed head weevil has been moving up in elevation in response to climate change. The results indicate that the proximity between musk thistle and wavyleaf thistle had no significant effect on thistle seed head weevil abundance on the native thistle. Moreover, the invasive thistle seed head weevil is still restricted to elevations below 3100 meters, meaning the tree line population of fringed thistle are protected from attack. Although both species of native thistle seem to have little risk of local extirpation due to the invasive seed head weevil, continued monitoring is advised to assess the indirect impacts of this invasive species through time and space.

Introduction

Biodiversity loss is occurring in all regions on Earth due to a wide array of different reasons (Pievani, 2014), and conservation is a priority. There are a number of different forces that have been causing reductions in biodiversity, such as land transformations, massive amounts of pollution, and the over-harvest of wild animals (Pievani, 2014). Another important factor that has a negative affect on biodiversity are invasive species (Gurevitch & Padilla, 2004). A species becomes invasive once it has left its original home range and begins colonizing a new habitat, and this process is common (Myers & Brazely, 2003; Davis, 2009). Unfortunately, invasions are becoming more common due to human-facilitated dispersal, along with the collective changes in climate, atmospheric chemistry, and fire frequency (Richardson, 2011).

Procedures to limit damage by invasive species are both diverse and controversial (Davis, 2009). Among these, biological controls agents that serve as introduced herbivores of invasive plants, have been released on a number of introduced plant species identified as problems to crops and pastures. Biological control agents (bio-controls) have been implemented all over the world, and for the most part they have been very successful in reducing the number of invasive species (Pemberton, 2000; Suckling & Sforza, 2014). Unfortunately there is not a 100% success rate when it comes to bio-controls, and a very few of theses non-native species introduced in order to control an invasive species eventually end up turning into their own invasive species, and negatively affect the native biodiversity in its new habitat (Simberloff, 2012). While these types of mistakes are rare and becoming more so due to better testing procedures, accidental releases of insects that function as bio-controls are likely to continue (Seastedt, 2015).

One of the most notorious bio-controls that has produced undesirable non-target effects is *Rhinocyllus conicus* (Suckling & Sforza, 2014). Also known as the Thistle Seed-Head Weevil, *R. conicu*s was introduced to America from France in 1969 in Virginia, California, and Montana in order to control the noxious weed *Carduus nutans,* or commonly known as the musk thistle or nodding thistle. After overwintering in the soil, adult *R. conicus* mate in the spring and early summer, and then deposit their eggs on the involucral bracts of young flower heads. Once the eggs hatch, the larvae burrow into the flower head and feed upon the thistle's developing seeds until the larvae form into a black ovoid cell and pupate into adults within the seed head (Gassman & Louda, 2001). The initial results with *R. conicus* were promising, since it was able to reduce *C. nutans* density by over 90% (Kok & Surles, 1975). *Carduus. nutans* is a fast growing and resilient plant that has been in America as early as the 1850's, and it has constantly been an annoyance to farmers because it is unpalatable to grazing livestock (Beck, 1999), so the demand for this seemingly effective bio-control was high. Musk thistle is currently listed as a B list species by the Colorado Noxious Weed act, which means this weed occurs in populations with varying distribution and density across the state of Colorado, and the level of control necessary for limiting the range of this noxious weed is based on its local conditions (CWMA, 2015). While bio-control methods are utilized for reducing the densities of *C. nutans,* other methods such as mechanical removal and herbicides have been used across the state of Colorado.

Soon after *R. conicus* was released into the wild, scientists observed it preying upon native thistles, though often at a magnitude smaller than its predation upon *C. nutans* (Rees, 1977). Unfortunately due to the erroneous mindset of the time, all thistles were viewed as impediments to grazing land, and land managers did not care to stop *R. conicus* from harming native thistles. Eventually, researchers began to realize the integral role thistles play in their environment, since they are important food source for a variety of organisms, and also play important role in pollination ecology (Kearns et al. 1998; Panjabi & Ackerfield, 2012). After *R. conicus* was released, it was found that there could have been six possible bio-controls released for *C. nutans* in America, some of which could have had potentially much more minor impacts (Schroder, 1980). Further research has also pointed out four ecological roles that *R. conicus* failed to meet when introduced as a bio-control that lead to it becoming a negative invasive species. These include an assessment of the ecological similarity of the target plant relative to native plants, synchrony of critical stages between bio-control and the target and native plants, and any potential overlap of feeding niches within the native group of species that depends on the plant that the bio-control infects (Louda & Arnett, 1999). Due to the various negative ecological effects of *R. conicus*, and its widespread distribution across the majority of the USA, this weevil is no longer available as a bio-control to purchase, nor do most state weed managers release it (Colorado Department of Agriculture, 2015).

R. conicus has been present in the Rocky Mountains of America since the 1970's (Hodgson & Rees, 1976) and has been continuing to expand into its new home range. It was reported in the Front Range of Colorado in the late 1990's (Louda et al., 1997) and the eastern plains of Colorado in the early 2000's (Louda, 2000). Previous research has found that the negative impact of *R. conicus* on native thistles tends to decrease as the thistles increase in altitude (Hicks et al., 2013), but as the earth's global temperatures continue to rise, *R. conicus*'s range has the potential to expand higher. It is crucial to intimately understand how *R. conicus* interacts with its environment and the other plants and animals in order recognize how it is affecting the ecological community. Only then can corrective action be taken to help mitigate the negative effects of this bio-control. Data on *R. conicus* has been collected in the Colorado Front Range since 2004, and this current research is expanding upon this large base of knowledge (Korth et al., unpublished). By looking at *R. conicus* through time, its patterns of movement and attack of native plants can be mapped and understood over a larger temporal and spatial scale.

Key study questions

By tracking *R. conicus* though space, its movements away from *C. nutans* can be analyzed, and its impact upon native thistles can then be assessed. In this study, the impacts of *R. conicus* were measured in two different locations, and on two different native species of *Cirsium* thistle. The first part of this study examined if the native wavyleaf thistle's (*Cirsium undulatum*) distance from *C. nutans* mediates the rate of seed predation by *R. conicus*. It is predicted that the closer *C. undulatum* grows to *C. nutans*, the more likely the plant is to be attacked by *R. conicus* due to their proximity. The second part of this study investigated if the type of mountainous habitat influences the attack rates of *R. conicus* upon the native fringed thistle (*Cirsium centaureae*). Measurements from previous studies were repeated in order to see if *R. conicus* was moving higher up in elevation in response to climate change, due to the strong evidence of warming at or near the Colorado tree line. (Kittel et al., 2015). The three types of habitats that were examined were montane meadow, montane riparian habitat, and tree-line habitat. It was predicted that the attack rates will be lowest in the coldest region, the tree-line habitat, and highest in the warmest region, the meadow habitat, since it most closely resembles the favored habitat of *C. nutans*. This trend has also been supported by previous research in the Colorado foothills (Hicks et al., 2013).

Methods

For the first part of the study, a section of private property in Left Hand Canyon was surveyed for *C. undulatum* plants. The vegetation of this site has been described in detail by a series of papers by Knochel and Seastedt (2010) and Prevey et al. (2014). This site is a transition zone where the mixed grass prairie of the plains integrates into Ponderosa pine savannah and closed canopy forest generated by fire reduction. Data was collected in Left Hand Canyon from 8/9/2016 to 9/9/2016 in order to find *C. undulatum* that contained developed seeds ready for dispersal before the plans underwent senescence. Each individual plant was located by hiking across this private property. When a *C. undulatum* was observed, its habitat was recorded, along with the approximate density of *C. nutans* around it, and a tape measure was used to measure the distance to the closest *C. nutans*. The approximate density of surrounding musk thistle was determined by counting the number of musk thistle surrounding the individual *C. undulatum* by a radius of approximately 30 meters. Ten or more patches of *C. nutans* indicated high surrounding musk thistle density, while less than ten indicated low surrounding musk thistle density. All the seed heads from each recorded *C. undulatum* were collected and taken back to the lab to be refrigerated for later dissection, in order to count seeds and any insects within the seed heads.

For the second part of this study, of comparing habitat type and *R. conicus* prevalence, several transects were conducted looking for *C. centaureae* at the University of Colorado Mountain Research Station. Data was collected from all sites between 9/16/2016 and 9/30/2016 in order to locate any *C. centaureae* that contained developed seeds ready for dispersal before the plants underwent senescence. The location of the meadow site is known as Elk Meadow and is located at an elevation of 2950 m. This habitat was characterized by full sunlight, well-drained soils, and a plant community composition typical of Colorado subalpine meadow. The meadow was dominated by native grasses and forbs, but also had abundant patches of the European grass timothy (*Phleum pretense*), which is now very common in the site. The riparian site was along Como Creek at the same elevation as the meadow site, whose plant community was composed of mostly willow, aspen, subalpine fir, and lodge-pole pine, which come together to create a closed canopy forest that allows only small bits of sunlight to hit the riparian forest floor. The tree-line site is known as the East Knoll, which is situated around 3150 m in elevation near the apex of Niwot Ridge on the eastern edge of the alpine tundra. A shorter growing season and cooler mean monthly temperatures, as compared to the lower study sites, characterize this environment. As many as six 50-100 meter transects were conducted in all three habitat types, and the density of all plants was recorded, as well as the number of rosettes and bolting stems in each square meter where there was a plant. Any *C. centaureae* that were missing all of their seed heads, usually assumed to be due to predation, were not recorded. All the seed heads from each plant that were recorded were collected and taken back to the lab to be refrigerated for later dissection.

Once back in the lab with all of the seed head samples, each seed head was dissected to recorded the number of viable seeds, any evidence of *R. conicus*, and any other relevant information that could be extracted, which included quantifying other insects inside the seed heads. When an unfamiliar insect was recorded while dissecting the seed heads, a few samples were saved and cross referenced with the CU Entomology Collection to accurately determine the species. In order to measure viable seeds as objectively as possible, each seed was mentally rated on its thickness, color, and straightness as measures of viability. A perfectly viable seed would be filled in and thick, a light tan color, and a straight oval kind-of shape.

After collecting the data from the seed head dissections, patterns and relationships were assessed with a series of statistical procedures using Statistical Analysis Systems (SAS 2002). In order to assess correlations among variables in Left Hand Canyon, the CORR procedure was used to produce Pearson Correlation coefficients. Relationships between variables with continuous distributions were assessed with regression techniques through the REG Procedure. Means and standard deviations of all variables associated with the CU Mountain Research

Station were obtained with the MEANS procedure, and statistically significant ($P<0.05$) differences among mean values were assessed using the GLM procedure, which uses an ANOVA procedure to handle differential sample sizes. When statistical differences were identified between more than two treatments, a follow up test (Student Newman Keuls, SNK procedure) was used to identify which treatments were different from one another. In most cases, the variances of treatments differed, so a log transformation was used to homogenize variances. In cases were treatments produced zero values (i.e. for seeds or for insects), these procedures were not used. Nonparametric tests were then used as needed.

Results

Left Hand Canyon

Seed heads from 30 *C. undulatum* were collected from Left Hand Canyon. After dissecting all of the collected *C. undulatum* seed heads (n=157), the measures of central tendency for the variables measured were evaluated, and any significant associations between the variables were assessed. The average seed count per plant was 61.6 seeds, with a range from 0 to 281 seeds. While dissecting the seed heads, dipteran larvae were found, and these were identified as the picture-winged fly *Paracantha culta* based upon previous collection of adults referenced to the CU Entomology Collection. There were on average many more *P. culta* flies inside the thistle seed heads than *R. conicus*, with an average of 10.3 *P. culta* per plant, compared to an average of .2 *R. conicus* per plant (see Figure 1). There were no significant associations between either insects' presence and the plant's seed count or its distance to the closest *C. nutans*. Despite the much higher abundance of observed *P. culta*, there were no significant correlations between fly numbers and seeds produced per seed head (see Figure 2).

The average distance between *C. undulatum* and *C. nutans* was 18.1 meters, but had a range of 1.2 to 51 meters. Despite having no significant differences (P< 0.05) between high and low density surrounding *C. nutans*, there were slightly more *P. culta* and *R. conicus* in areas with higher density of *C. nutans* around the *C. undulatum* (see Figure 3). The mean number of *P. culta* in *C. undulatum* surrounded by low density *C. nutans* was 9.8 flies per plant, and the mean number of *R. conicus* surrounded by low density *C. nutans* was 0.13 weevils per thistle. This is compared to the average number of *R. conicus* per *C. undulatum* in the high density *C. nutans* plants, which was doubled at 0.26 weevils per plant, while the average number of *P. culta* in high density *C. nutans* plants was only slightly higher than low density at 10.7 flies per plant. The average seed count in both densities only differed by two seeds. There were substantially more plants infected with *P. culta* than *R. conicus*. Only four plants contained *R. conicus*, while 28 plants contained *P. culta*.

There was no significant association (P> 0.05) between the distance of the closest *C. nutans* and the attack rates of *R. conicus* upon *C. undulatum*. By looking at the seed production of *C. undulatum*, the impact of *R. conicus* can be assessed. By correlating each *C. undulatum's* proximity to the closest *C. nutans* with the number of seeds produced by each *C. undulatum*, it is clear that there is no association between the two variables (see Figure 4).

<u>CU Mountain Research Station</u>

Thirty *C. centaureae* plants from the meadow and riparian habitats were collected, along with 26 thistles from the tree line habitat. The habitat type with the highest density of *C. centaureae* was the meadow with 0.11 thistles per square meter, and the riparian habitat followed in closely behind at 0.10 thistles per square meter. The habitat type with the lowest density of *C. centaureae* was the tree-line habitat with a density of 0.05 thistles per square meter. The GLM procedure was run to see if there were any significant associations between density and habitat type, but there were no significant results. High local variance in plant densities within each habitat type obscured any potential differences in plant densities among habitats.

Each habitat type differed in the amount of seeds produced by the thistles in their respective habitats. The habitat type that had the highest average seed count was the tree-line habitat with an average of 29.6 seeds per thistle, and the tree-line habitat also had the highest average number of seed heads per plant, at 8.8 seed heads per thistle. The meadow habitat had the second highest average seed count at 7.1 seeds per thistle, and also had the second highest mean seed head count at 6.2 seed heads per thistle. The riparian habitat had the lowest average seed count at 2 seeds per thistle, and also had the lowest average number of seed heads at 3 seed heads per thistle (Figure 5). After combining all the seed and seed head data from each location, the GLM procedure found a significant difference ($P<.0001$, $F=11.63$) between average seed-count and habitat type, and there was also a significant difference ($P<.0001$, $F=19.86$) between average seed-heads present on a plant and habitat type.

Rhinocyllus conicus was only found in the meadow habitat, with an average of 4.1 weevils per thistle; the weevils were not found in either the riparian or tree-line habitats (Figure 6). Unlike *R. conicus*, *P. culta* was found in two habitat types. The meadow had the highest average *P. culta* count at 1.3 flies per plant, while the tree-line habitat followed behind at an average of .5 flies per plant. There were no flies collected in the riparian habitat. There was a significant association (P=.0074, r=.29) between *P. culta* presence and the number of seed-heads per thistles, and there was also a significant difference (P=.0224) between *P. culta* presence and habitat type. After combining all of the data from *P. culta* and *R. conicus* presence, it was found that insect presence was significantly and positively correlated (P=.0039, r=.31) with the number of seed-heads per plant.

Discussion

Left Hand Canyon

The first study question sought out to see if the proximity of *C. undulatum* and *C. nutans* influenced the attack rates of *R. conicus* upon the native *C. undulatum*. The results indicate that the distance between these two different types of thistles does not influence the attack rate of *R. conicus* upon *C. undulatum*, and that this attack rate is, in general, very low. This finding is 'good news' for this species of native thistle. Although it was found that *R. conicus* and *P. culta* were slightly more abundant in areas of denser *C. nutans,* there were no significant effects on *C. undulatum* seed production. The long-term survival of *C. undulatum* in this transitional zone between the mountains and prairies is not likely to be heavily influenced by the invasive *R. conicus*.

Based on previous research, the results obtained by examining the relationship between *R. conicus* and *C. undulatum* are not surprising. While *R. conicus* has been observed feeding upon the seeds of *C. undulatum* in the prairies of Nebraska, it was found that attack rates were significantly higher for Platte Thistle (*Cirsium canescens*) when compared to *C. undulatum* over a timespan of two years (Louda, 1998). Louda hypothesized that the difference between *R. conicus* oviposition activity period and the synchronicity of flowering helped explain the lowered attack rates upon *C. undulatum*. Although the relationship between *R. conicus* and *C. undulatum* has not been studied extensively, research from Gassman & Louda (2001) & Louda (2000; 1998) are consistent with these findings. Due to the lack of significant associations between *R. conicus* and seed production in *C. undulatum* found in this study, it could be inferred that *R. conicus* does not have a detrimental effect on *C. undulatum* at this moment in time, and that *C. undulatum* is at a relatively low risk of local extirpation by *R. conicus*.

Another factor that could potentially increase the resilience of *C. undulatum* from *R. conicus* is its relationship with the native picture winged flies. Out of the 30 thistles collected in Left Hand Canyon, 28 of them were infected with native *P. culta*. Due to the surprisingly high numbers of *P. culta* found while surveying the seed-heads of *C. undulatum*, it can be inferred that they potentially compete with *R. conicus* for food and space. Also, it has been previously observed that *P. culta* feeds upon *C. undulatum* seeds before *R. conicus* are able to access the seeds (Louda, 2000). Fortunately for *C. undulatum,* there are no significant associations between the presence of the native *P. culta* and seed production from *C. undulatum* (see Figure 2). This potential interference, and lack of harmful effects upon *C. undulatum* seed production, has the potential to increase the thistle's resilience against *R. conicus* without seriously reducing the number of seeds produced. Coupled with the other factors specific to *C. undulatum* and the strong prevalence of native picture-winged flies competing with *R. conicus*, *C. undulatum* is at a very low risk of extinction in the Front Range due to *R. conicus*.

<u>CU Mountain Research Station</u>

The initial question that sought to be answered by examining *R. conicus* in varied habitats at the CU Mountain Research Station was to examine if they have been moving up in elevation in response to climate change warming the alpine habitat (Kittel et al., 2015). Based of the data collected in this study, it can be definitively stated that *R. conicus* is still restricted to elevations below 3150 m, as previously reported at the CU Mountain Research Station and in Rocky Mountain National Park (Korth et al., unpublished; Hicks, 2011). Of all the 26 *C. centaureae* collected at the tree-line habitat, none contained any evidence of *R. conicus*. While there is evidence that *R. conicus* can successfully reproduce in *C. centaureae* in the tree-line habitat (Hicks, 2011), the cold temperatures in the soil during the winter apparently kill any overwintering adults. Further, dispersal of adults into plants during the spring season from lower elevation populations has not occurred.

Due at least in part to the lack of *R. conicus* at the tree-line habitat, the thistles in this location had both the highest average viable seed count and highest number of average seed heads per thistle, which is consistent with previous research conducted on this population of thistles (Korth et al., unpublished). The significant differences between both average seed count and average seed heads per thistle and habitat type indicate that open habitats (i.e. meadow and tree-line) outperform the closed habitat of the riparian zone in terms of reproductive output by thistles. Also, less than half the average number *P. culta*, were found in the tree-line thistles relative to the subalpine meadow thistles. Due to the harsh cold winters that place a selective pressure on what can or cannot overwinter in a tree-line habitat, *C. centaureae* is presumably safe from attack from *R. conicus* as long as warming in this habitat does not increase to the threshold where the insect can successfully colonize the plant.

The habitat that fostered the greatest attack rate of *R. conicus* upon *C. centaureae* was the meadow habitat, with an average of just over 4 weevils per thistle. These results are unsurprising based on previous studies conducted at the CU Mountain Research Station (Korth et al., unpublished; Hicks, 2011). By using the number of viable seeds produced per seed head of all thistles, a long-term index of *R. conicus* attack can be assessed (Table 1). In 2004, the number of viable seeds produced per seed head in the meadow habitat was around 2.6, but has slowly been decreasing to the currently observed 1.1 seeds per seed head. This habitat has classically fostered the greatest *R. conicus* numbers, which explains the low viable seeds per seed head, and the small range of variation across more than 10 years. In contrast, ever since Korth et al. initially collected data in 2008, the tree-line habitat has almost always had the highest number of viable seeds per seed head. The perpetual lack of *R. conicus* in the tree line habitat, and drastically reduced numbers of other seed predators (i.e. *P. culta*) allow this population of thistles to experience very low seed herbivory, and therefore consistently produce the highest number of viable seeds per seed head. This long-term data supports the contention that the meadow habitat fosters the greatest attack rates by *R. conicus*, while the absence of this weevil at tree line allows that population of *C. centaureae* to produce the highest number of viable seeds as a function of seed predation.

One aspect of the data collected in this experiment that was unexpected was the results from the riparian habitat along Como Creek. Previous examination (Korth et al., unpublished) of this population of *C. centaureae* along Como Creek found these thistles in either equal or greater density relative to the meadow population from 2004 to 2009. This same study also showed initially much greater seeds per seed head in the *C. centaureae* population in 2004 compared to the meadow population, but then their seeds per seed head were equal to the meadow population in 2009 (Korth et al., unpublished). The trend from Korth et al.'s results indicate the *C. centaureae* population located in the riparian habitat at the CU Mountain Research Station has been decreasing in the number of viable seeds produced per seed head since 2004, and the data from this current study further supports that trend. While it seems intuitive that this could be explained by the presence of the invasive seed predator *R. conicus*, no weevils or indicators of weevil presence were found in the 30 sampled thistles along Como Creek. A different variable that could be influencing the health of this thistle population is the microclimate in the riparian habitat. Climate data has been collected at the CU

Mountain Research Station since 1952 in both the alpine and subalpine habitats, and the general trend since then has been an increase in temperature, and a decrease in the amount of precipitation (Kittel et al., 2015). However, in the past 4 years since 2010, there has been a decrease in the maximum yearly temperatures, and an increase in precipitation. While *C. centaureae* appear to prefer open habitats, they can still manage to live in the riparian habitat with limited sunlight and wetter, cooler soils. Although, with the addition of increased levels of precipitation over several years, the already dense canopy of the riparian habitat may have thickened and restricted the sunlight that reaches the forest floor. Coupled with the addition of several years of cooler weather, these climate variables could have lead to the observed reduction in density and seeds produced per seed head in the *C. centaureae* riparian population. Due to the inadequate growing conditions, the riparian *C. centaureae* appear not to have produced enough seeds, thereby precluding any herbivory. Further experimentation and monitoring is needed to resolve this question.

Conclusion

Based on data documenting *R. conicus* damage to non-target species of *Cirsium* thistles, *C. undulatum* is at a very low risk of local extirpation due to *R. conicus*, and that *C. centaureae* growing above 3100 m in elevation are currently safe from attack. This study has shown that under conditions that best favor high *C. nutans* densities, the prairies and foothill environments, densities of their introduced bio-controls do not appear to be harming the common native thistle found there. In contrast, *C. nutans* is extremely rare in higher elevation meadows such as those found at the CU Mountain Research Station, but the bio-control is present and has established itself on the native thistles. Temperature limitations appear to still be protecting the highest elevation populations of *C. centaureae*. For the first time since 2004, the weevil was also found to not exist in adjacent riparian habitats. However, changes in the microclimate, such as sunlight and soil conditions, also appear to limit the native thistle in riparian habitats. Continued studies using *C. centaureae* as a 'model system' for biota affected by both direct climate effects (i.e. controls on microhabitats) and indirect effects (i.e. controls on herbivore success) are appropriate to understand how plant species are being influenced by the collective interactions generated by climate and species introductions. As more evidence points towards the warming at the Colorado tree-line habitat (Kittel et al., 2015), monitoring of *R. conicus* above 3100m in high alpine thistles should be continued. It has been observed that *R. conicus* not only will feed upon the seeds of *C. centaureae*, but also *C. scopulorum* (Louda, 2000), so both species of alpine native thistle should continue being monitored in order to track *R. conicus* movements through time and space.

Musk thistle is currently listed as a B species by the Colorado Noxious Weed Act (CWMA, 2015), which demands control but not eradication of the species. The data gathered in this current study supports *C. nutans's* position on the Colorado Noxious Weed Act. Arguably, if this species directly or indirectly (i.e. through its bio-control agents) threatened the existence of a native species, it would deserve an A listing, which then requires eradication. Since the bio-control released to manage this crop has not significantly impacted native thistles to date, it should remain a B list species. The continued management of *C. nutans* in the Colorado Front Range is still an important task for Colorado land managers, but the elevation of *C. nutans* to an A list noxious weed based on its invasive bio-control's impact on native thistles is not warranted at this time.

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Figures & Tables:

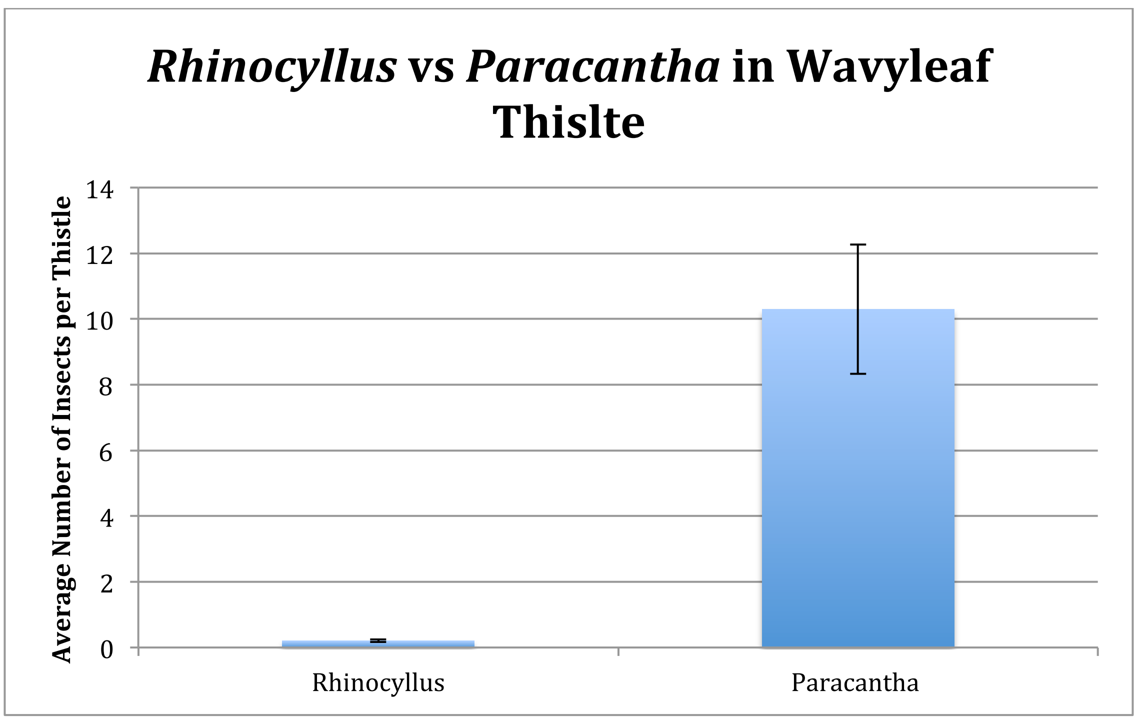

Figure 1: The average number of *Rhinocyllus* and the average number of *Paracantha* found in all 30 thistles collected in Left Hand Canyon. The standard error for each insect is graphed as well.

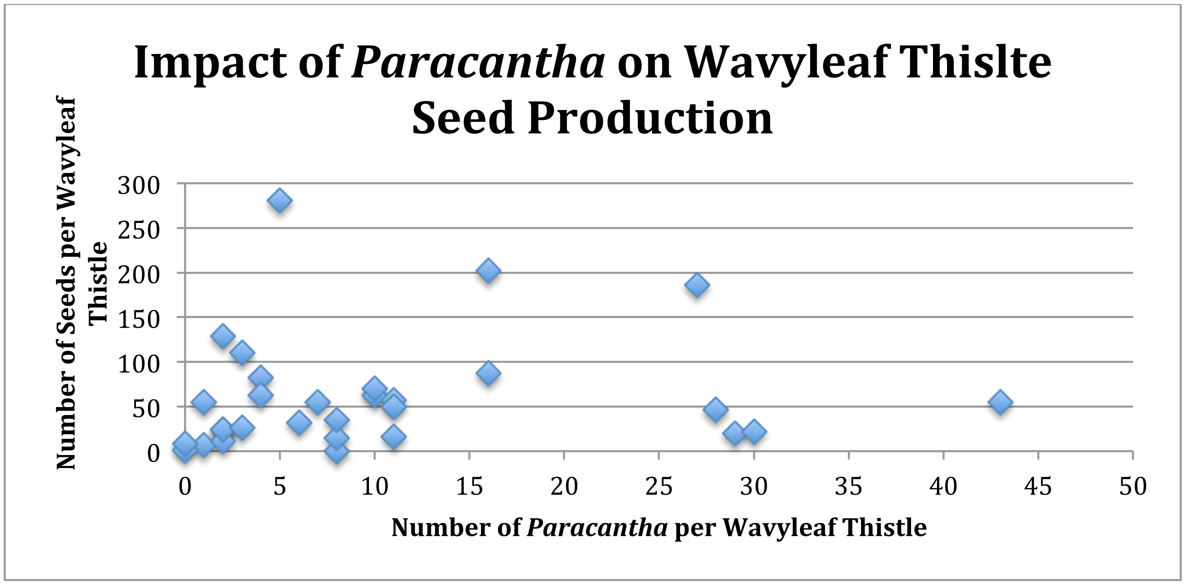

Figure 2: The number of seeds per *C. undulatum,* a measure of attack by seed-predators, in relation to the number of native *P. culta* found in each thistle. There was no significant correlation between the two variables.

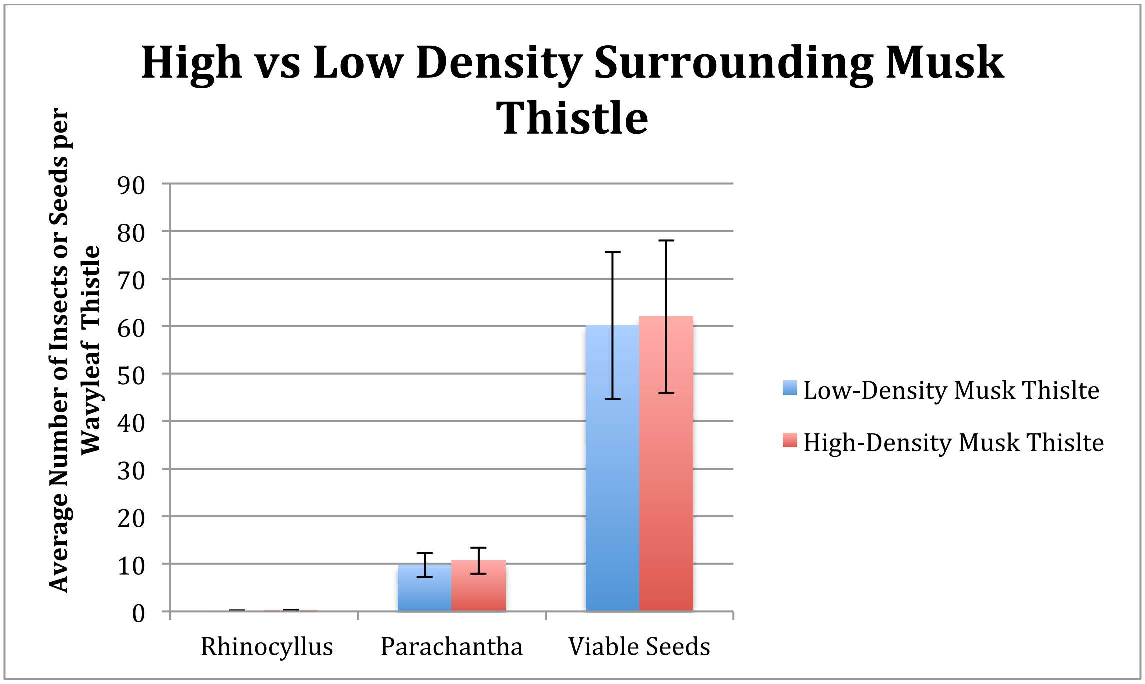

Figure 3: The average number of *Rhinocyllus, Paracantha,* and number of seeds produced in all thistles are broken down by areas of high and low density surrounding musk thistle. There are no significant differences between each condition of surrounding musk thistle.

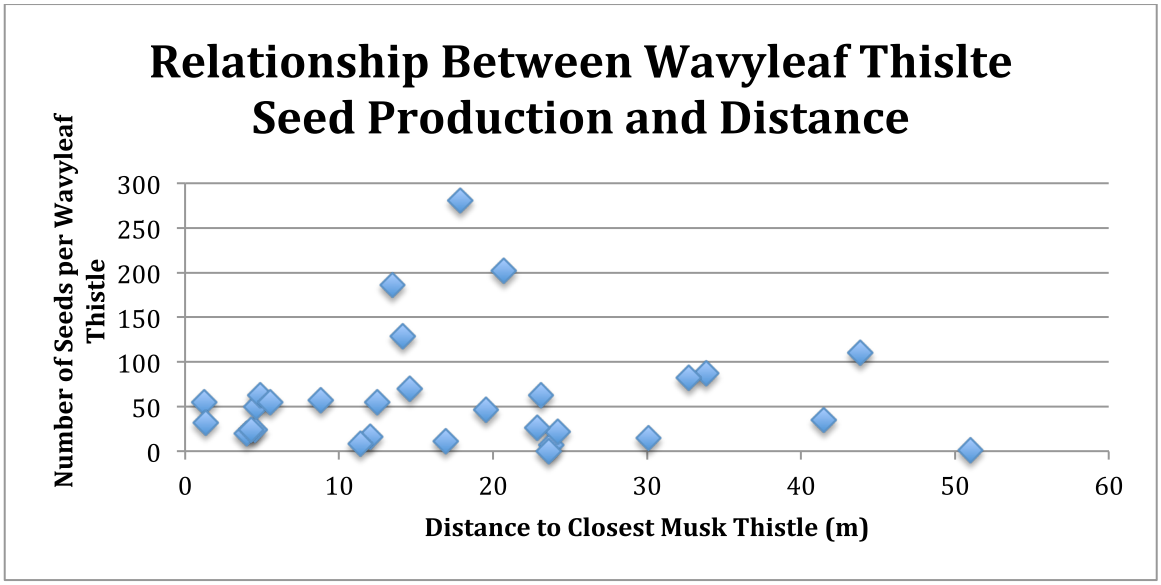

Figure 4: Plot of the number of seeds produced by *C. undulatum* and its distance to the closest *C. nutans* is illustrated above. There is no significant correlation between the two variables that were measured.

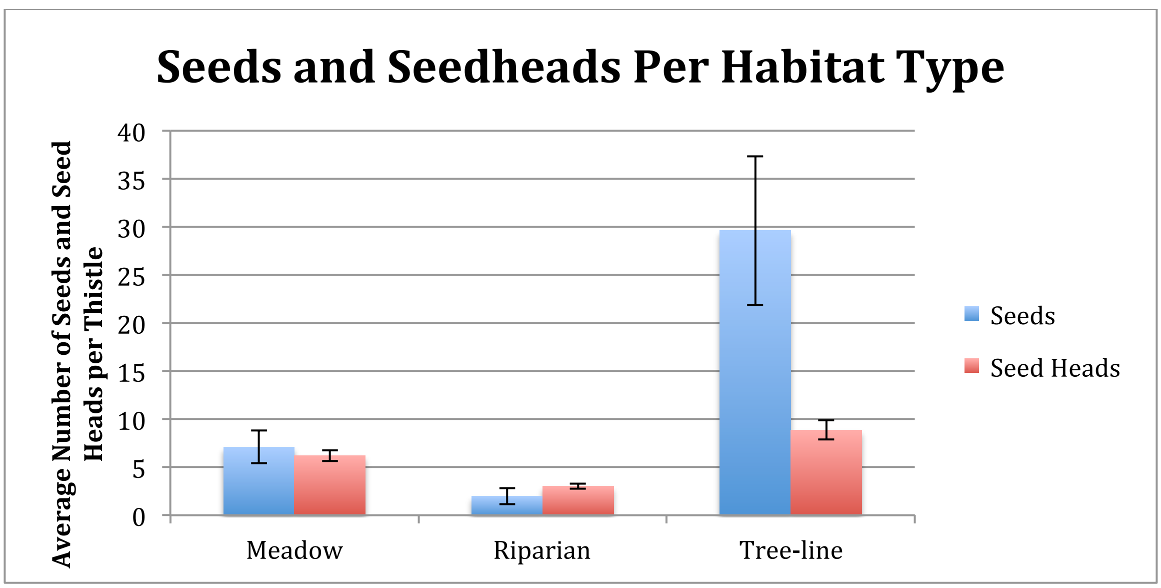

Figure 5: The average number of seeds and seed-heads from *C. centaureae* in each habitat type is illustrated in the graph above. Standard error bars were calculated for each condition.

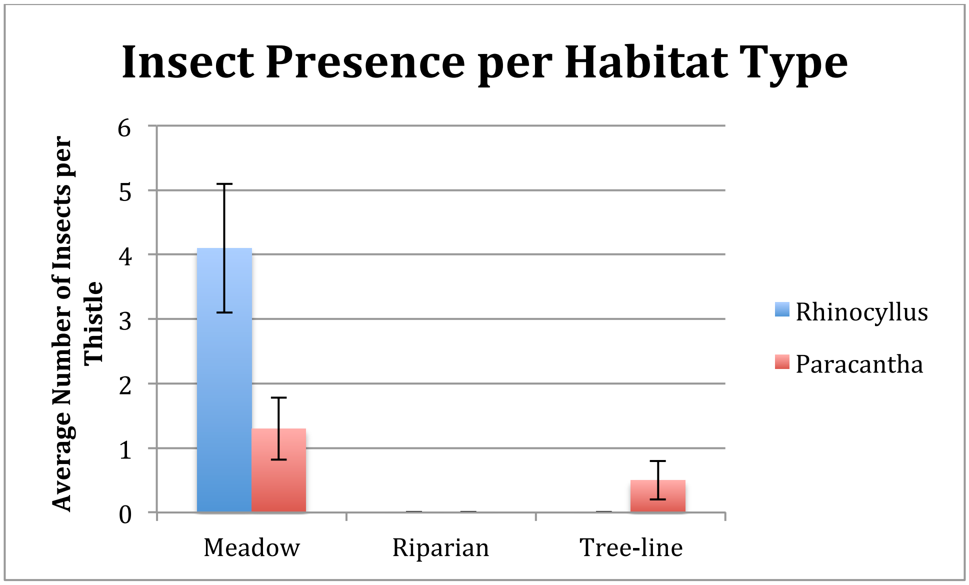

Figure 6: Number of *R. conicus* and *P. culta* found in each habitat type; error bars are standard errors. No insects were found in the Riparian zone, and no *R. conicus* in were found the tree-line habitat.

Habitat	2015	2011	2009	2008	2004
Meadow	1.13	1.221	1.8	2.2	2.6
Riparian	.66	N/A	1.8	3.6	8.1
Tree-line	3.35	.368	13.1	8.4	N/A

Table 1: The ratio of viable seeds per seed head for *C. centaureae* is depicted in the table above for each of the three habitats observed from 2004 to 2015. No tree-line data was collected in 2004, and no riparian data was collected in 2011, which is denoted by "N/A".