The Effects of Caging to Prevent Herbivory on the Pollination Ecology of the Endangered Plant *Astragalus bibullatus*

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For the love of my life, who has always shared and supported my dreams and passions,

and all those who care for our beautiful planet.

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ABSTRACT

Astragalus bibullatus is an endangered perennial herb endemic to the limestone cedar glades of Rutherford County, Tennessee. The species has been introduced into new sites; however, herbivory by mammals has led to loss of biomass of *A. bibullatus* after reintroduction. To prevent herbivory, 1.2-m-tall open-topped metal cages with mesh openings of 0.5 cm^2 (hereafter, referred to as small mesh cages) were placed around the immature plants. Initial observations from 2021 showed that pollinators would not enter the cages, either through the mesh or from the top. The goal of my study was to examine whether increasing the mesh size would increase pollination success by allowing access to insects and preventing herbivory by exclusion of mammals. A field experiment was conducted from 2022-2023 that utilized two different sizes of mesh for caging plants to compare to no caging as a control. The size of the mesh in large cages was selected based on the average wingspan of insects visiting uncaged plants. Pollinator visitation and fruit and seed production were recorded, and trail cameras captured images of herbivores visiting plants. We observed eight species of insects visiting flowers in 2022 and the large mesh size allowed access to plants for all pollinators. Only one fruit and a single seed was produced from plants in small mesh cages during both seasons; however, plants in large mesh cages and uncaged plants produced similar numbers of fruits and seeds. Signs of herbivory observed on the uncaged plants were likely from common herbivores such as white-tailed deer (*Odocoileus virginianus*) and eastern cottontail rabbits (*Sylvilagus floridanus*). Based on the effects of caging on pollination and herbivory, I recommend that large mesh cages be used in the reintroduction of *A. bibullatus* to allow effective pollination and prevent herbivory.

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Chapter I: Introduction

Astragalus bibullatus is an endangered perennial herb that is endemic to the cedar glades of middle Tennessee which are made up of mostly flat, thin-bedded, flaggy, fossiliferous limestone (Barneby and Bridges, 1987). This distribution of *A. bibullatus* is limited to an area encompassing approximately 235 km^2 due primarily to loss, alteration, or degradation of habitat through development, grazing, and encroachment of vegetation (Call et al., 2011). Because of the reduction in the population size of *A. bibullatus* due to these threats, the species was listed as endangered on September 26, 1991 (USFWS, 1991). The U.S. Fish and Wildlife Service developed a recovery plan in 2011 after recognizing that the entire range of the species was highly fragmented within the Stones River watershed (Call et al., 2011). For the past 20 years, the conservation research by biologists at the Missouri Botanical Garden (MBG) has established protocols to monitor the population dynamics of the species in conjunction with conservation management protocols such as tree removal and reintroductions (Albrecht, 2021). Some of these reintroductions have failed for reasons that are unknown, but the most probable causes include poor habitat suitability, herbivory, small founder size and possibly a soil microbiome that does not match native sites (Albrecht and McCue, 2009; Becknell et al., 2021).

One solution to increase the success of MBG reintroductions was to use cages to protect *A. bibullatus* from herbivores such as white-tailed deer. The primary purpose of the cages is to protect plants from herbivory and increase chances of long-term survival (Albrecht and Long, 2018). However, the cages are limiting pollinator access which reduces reproduction. MBG uses a 0.5 cm^2 mesh cage (Figure 1) and a 1.5 cm^2 mesh cage (Figure 2) that are too small for access by most pollinators. During the 2021 field season, it was observed that hummingbird moths would not visit caged flowers even though they actively visited uncaged flowers (Herlihy, Knorp, and Walck, unpubl. data). Hummingbird moths are too large to fly through the mesh and only fly about 30 cm above the ground, well below the top of the 1.2 m tall cage. Additionally, small bees did not fly through the mesh even though they were small enough to do so (i.e. they were observed flying up the mesh and sitting on the mesh but not flying into the cage). Only once was a solitary bee observed to flyover the cage and enter from the top.

Figure 1. The Missouri Botanical Garden's 0.5 cm^2 cage (top view). This was used as the small mesh cage in this experiment.

Figure 2. The Missouri Botanical Garden's 1.5 cm^2 cage (top view). This cage was not used in this experiment.

Previous studies done using cages or exclosures to prevent herbivory have had widely varying results at the community or organismal level. Exclosures in a meadow in Canada led to increased diversity and species richness and higher primary productivity as compared to uncaged plots (Fraser and Madson, 2008). For *Ranunculus aestivalis,* grazing was required for survival (Skopec et al., 2017). In the rare *Astragalus sinuatus,* seed predation was higher than in other common sympatric congeners, suggesting seed predation could be a driver of rarity (Combs et al., 2013). Caging to reduce granivory in the endangered legume *Crotalaria avonensis* increased survival rates, relative growth rate, and flowering (Menges et. al., 2016). In previous studies on *A. bibullatus*, uncaged plots showed that larger plants exhibited lower survival probabilities than smaller plants since larger perennial forbs were preferred (Albrecht and Long, 2018). While herbivores have the direct effect of removing biomass from plants, they may also cause indirect effects by modifying resource availability or by differential grazing on neighboring plants (Van Der Wal et al., 2000). To this end, reintroductions with perennials often require a decade or more to accurately assess the effects of different management protocols, such as caging to prevent herbivory (Bell et al., 2003).

Although the cages have reduced herbivory and increased the survivorship of out-planted seedlings, observations suggest that the cages may be having unintended consequences on pollinator visitation (Albrecht and Long, 2018). Many factors, such as climate change, habitat fragmentation, agricultural infestation, urbanization, pollution, pesticides, and invasive species have the potential to affect plant-pollinator interactions directly and indirectly (Mitchell et al., 2009). However, for the case of *A. bibullatus* an immediate concern is to evaluate the effects of the cages on pollinators. To do this, my study was conducted in the springs of 2022 and 2023. This species flowers from April to May and produces fruit from May to June (Call et al., 2011).

The objectives of the 2022 field season were to monitor pollinator visitation to *A. bibullatus* flowers using cages of different mesh sizes in comparison to uncaged flowers and to estimate herbivory and identify herbivore interactions by image analysis. In 2023, data on insect visitation and pollination and herbivore interactions at a second site was compiled. The overall goals of these experiments were to determine whether there was a mesh size that allows pollinator access while also excluding herbivores. Results from this project are intended to be used to inform management decisions for *A. bibullatus,* specifically the mesh size that should be used for caging plants.

Research Questions

The first question focused on determining whether caging the *A. bibullatus* plants affected potential pollinator visitation behavior. I collected data for this question by observing pollinator interactions with the plots and comparing the differences in interactions with the three treatments. I hypothesized that there would be lower visitation as a result of limited pollination access to caged plants with smaller mesh size.

The second question focused on whether the mesh size of the cages influenced pollinator behavior. This question was answered by recording pollinator flower visitation regarding treatment type and quantified with flower, fruit, and seed set data. I hypothesized that the small mesh cages would have reduced fruit and seed sets when compared to the large mesh cages and uncaged plots.

The third question focused on the cage’s ability to prevent herbivory of *A. bibullatus*. I collected data for this by analyzing differences in herbivory rates of the small and large caged plants as compared to the uncaged plants. I hypothesized that the caged plants would have reduced herbivory relative to the uncaged plants, and that the large mesh cages would be an effective size in reducing herbivory.

Preliminary Data

In November 2021, we located populations of *A. bibullatus* to include in this research project. I determined that Hall Farm in Rutherford County, TN, a population near Flat Rock Cedar Glade and Barrens State Natural Area, would best suit my research needs. Cages of two sizes, 0.5 cm^2 mesh and 1.5 cm^2 mesh, have already been placed at this site by MBG, and I utilized the 0.5 cm^2 mesh size in the experiment. This site had a sizable population of plants to choose from and was easily accessible by herbivores making it an ideal choice for the experiment.

Previous observations of insect visitation to *A. bibullatus* (Knorp unpubl. data, Table 1) were used as a reference to get an initial idea of the expected visitors and to estimate their size for construction of cages with an appropriate mesh size (Table 1). I also used the data collected to estimate the average wingspan of the insect visitors by comparing the size of museum specimens of the sample species and calculating a mean size to ensure that pollinators could access the plants within the larger mesh size (University of Alberta E.H. Strickland Entomological Museum, 2021).

Table 1. A list of insect visitors to *Astragalus bibullatus* observed during spring 2021 (Knorph, unpubl. data).

Family	Scientific Name	Common Name
Andrenidae, Halictidae, Megchilidae	N/A	Various Solitary Bees
Apidae	*Apis melifera*	Honeybee
	Bombus auricomus	Black and Gold Bumblebee
	Bombus impatiens	Common Eastern Bumblebee
	Bombus pensylvanicus	American Bumblebee
	Bombus spp.	Various Bumblebees
	Xylocopa virginica	Eastern Carpenter Bee
Hesperiidae	*Erynnis icelus*	Dreamy Duskywing
Lycaenidae	*Celestrina ladon*	Spring Azure
	Callophyrys gryneus	Juniper Hairstreak
Nymphalidae	*Phycoides tharos*	Pearl Crescent
Papilionidae	*Battus philenor*	Pipevine Swallowtail
	Papilio polyxenes	Black Swallowtail
	Papilio glaucus	Eastern Tiger Swallowtail
	Papilio troilus	Spicebush Swallowtail
Pieridae	*Anthocharis midea*	Falcate Orangetip
	Colias philodice	Clouded Sulphur
	Phoebis sennae	Cloudless Sulphur
Sphingidae	*Hemaris diffinis*	Snowberry Clearwing
	Hemaris thysbe	Hummingbird Clearwing

Using the list of insect visitors, I compiled the average wingspan for a subset of species that represented a broad range of sizes (Table 2). Using this information, I decided to use a mesh cage that had openings of 5.08 cm x 7.62 cm, since it would accommodate most pollinators and it was readily available at a garden-supply center; hereafter, referred to as the large mesh cage.

Table 2. Observed insects near *Astragalus bibullatus*, and their wingspan, at the Hall Farm study site in 2022.

All Observed Insects	**Average Wingspan (cm)**
Andrenidae, Halictidae, Megchilidae (Various Solitary Bees)	0.6
Apis melifera (Honey Bee)	1.0
Xylocopa virginica (Eastern Carpenter Bee)	1.8
Celestrina ladon (Spring Azure)	2.9
Phycoides tharos (Pearl Crescent)	3.8
Hemaris diffinis (Snowberry Clearwing)	4.1
Papilio polyxenes (Black Swallowtail)	10.4
Papilio glaucus (Eastern Tiger Swallowtail)	11.0

In the 2022 field season, there was minimal herbivory of *A. bibullatus* at the Hall Farm site. In the 2023 field season, I included a second site known as Savanna, which is also in Rutherford County, TN, at Flat Rock State Natural Area. Although the Savanna population is smaller than Hall Farm, higher rates of herbivory on *A. bibullatus* have been observed in previous years at this site (Herlihy, Knorp, and Walck unpubl. data).

Chapter II: Experimental Methods

Materials and Preparation

The small mesh cages, already present at the Hall Farm and Savanna sites, were used as a guide to construct a similarly shaped and sized exclosure with larger mesh for this experiment (Tetreault and Aho, 2021). After cutting pieces of the mesh to 152 cm lengths, cylindrical exclosures with open tops (n=12) were constructed by securing the circular metal frame with zip ties. Once the cages were constructed, metal stakes were used to anchor each one into the ground.

Figure 3. The exclosure constructed was based on the MBG's original design using 5.08 cm x 7.62 cm (38.7 cm^2) mesh. This design is referred to as the large mesh cage.

Trail cameras (Natureview HD, Bushnell Inc, Overland Park, KS) were programmed prior to the placement in the field prepared in the laboratory with the. Each camera was programmed to capture images every 5-10 minutes unless motion was detected, and the camera was triggered. This trigger mechanism was set to "field scan" mode and the sensitivity was set to "high". Once the cameras were programmed, they were placed at each site in a position that ensured that the majority of plants were in the field of view. Cameras were secured by either strapping to buried rebar or nearby trees.

Methodology

At the Hall Farm site in 2022, small, circular plots were established where 1-3 plants of *A. bibullatus* were found. Thirty plots were selected, and each plot was assigned to one of three experimental treatments: i) one uncaged plot as a control; ii) one small mesh plot (0.5 cm^2) and iii) one large mesh plot (38.7 cm^2), resulting in 10 treatment groups. The cages were constructed and established in early March before the flowering season began. Each treatment plot (1-10) and each treatment type (s-small mesh, l-large mesh, and u-uncaged) was labeled on an impressionable aluminum tag (AL Tag, Forestry Suppliers Inc, Jackson MS). Labels for caged plants were secured to the cages with a zip tie, while the labels for the uncaged plots were secured into the ground with a nail. Initial photos of the plants were taken to identify signs of herbivory and to monitor weekly plant growth throughout the season.

To observe pollinator interactions, I spent 3-6 hours per week during the flowering season recording the species that visited the plants. I noted the behavior of insects that visited flowers including how they approach the flower, whether or not they visit the same plot of flowers, if there was pollen or nectar collection, grooming behaviors on the flowers, and any other significant interactions. I also noted their interactions with the cages including avoidance of the cages, whether they fly through the mesh, how they move between different plots of flowers, and any other interactions with the cages themselves. Each day, one treatment group (one large mesh cage, one small mesh cage, and one uncaged plot) with flowers present was selected to observe for approximately one hour at a time. I recorded data for that hour during peak pollinator activity between 10 am and 2 pm, then switched to another treatment group containing flowers. I would stay long enough to observe each flowering treatment group for one hour. Binoculars (8 × 32 Celestron Nature DX) and a camera (Nikon D80 with 70-300 mm zoom lens) aided in observing interactions and identifying insects.

Once the flowering season began, the inflorescences were counted on each plant every week and monitored for changes throughout the growing season. Fruits were continuously counted in June and each week the fruit would be counted, inspected for damage, and photographed. When the fruits were mature, I would open each one and count the seeds. The seeds were scattered near the plant that they originated from.

The time-stamped photographs from the trail cameras were used to identify herbivores and their interactions with the plants. Once a week, the photographs were downloaded from each of six trail cameras. Each photograph was examined and the herbivore and interactions with plants were identified, and the time and date were noted. Additionally, the plants were inspected for damage that could be linked to a potential herbivore.

The same experiment conducted at the Hall Farm site was expanded to the Savanna site in 2023. Three plots each possessing one of the three treatment types (uncaged, small mesh cage, and large mesh cage) were established at the Savanna site. Each plot had a trail camera. At Hall Farm, nine plots were established, and five trail cameras were set up. Pollinator observations followed the same protocols as in 2022, but I had to visit both field sites each week for herbivore monitoring purposes. Because the

Savanna site did not produce any flowers in 2023, pollinator observations were not performed for plants at this site. The same counting and monitoring protocols were also followed when the flowering and fruiting seasons began.

Data Analysis

All analyses were conducted in R version 4.2.2. The association between recorded insect interactions of *A. bibullatus* were analyzed with contingency table analysis. The three types of interactions (flying around the cage, flying through the cage, and flying over the plot) and the three treatment types were analyzed for association and a Pearson's chi-squared test was used to determine the significance of the effects.

A two-way ANOVA was used to analyze the association between pollinator size and visitation of small mesh caged, large mesh caged, and uncaged *A. bibullatus* plants. The assumptions of normality and homogeneity of the variances were tested using a Shapiro-Wilk normality test and a Bartlett test for homogeneity of variances. If the data did not meet the assumptions of the test for equal variance, an arcsine transformation was performed. Since transformed data did not meet the assumption after transformation, the two-way ANOVA was analyzed; however, the results should be interpreted with caution.

A one-way ANOVA was used to analyze the association between flowering and fruiting ratios and small mesh caged, large mesh caged, and uncaged *A. bibullatus* plants. The assumptions of normality and homogeneity of the variances were tested using a Shapiro-Wilk normality test and a Bartlett test for homogeneity of variances. Since the data did not meet normality or equal variance assumptions, a nonparametric Kruskal-

Wallis test was used to determine the significance of the data. Pairwise differences between means were tested with a Wilcox signed-rank test.

Chapter III: Results

Pollinator Observations from 2022

Eight taxa of insects were observed at the Hall Farm site of *A. bibullatus* on at least one occasion including the honeybee (*Apis melifera*), eastern carpenter bee (*Xylocopa virginica*), spring azure (*Celestrina ladon*), pearl crescent (*Phycoides tharos*), black swallowtail (*Papilio polyxenes*), eastern tiger swallowtail (*Papilio glaucus*), snowberry clearwing (*Hemaris diffinis*), and various solitary bees (of the families Andrenidae, Halictidae, and Megchilidae). Of these insect visitors, none visited plants in the small mesh cages, four taxa were observed on plants in the large mesh cages (eastern carpenter bee, pearl crescent, snowberry clearwing, and the various solitary bees) and two taxa were observed on uncaged plants (eastern carpenter bee and snowberry clearwing) (Table 3).

Table 3. Potential pollinators of *Astragalus bibullatus* at the Hall Farm study site in 2022. Visitation is shown for each treatment group (S-small mesh, L-large mesh, and U-uncaged). The carpenter bee is a nectar robber, whereas all other insects are potential pollinators.

	Times Observed	Flower Visitation (S)	Flower Visitation (L)	Flower Visitation (U)
Andrenidae, Halictidae, Megchilidae (Various Solitary Bees)	2	-	2	-
***Xylocopa virginica* (Eastern Carpenter Bee)**	7	-	3	5
***Phycoides tharos* (Pearl Crescent)**	2	-	1	-
***Hemaris diffinis* (Snowberry Clearwing)**	2	-	2	3

Insects entered the large mesh cages through the mesh on the sides of the cage, and never through the open top. Insects that visited plants in large mesh cages were not observed to interact with plants differently from those that visited uncaged plants. In contrast, insect interactions with small mesh cages were limited to behaviors associated with avoidance (e.g. flying around a small mesh cage in their direct path from one patch of flowers to another) (Table 4). Our analysis revealed a dependence in visitation success for plants in large mesh caged, small mesh caged, and uncaged plots ($\chi 2 = 52$, df = 4, p-value = 1.379e-10).

Table 4. Recorded insect behaviors with plots of *Astragalus bibullatus* at the Hall Farm study site in 2022. Interactions are shown for each treatment group.

	Small mesh cages	**Large mesh cages**	**Uncaged plots**
Flying around the cage	8	0	-
Flying through the cage	0	10	-
Passing over	-	-	8

The average wingspan for the recorded insect visitors was 2.6 cm. Thus, values less than 2.6 were considered small insect visitors (e.g. solitary bees) and those greater than 2.6 were considered large insect visitors (e.g. *Hemaris diffinis,* Snowberry Clearwing). Both large and small insects visited uncaged plants and plants in large mesh cages, but a greater percentage of insects in large class size ($P < 0.05$) did so for both uncaged and large mesh caged plants (Figure 4).

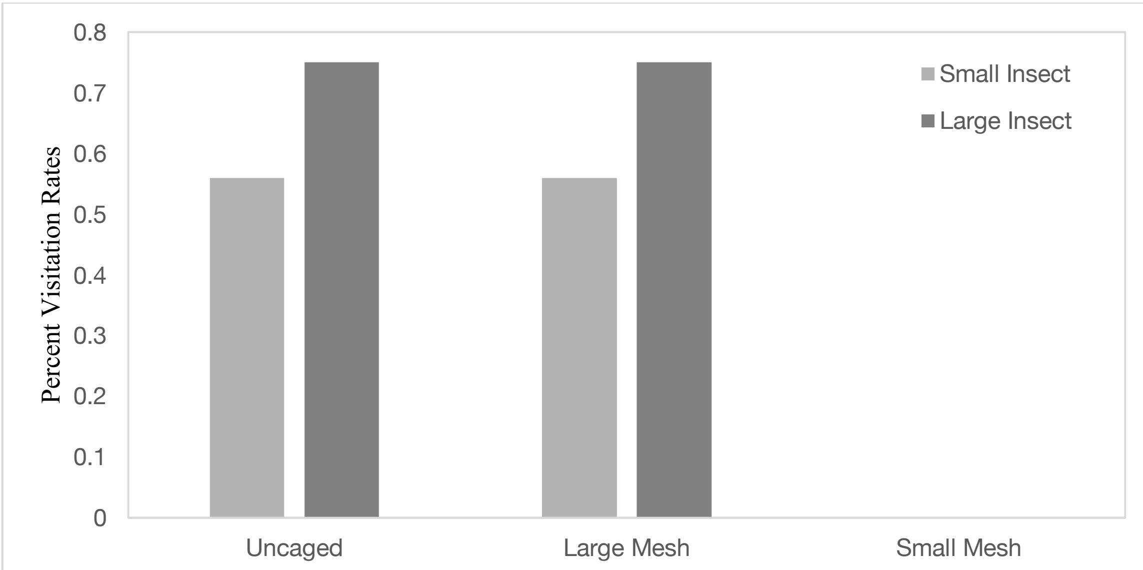

Figure 4. Average visitation rates for large (wingspan >2.6 cm) and small (wingspan < 2.6 cm) insects that visited plants expressed as a percentage of the number of times that the insect was observed at the study site.

The interaction between caging style and insect size did have a significant effect on visitation rates ($P < 0.05$). Mean flower visitation rates for small mesh size caged plants were nonexistent for either size of insect visitor (Figure 5). Both the large mesh caged plants and uncaged plants had a higher mean visitation rate from the larger class size of insects, but the same mean visitation rate for both class sizes of insects (Figure 5).

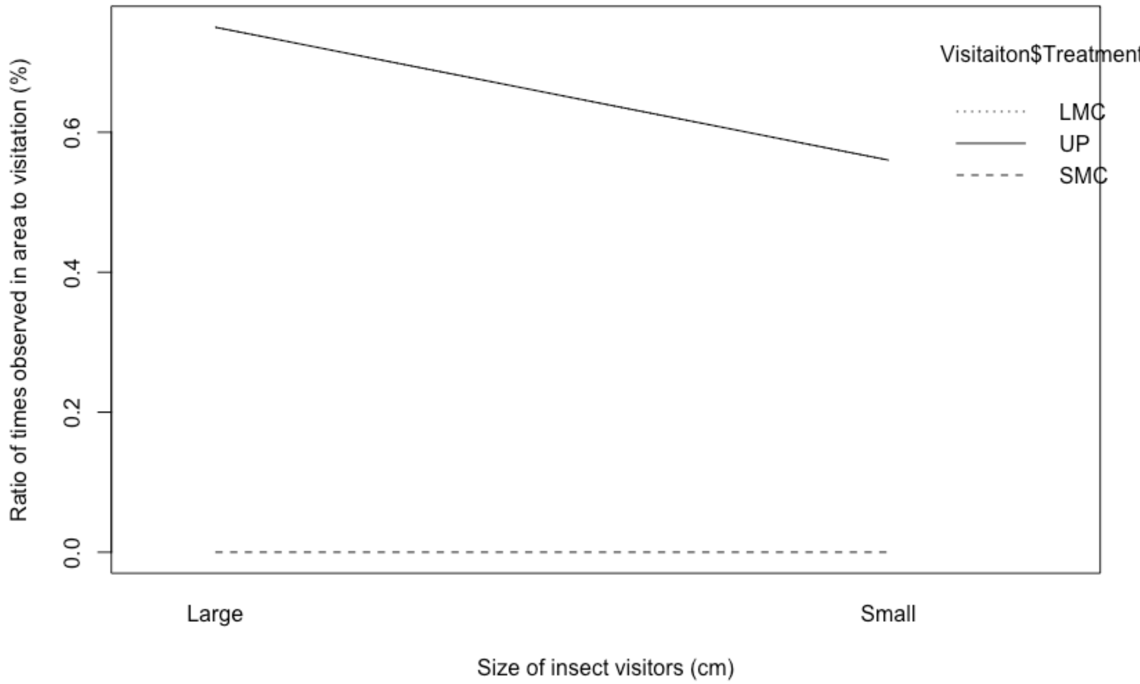

Figure 5. Interaction plot for the percent visitation rates of observed insect visitors and the size class of those insect visitors (large insects have a wingspan >2.6 cm and small insects have a wingspan < 2.6 cm). The interaction is shown for each of the three treatment types (LMC= large mesh cage, UP= uncaged plants, and SMC= small mesh cage) based on data gathered at the Hall Farm site in 2022.

Seed Sets from 2022

In small mesh cages, five plants produced flowers. At the peak of flowering, there were 27 inflorescences; however, no pollinators were observed visiting them. No fruits were produced from any flowering plants (Figure 6).

In the large mesh cages, six plants produced flowers. At the peak of flowering, there were 28 inflorescences and some visitation by pollinators. Of the six plants that flowered, three produced fruit (Figure 6). These plants produced 87 fruits, with an average of 8.5 seeds per fruit with a standard error of 28.6 seeds.

Of 18 uncaged plants, seven of them flowered. At the peak of flowering, there were 31 inflorescences and some visitation by pollinators. Of the seven plants that flowered, all seven produced fruit (Figure 6). These plants produced 80 fruits, with an average of 8.9 seeds per fruit with a standard error of 28.6 seeds.

Our analysis determined that there was not a significant difference between the flowering to fruiting ratios of the three treatment types ($\chi2 = 2$, df = 2, $P > 0.05$).

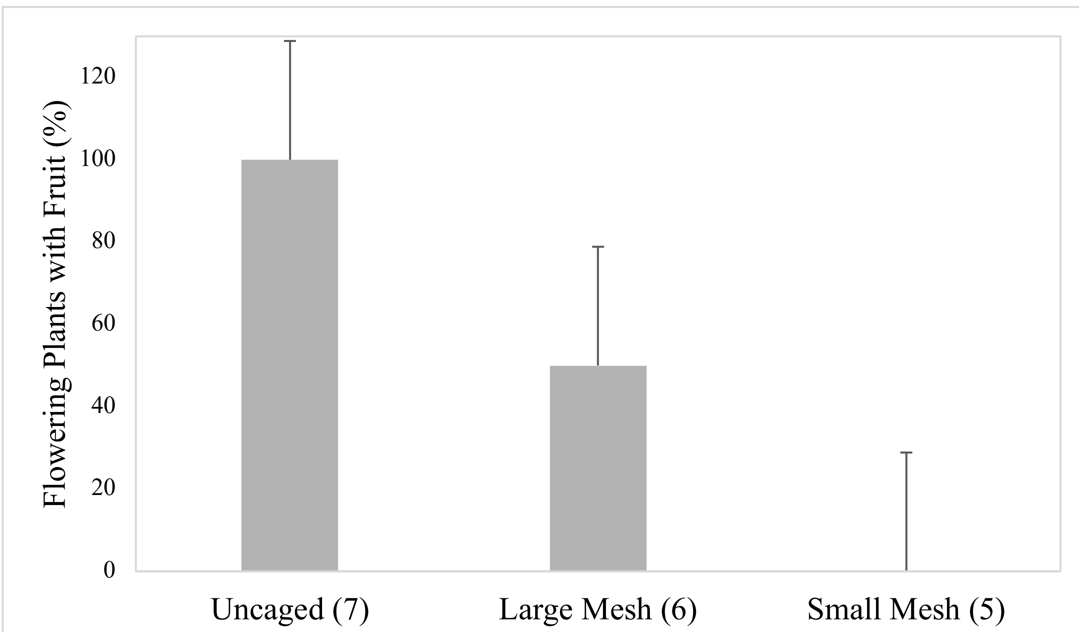

Figure 6. Percentage of plants that produced fruit in uncaged and cages with large and small mesh sizes during the 2022 field season. The values in parentheses refer to the number of flowering plants within the given treatment group. The error bars represent one standard error.

Herbivore Observations from 2022

Several potential herbivores were observed on the trail cameras. More often, white-tailed deer (*Odocoileus virginianus*) and eastern cottontail rabbit (*Sylvilagus floridanus*) were observed. White-tailed deer were imaged on 14 separate occasions and eastern cottontail rabbits were imaged on 39 separate occasions between April-May of 2022. Various other birds and other animals were also imaged but not nearly as consistently as the deer and rabbits. Although the potential herbivores were observed on the trail cameras at several points throughout the flowering season, no evidence of herbivory was found on *A. bibullatus* plants.

Pollinator Observations from 2023

Plants at the Savanna site produced no flowers, and thus, no pollinator observations were made. Plants at the Hall Farm site produced 15 inflorescences. Five pollinators were observed; however, there were few pollinator species, and no visitation of *A. bibullatus* was observed. Some insects were seen flying through the large mesh cages, over the uncaged plants, and around the small mesh cages, but no pollination behaviors were evident (Table 5). Our analysis revealed a dependence in visitation success for plants in large mesh caged, small mesh caged, and uncaged plots ($\chi 2 = 26$, df $= 4$, p-value = 3.164e-05).

Table 5. Recorded insect behaviors with plots of *Astragalus bibullatus* at the Hall Farm study site in 2023. Interactions are shown for each treatment group.

	Small mesh cages	**Large mesh cages**	**Uncaged plots**
Flying around the cage	4	0	-
Flying through the cage	0	6	-
Passing over	-	-	3

Seed Sets from 2023

Of the 26 plants at both sites in small mesh cages, three plants produced flowers. At the peak of the flowering season, they had five inflorescences; however, no insects visited the flowers during the observation periods. One fruit was produced from one of the plants that flowered within this treatment group and only one seed was collected (Figure 7).

Of the 25 plants at both sites in large mesh cages, three plants flowered. At the peak of the flowering season, they had five inflorescences and no insects visited flowers during the observation periods. Of the three plants that flowered, only one of them produced fruit with two seeds (Figure 7).

Of the 24 uncaged plants at both sites, four plants flowered. At the peak of the flowering season, the had six inflorescences and no insects visited the flowers during the periods of observation. Of the four plants that flowered, only two produced fruits. For one plant, all fruits were consumed and did not produce seeds. Another plant produced 10 fruits, with an average of 6.5 seeds per fruit (Figure 7). We observed no differences in seed set among uncaged and caged plants with different mesh sizes ($\chi2 = 2$, df = 2, $P < 0.05$)

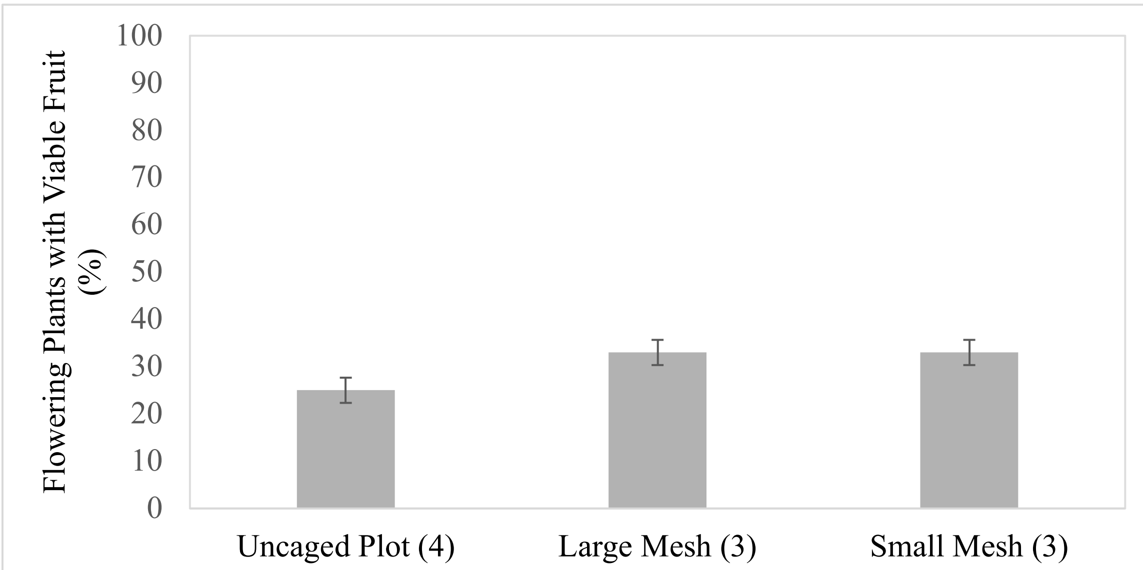

Figure 7. Percentage of plants that produced viable fruit in uncaged treatment and in large and small mesh cages during the 2023 field season. A fruit was viable if it matured and produced seeds. The values numbers in parentheses refer to the number of flowering plants within the treatment. The error bars represent one standard error.

Herbivore Observations from 2023

The same two potential herbivores seen in 2022 were observed on the trail cameras in 2023. While no damage was found on any of the small or large mesh caged plants, significant evidence of herbivory was noted on three separate uncaged plots (one at the Savanna site and two at the Hall Farm site). The damage was near the base of the plant and the missing stem appeared frayed (Figure 8). This is consistent with herbivory from a white-tailed deer (*Odocoileus virginianus*), which was observed in the images from the trail cameras. White-tailed deer were in the area at the time the damage would have taken place and can be seen in feeding postures in the image.

Figure 8. Damage found on an *Astragalus bibullatus* plant on March 9, 2023 and suspected herbivore caught on trail cameras near the damaged plant on March 6, 2023.

During both field seasons, I observed ants eating the fruit before seeds could be produced. Damage to the fruit would be made in small circular formations and the fruit would be eaten from the inside out (Figure 9). Ant damage occurred to at least two fruits in the 2022 field season and to all six fruits on one plant in the 2023 field season; ant damage was observed only on uncaged plants.

Figure 9. Damage found on an uncaged *Astragalus bibullatus* plant thought to be caused by ants.

Chapter IV: Discussion

My research goal was to determine if small mesh cages, currently used to prevent herbivory on plants during re-introductions, affected the pollination of *A. bibullatus.* To test the effects of the small mesh cages, I compared pollinator behavior, seed set, and herbivory on plants in small mesh cages with those on plants in large mesh cages and on uncaged plants. Data collected during two field seasons and from two sites were used to inform conservation management strategies for *A. bibullatus*.

I found that pollinator behavior and interactions with the three treatment types were significantly influenced by the presence of the cages. Potential pollinators avoided the small mesh cages, and the plants in these cages produced only one fruit during the two-year experiment. When the small mesh cages were in the near vicinity of uncaged plants, insects would avoid these plants. The reduced fruit production of plants in small mesh cages was probably due to the lack of pollinator visitation and pollination.

Many species in the genus *Astragalus* rely on outcrossing for effective pollination (Watrous and Cane, 2011; Sontani et al., 2021). The MBG acknowledged a similar problem when using a cage with a small square mesh that completely covered each out-planted seedling (Albrecht and Long, 2018). Due to concerns of access to pollinators, a cage with a circular open top was designed to allow pollinator access to the plants (Albrecht and Long, 2018). Since the potential pollinators did not enter through the open top of this later design, it would be logical to adjust the size of the mesh. In contrast to the small mesh cages, insects flew into and out of the large mesh cages directly through the mesh. As such, plants in the large mesh cages were cross-pollinated and produced more fruit and seeds than those in small mesh cages. Insect interactions with plants in the large mesh cages were similar to uncaged plants for both insect class sizes.

While plants in the large mesh cages did not have the same fruiting success as the uncaged plants, they produced approximately the same number of seeds. It was also determined that there was no significant difference in flowering to fruiting ratios of the three treatment types during the two-year experiment. A previous study done with *A. bibullatus* plants in small mesh cages found that over a four-year period, the caged plants had over 20% of the survivors transition into flowering adults while only a single flowering plant produced fruit outside of cages (Albrecht and Long, 2018). In addition, selective grazing by herbivores on larger, uncaged *A. bibullatus* plants may be a factor contributing to the reduced reproduction and illustrates that the cages are a necessary means to reduce grazing by herbivores (Albrecht and Long, 2018). If the plants are large enough to flower and are then selected by herbivores, reproduction will be severely curtailed.

In addition, my data supports previous findings that herbivores, particularly white-tailed deer (*Odocoileus virginianus*) and eastern cottontail rabbits (*Sylvilagus floridanus*), are present at these sites and will consume *A. bibullatus* plants (Albrecht and Long, 2018). Although my data is limited, herbivores did not consume caged plants as was observed for uncaged plants. My results also indicate deer are the primary herbivore of *A. bibullatus* which contrasts with an earlier study which found that it was likely rabbits causing most of the damage (Albrecht and Long, 2018). In previous studies, legumes tended to be more susceptible to herbivory than grasses, and the inability of plants to compensate for lost tissue only exaggerates the effects of herbivory (Hulme, 1996).

Long-term observations of the effects of caging on both pollinators and herbivores is necessary to determine effective protocols for management, it could take several years to understand the effects of caging on reproduction (Albrecht and Long, 2018). Plants at the Hall Farm site produced more flowers and fruits in 2022 than in 2023 with no clear explanation except the historical variation in the rates as shown in previous studies (Albrecht and Long, 2018).

The effects of herbivory at different stages of growth, flowering, and fruiting should also be addressed. I did not find any signs of herbivory on flowers, but I did find signs of herbivory on the vegetative portion and fruits of *A. bibullatus*. A previous study on *A. bibullatus* found that the vegetative stems were consumed by herbivores both before and after flowering and seed set (Albrecht and Long, 2018). Beyond that, frugivory should also be considered as a possible concern. One study comparing arthropod, mollusk, and rodent herbivory found that while rodents and lagomorphs tended to have the largest effect on plant biomass and survival, arthropods play only a minor role (Hulme, 1996). Here, ants encountered in my study likely affected reproduction during each field season. For example, 33% of the fruits produced in 2023 failed to produce seeds due herbivory, while only 0.02% of fruits produced (4 out of the total 196) in 2022 were affected. While this is a relatively low percentage of fruits destroyed by ants, seasons with low flowering and fruiting rates could be heavily impacted by this fruit loss to herbivory.

Adapting and updating the recovery plan is essential for the long-term survival of the endangered species, *A. bibullatus*. While herbivory did not drastically reduce populations, vertebrate herbivores have been found to selectively graze grassland legumes (Albrecht and Long, 2018; Hulme, 1996). Ensuring that plants mature to flower and protection from herbivores could help to improve the successful plant reproduction of *A. bibullatus.* When *A. bibullatus* plants were uncaged, their population structure was skewed toward smaller plants with fewer flowers; however, when the plants were caged, 20% of surviving plants matured into flowering adults over 4 years (Albrecht and Long, 2018). While caging is beneficial for success in out-planting, using a mesh size that allows for pollinator access will greatly augment reproduction of caged plants.

Chapter V: References

Albrecht, M., & McCue, K. (2010). Changes in demographic processes over long-time scales reveal the challenge of restoring an endangered plant. Restoration Ecology, 18, 235-243.

Albrecht, M., & Long, Q. (2018). Habitat suitability and herbivores determine reintroduction success of an endangered legume. Plant Diversity, 41, 109-117.

Albrecht, M. (2021). Learning from monitoring: improving plant reintroductions over time. Missouri Botanical Garden - Center for Plant Conservation. Retrieved 9 December 2021.

Barneby, R. & Bridges, E. (1987). A new species of *Astragalus* (Fabaceae) from Tennessee's Central Basin. Brittonia, 39, 358-363.

Becknell, R.E., Showalter, K.G., Albrecht, M.A., & Mangan, S.A. (2021). Soil mutualisms potentially determine the reintroduction outcome of an endangered legume. Restoration Ecology, 29, e13355.

Bell T.J., Bowles M.L., & McEachern A.K. (2003). Projecting the success of plant population restoration with viability analysis. In: Brigham C.A., Schwartz M.W. (eds) Population viability in plants. Ecological Studies (Analysis and Synthesis), vol. 165. Springer, Berlin, Heidelberg.

Call, G., Lincicome, D., & Bishop, A. (2011). Recovery Plan for *Astragalus bibullatus*. U.S. Fish and Wildlife Service, Atlanta, Georgia. Retrieved 17 October 2021.

Combs, J.K., Lambert, A.M., & Reichard, S.H. (2013). Predispersal seed predation is higher in a rare species than in its widespread sympatric congeners (*Astragalus*, Fabaceae). American Journal of Botany, 100, 2149-2157.

Fraser, L., & Madson, E. (2008). The interacting effects of herbivore exclosures and seed addition in a wet meadow. Oikos, 117, 1057-1063.

Hulme, P. E. (1996). Herbivores and the performance of grassland plants: A comparison of arthropod, mollusc and rodent herbivory. Journal of Ecology, 84, 43-51.

Menges, E.S., Pace-Aldana, B., Haller, S.J., & Smith, S.A. (2016). Ecology and conservation of the endangered legume *Crotalaria avonensis* in Florida scrub. Southeastern Naturalist, 15, 549-574.

Mitchell, R., Irwin, R., Flanagan, R., & Karron, J. (2009). Ecology and evolution of plant–pollinator interactions. Annals of Botany, 103, 1355-1363.

Skopec, M., Lewinsohn, J., Sandoval, T., Wirick, C., Murray, S., Pence, V., & Whitham, L. (2017). Managed grazing is an effective strategy to restore habitat for the endangered autumn buttercup (*Ranunculus aestivalis*). Restoration Ecology, 26, 629-635.

Soltani, E., Benakashani, F., Baskin, J.M. & Baskin, C.C. (2021). Reproductive biology, ecological life history/demography and genetic diversity of the megagenus *Astragalus* (Fabaceae, Papilionoideae). The Botanical Review, 87, 55-106.

Tetreault, T., & Aho, K. (2021). An updated insect exclosure design for pollination ecology. Journal of Pollination Ecology, 29, 249-257.

University of Alberta E.H. Strickland Entomological Museum. (2021). *University of Alberta Museums*, Entomology Database. Accessed 9 Dec. 2021.

U.S. Fish and Wildlife Service (USFWS). (1991). *Astragalus bibullatus* (Guthrie's ground-plum) determined to be endangered. Federal Register, 56, 48748-48751.

U.S. Fish and Wildlife Service (USFWS). (2009). Technical/Agency Draft Recovery Plan for *Astragalus bibullatus* (Pyne's Ground-plum). Atlanta, Georgia. 39 pp.

Van Der Wal, R., Egas, M., Van Der Veen, A., & Bakker, J. (2000). Effects of resource competition and herbivory on plant performance along a natural productivity gradient. Journal of Ecology, 88, 317-330.

Watrous, K.M. & Cane, J.H. (2011). Breeding biology of the threadstalk milkvetch, *Astragalus filipes* (Fabaceae), with a review of the genus. The American Midland Naturalist, 165, 225-240.