The Relationship Between Carpenter Bees and Pyne's Ground-plum (*Astragalus bibullatus*)

By

Marian Medley

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Thesis Committee:

Dr. Jeffrey Walck, Thesis Director

Dr. Chris Herlihy, Second Reader

Dr. Stephen Howard, Thesis Committee Chair

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APPROVED:

Dr. Jeffrey Walck, Thesis Director

Professor, Department of Biology

Dr. Stephen Howard, Thesis Committee Chair

Professor, Department of Biology

Abstract:

Astragalus bibullatus is a federally endangered species, endemic to the limestone cedar glades of Middle Tennessee. Cedar glades are an endangered ecosystem, and the plant is found in only a few naturally occurring populations, all within Rutherford County, Tennessee. Insects in the genus *Xylocopa*, known as carpenter bees, perform a phenomenon referred to as nectar robbing on *A. bibullatus*. This method of nectar collection bypasses pollination and thus poses a threat to the reproductive fitness of the plant. Scant data on nectar rubbing by carpenter bees exists, especially regarding *A. bibullatus*.The goal of this research project has been to determine the level of interference that carpenter bees have on the pollination and reproduction of *A. bibullatus*. Carpenter bees started to visit plants of *A. bibullatus* 4 weeks after flowering started in the species. The bees remained constant to *A. bibullatus* and did not move to the co-flowering *Pediomelum subacuale*. No other insect species visited the holes made by carpenter bees to collect nectar, in contrast to other researcher's observations in previous years. While no fruit production was observed on plants visited by carpenter bees, additional studies are needed to determine the degree that carpenter bees impact the reproduction of *A. bibullatus*.

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Cedar glades are endangered ecosystems that are primarily located in the southeastern United States, with the most significant region being the Nashville Basin. Cedar glades are considered a hotspot for biodiversity and host a range of rare and endemic species (Harper, 1926; Estill and Cruzan, 2001). The geology that is responsible for the formation of cedar glades is also a key factor in determining what types of plants can occur in this environment. This ecosystem can be characterized by limestone bedrock with exposed bedrock and thin, rocky soils, giving way to a host of specialized plant species that are adapted to challenging conditions, including drought and heat tolerant species (Quarterman, 1989; Quarterman et al., 1993; Ware, 2002; Baskin and Baskin, 2005). Cedar glades are also characterized by karst limestone topography, and usually can be found in areas where sinkholes and caves are common (Baskin et al., 1994).

Cedar glades have a large ecological significance due to hosting a variety of rare and/or endemic species (Baskin and Baskin, 1999; Baskin et al., 2007).

This ecosystem is especially vulnerable to threats such as habitat loss due to housing and industrial developments, fire suppression, invasive species, and climate change. As a result, conservation efforts are underway to protect and preserve them, recognizing their educational, recreational, and aesthetic value (Ware, 2002). Conservation efforts include establishing protected areas, conducting research, and implementing measures like controlled burns and invasive species control. Preserving the integrity of cedar glades is crucial for maintaining biodiversity and ensuring the survival of specialized plant and animal life.

One such rare and endemic cedar species is *Astragalus bibullatus,* commonly known as Pyne's ground-plum. It is a perennial flowering plant native to the limestone cedar glades of the Central Basin, particularly sites found in Rutherford County, Tennessee. Despite the name, the plant is unrelated to plums and is a member of the legume family (Fabaceae). The plant was first described as a new species in 1987 by Rupert Barneby and Edwin Bridges (Barneby and Bridges, 1987; Baskin and Baskin, 2005). The herb grows along the deeper-soiled margins or partially shaded areas of a cedar glade (Albrecht and Long, 2019). The stems grow from 5 to 15 cm and support around 10 leaves

each, ranging from 5 to 10 cm. The inflorescence supports a range of 10-16 flowers, which are purple in color. The flowering season is from early April through May, with fruiting occurring throughout May and June (Barnaby, 1987). The flowers of *A. bibullatus* have a wide opening that progresses into a tubular shape towards the corolla. The fruit of *A. bibullatus* is reddish with yellow undersides and an oblong shape. *Astragalus bibullatus* is similar to the Tennessee milkvetch, *A. tennesseensis,* but can be mainly distinguished by the difference in the colors of the flowers: *A. tennesseensis* being cream-colored and *A. bibullatus* being purple. *Astragalus bibullatus* is listed as a federally endangered species, and threats to it include climate change, encroachment of vegetation and fire suppression, herbivory, and moderate genetic diversity (Bernardo et al., 2016; Albrecht et al., 2016; Albrecht & Long, 2019; Morris et al., 2021).

One insect that frequents flowers of *A. bibullatus* is carpenter bees (genus *Xylocopa*), which are large, diurnal, solitary bees. Their foraging habits include collecting nectar and pollen from several flowers, including those with tubular corollas like that of the flowers present on *A. bibullatus*. Carpenter bees can effectively pollinate, however, they have been

observed participating in a phenomenon known as nectar robbing. Nectar robbing is foraging behavior, in which nectar collection bypasses the typical pollination process (Gerling, 1989; Elisante et al., 2020). Usually, a pollinator would need to come into contact with a flower's reproductive structures in order to access nectar at the base of the structures. However, in the case of nectar robbing, a hole is made at the base of the corolla, either by the mandibles of an insect or the beak of some bird species, and the nectar is extracted from this hole. This action poses a potential threat to the reproductive fitness of a flower by reducing its attractiveness to other pollinators or reducing the lifespan of the flower (Zhang, 2007), although it cannot be assumed that the act of nectar robbing is entirely negative or that zero pollination has occurred (Maloof, 2000).

The purposes of the present study were to (1) record the phenology of flowering in *A. bibullatus* in relation to that of the presence of carpenter bees, (2) track the visitation of carpenter bees to *A. bibullatus* and to other co-flowering species, and (3) document whether other insects visited the holes made by carpenter bees in flowers of *A. bibullatus*, and (4) verify if fruits are produced by *A. bibullatus* flowers that have been rubbed of nectar by carpenter bees.

Section II: Methods

The following aspects were documented during the study:

First, the phenology of carpenter bee visitation and flowering was tracked during the 2023 growing season. A transect was established at three sites, the length of which varied but was placed to cover the largest part of the population. All sites were found within the Flat Rock

Cedar Glade and Barrens State Natural Area in Rutherford County, Tennessee. The site names were as follows: Airport Front, Airport Back, and Alexander. Starting in early March 2023, the transects were walked at least three times a week on non-rainy days and the number of infloresences and the number of carpenter bees were counted that were within 0.5 m on each side of the transect.

Then, visitation to the infloresences by carpenter bees and other insects was monitored. A small patch (about 1 x 1 m) of ground-plum plants with mature infloresences (i.e. with nearly all flowers fully opened) were selected at the three

sites. The patches were observed for 1 h each day during the flowering period, and the number of times that carpenter bees visited an infloresence was recorded. In addition, visitors to holes that carpenter bees made were also recorded. Insects were identified to the lowest taxon possible.

Next, insect visitation following carpenter bee robbing was recorded. Once a carpenter bee had visited a damaged flower, the flower was marked by placing thread at its base. Then, this flower was observed for visitation by other insects with particular interest in the subsequent pollination attempts from those insects. The identity and number of visitations were recorded. These marked flowers were followed for fruit development and then the number of seeds was counted once the fruits matured.

Section III: Results

Two difficulties occurred when conducting research during the 2023 growing season. First, substantially less plants of *A. bibullatus* flowered than in previous years (Walck, pers. comm.). This flowering pattern, in which many plants flower for a few years followed by low flowering for one year, is usual for the species. Second, most of the plants in the largest population of *A. bibullatus* were used by a researcher for another project. As such, both of these difficulties led to the present studies' sample size being low.

Sightings of carpenter bees began approximately 4 weeks after flowering of *A. bibullatus* started at the three study sites (Table 1). The instances of nectar robbing by carpenter bees (Figures 1, 2) were limited to 10 infloresences. The carpenter bees were most active in the

afternoon, on clear and warm days; none were seen on rainy or chilly days. Bumblebee activity was limited when carpenter bees were present in the area. Only one time was a bumblebee observed in the area of carpenter bee activity. Other insects were identified at the sites; however, none were witnessed visiting flowers of *A. bibullatus* that contained holes made by carpenter bees. The carpenter bees were observed visiting either *A. bibullatus* or *Pediomelum subacuale*, a co-flowering and co-occurring fabaceous species, i.e., the bees did not go from one species to the other but remained constant with one species. During the fruiting period, no fruit was found from the 10 marked plants that had an observed nectar robbing occurrence.

Table 1. Number of infloresences of *Astragalus bibullatus* and number of carpenter bees visiting them at three sites and number of times that nectar robbing was observed by carpenter bees at all three sites over time.

Date	Airport Front: Flowers	Airport Front: Bees	Airport Back: Flowers	Airport Back: Bees	Alexander: Flowers	Alexander: Bees	Nectar Robbing Observed
03/21/23	0	0	0	0	0	0	0
03/28/23	33	0	19	0	11	0	0
03/31/23	27	0	17	0	8	0	0
04/02/23	27	0	19	0	13	0	0
04/04/23	29	23	10	1	15	1	7
04/09/23	30	0	11	0	12	0	0
04/12/23	21	12	5	1	22	0	3
04/15/23	16	4	6	2	10	0	0

Figure 1: Carpenter bee (genus *Xylocopa*) performing the act of nectar robbing on the corolla of an *Astragalus bibullatus.*

Figure 2: An example of a hole left by a carpenter bee after a nectar robbing event.

Section IV: Discussion

The data collection process of this research project required adjustments in the field based on the availability of specimens and the variability of the weather. There were many days during the flowering season that may have yielded some additional data, but the temperature would fluctuate too greatly or rain would erase the chances of a pollinator sighting at any of the sites. In addition to the changeability of the weather, there was parallel research utilizing the same populations that were being studied in this project. Another research project that was in progress at the same time as mine required that infloresences be bagged, thus rendering them unavailable for use in my study and reducing my sample size.

Although the sample size was less than desirable, some inferences can be made about the project. First, carpenter bees were not observed until about 4 weeks after flowering started in *A. bibullatus*. Spring emergence dates in carpenter bees are highly dependent on spring temperatures and weather conditions (Skandalis et al., 2011). Thus, plants of *A. bibullatus* probably started to flower at temperatures lower than required for carpenter bee emergence. It wasn't until spring temperatures warmed enough that carpenter bees emerged and were observed visiting flowers of *A. bibullatus*. Second, carpenter bees had relatively high constancy in visiting *A. bibullatus* flowers even when the co-flowering *P. subacuale* was present. Keasar et al. (2007) found that carpenter bees could be flower constant, but this behavior was highly dependent on whether the species was a solitary or social nester. The nesting behavior in the carpenter bees observed in the present study is unknown but may have influenced observations. However, other factors may have played a role in the constancy that I observed, especially given the low number of flowering plants of A. bibullatus, e.g., the position of a plant within a population (Kunin, 1997). Third, I was surprised that

no other insects were observed visiting the holes left by carpenter bees in flowers of *A. bibullatus* to collect nectar given that other researchers have observed this phenomenon (Walck, pers. comm.). However, this situation could have been due to the lack of availability for continued monitoring. Future studies could benefit from multiple researchers visiting more frequently and for longer periods of time, perhaps taking shifts so that additional activity can be logged.

No fruit was recovered from plants in this study in which carpenter bees visited; however, this does not signify that no fruit production occurred. Other plants in the study site produced fruit according to other researchers that were monitoring the sites. There is a potential that any fruit that may have been produced on the samples in this study may have been accidentally collected for other studies. Otherwise, the lack of fruit may suggest that the pollination of the *A. bibullatus* flower was unsuccessful and the overall fitness was reduced. However, it is worth noting that up to 90% of flowers have been affected by nectar

robbing in previous years (Walck, pers. comm.). This observation along with some fruit production suggests that pollinators have visited before nectar robbing occurred that nectar robbing did not affect subsequent pollination of the plant, or that pollination occurred during a nectar robbing event. At this time, the species would benefit from further studies examining the effects of nectar robbing on reproductive fitness.

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