

# An insect-pollinated species in a wind-pollinated genus: case study of the endemic plant, Laramie chickensage (*Artemisia simplex*)

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## Abstract

Rare and endemic plants preserve ecosystem functionality and niche differentiation by contributing novel plants to communities, yet very little is typically known about these species. Species level management strategies that protect the inherently small populations of these plants typically focus on population viability, population geography, surrogate species, and pollination mechanisms. Of all the vascular plant species around the world, 12.5% are threatened by extinction and case studies of specific species are limited. In North America, there are ~50 sagebrush species and 14 of those species are ranked as vulnerable, imperiled, or critically imperiled. A relatively new member to the sagebrush genus, *Artemisia simplex* (Laramie chickensage), is endemic to southeastern Wyoming, is a Bureau of Land Management Sensitive species and ranked imperiled by NatureServe. While other reports have mapped populations of this endemic plant, we are unaware of any studies addressing viability and pollination mechanisms. To provide much needed information on this endemic plant, we addressed how abundant *A. simplex* is in select populations, to what degree do this species self-pollinates, how many seeds it produces, and what pollination mechanisms this plant uses. We found that *A. simplex* abundance varied among sites and occupied 5.5% of the area on average. This unique plant did not self-pollinate, but instead needed pollen transported to flowers by an outside source. Both wind and animals transported pollen to *A. simplex*, but wind alone produced fewer seeds. Characteristics of the flower and pollen suggest that this plant is primarily animal pollinated. Members of the sagebrush genus are widely known to be wind-pollinated; however, we demonstrated that this species of sagebrush is largely animal pollinated. We demonstrated that assumptions of pollination mechanisms should not be based on taxonomy and plans may consider the mode of pollen transfers when managing species, especially when considering the decline of insect pollinators. Pollination provides information about the persistence of plant populations through sexual reproduction which produces the genetic variation needed for healthy organisms.

## Introduction

Globally, rare and endemic plant species are vital parts of many ecosystems by preserving functionality and niche differentiation (Dee et al., 2019; Flather & Sieg, 2007). These unique plants provide nutrition to a variety of animals (Schowalter, 2016), increase insect diversity (Hernández-Teixidor et al., 2020), and provide distinct contributions to the functional diversity within an ecosystem (Flather & Sieg, 2007; Jain et al., 2014). A plant is defined as rare when its abundance or frequency is low (Lyons et al., 2005), and a plant is endemic when the species has a small geographical range (Broennimann et al., 2005). Due to the nature of rare and endemic plants, these species have small populations and increased extinction risk (Flather & Sieg, 2007); therefore, base knowledge about rare and endemic plants is crucial for forming management strategies. Of the vascular plants around the world, many are considered rare (Dee et al., 2019) and 12.5% are threatened by extinction (Wilcock & Neiland, 2002). Extinction rates may be accelerated by human activities that reduce essential soil nutrients, change growing season lengths and temperatures, and limit available land and habitat (Tilman & Lehman, 2001). Species level management strategies for rare and endemic plants focus on population viability, population geography, and surrogate plant species (Marcot & Flather, 2007). Population viability studies provide an understanding of a plant's life history, habitat requirements, and potential threats. Geographic studies provide information concerning where rare and endemic plants grow, and surrogate species provide information in which inferences about other species may be drawn (Marcot & Flather, 2007). The preservation of rare and endemic species is important for

maintaining global biodiversity, and we suggest that pollination mechanisms is another key process to consider when managing species.

Pollination is essential for plant and animal life; it is necessary for fertile seed production, the continual growth of plants, and to produce forage for animals. Despite its importance, little is known about the pollination mechanisms or pollinators of rare plants (Gibson et al., 2006). Rare and endemic angiosperms can be biotically pollinated (e.g., animal-pollinated), abiotically pollinated (e.g., wind or water pollinated), or both (ambophily). There are ~352,000 angiosperm species in the world and ~87.5% of these plants are animal-pollinated while ~12.5% disperse pollen grains via wind or water (Ollerton et al., 2011). Animal-pollinated plants usually have simple stigmas, various stamen filament lengths, have rich nectaries, and showy perianths (flowers). These plants also produce small pollen loads of elaborate pollen grains with obvious pollenkits, sticky material that helps grains adhere to pollinators (Culley et al., 2002). Conversely, wind-pollinated plants typically have feathery stigmas, long stamen filaments, and reduced nectaries and perianths. They produce a large number of smooth pollen grains that are between 10-50 micrometers long and lack pollenkits (Culley et al., 2002). However, the distinction between abiotic and biotic pollination is unclear, as plants can transition between insect and wind pollination, or employ both mechanisms (Culley et al., 2002; Flamini, 2012). In changing environments, wind pollination may become more prominent when pollinators become scarce and pollen limitation occurs (Friedman & Barrett, 2009). Regardless of the method, pollination is a key process in maintaining plant populations.

*Artemisia* is a large genus of 350-500 sagebrush species globally (Hitchcock & Cronquist, 2018) and there are 10 endemic species ranked as vulnerable, imperiled, and critically imperiled in the United States alone (NatureServe, 2020). Sagebrush species occur in sagebrush steppe ecosystems and may be large, woody and shrub-like (e.g., *Artemisia tridentata*, Wyoming big sagebrush), or small, leafy and herbaceous (e.g., *Artemisia campestris*, field sagewort). Historically, shrub-like sagebrush is thought to be wind-pollinated (Laursen et al., 2007); however, few studies investigated the pollination mechanisms of herbaceous sagebrush that share characteristics with other plants in animal-pollinated genera. After recent phylogenetic studies, *Artemisia* went through taxonomic rearrangements and *Sphaeromeria* species within Asteraceae were transferred to *Artemisia* (Garcia et al., 2011). New members of *Artemisia*, such as *A. simplex*, *A. capitata* (rock tansy), and *A. argentea* (silver chickensage) have large, showy flowerheads that are unlike other sagebrush species and may attract insect pollinators. *Artemisia simplex* is a Wyoming endemic that only occurs in the foothills of the Laramie Range, Shirley Basin, and Shirley Mountains of southeastern Wyoming (Handley & Heidel, 2010). The plant is listed as a Bureau of Land Management Sensitive species and is ranked imperiled by NatureServe (NatureServe, 2020). Other studies mapped *A. simplex* populations (Handley & Heidel, 2010), but we are unaware of studies that investigated the viability and pollination mechanisms of this rare, endemic plant.

We measured the coverage of *A. simplex*, estimated the reproductive success of the plant by examining seed viability, and assessed how its flowers were pollinated. Our specific questions were: 1) What is coverage of *A. simplex* in each population?, 2) To what degree did *A. simplex* flowers self-pollinate, 3) To what degree did *A. simplex* produce developed and viable seeds?, and 4) Was *A. simplex* insect pollinated, wind-pollinated, or both? Results from our study will provide information on the coverage and reproduction strategies of *A. simplex*, creating baseline

knowledge for this plant's life history. Our study will provide information on which managers can base decisions.

## Study Area

*Artemisia simplex* populations grow in the foothills of the Laramie Range in southeastern Wyoming. The foothills to the west of the Laramie Range have elevations between 2,012 to 2,301 m (Knight & Brower, 2009). The average temperature in this area ranges from 1.7-7.2°C, and experiences 0-7 days above 32°C and 191-230 days below 0°C annually (Curtis, 2004). The mean annual precipitation in the area is 28-63 cm, with an average of 160-196 cm of snow in the winter. The Laramie Range foothills are windy, with daily average wind speeds between 6-9 m/s.

We selected six sites based on previous *A. simplex* surveys and on the plants' observed growing conditions (Figure 1a, b). We searched for *A. simplex* populations by following its preferred geology. *Artemisia simplex* grows on slopes, ridges, and rims, and prefers rocky or gravelly soils with limestone and calcium deposits (Handley & Heidel, 2010). This plant frequently grows in cushion plant communities dominated by *Phlox muscoides* (musk phlox), *Stenotus acaulis* (stemless mock goldenweed), *Tetraneuris acaulis* (stemless four-nerve daisy), and *Eremogone hookeri* (Hooker's sandwort). Although less common, *Artemisia simplex* may also occur in sparsely vegetated *Artemisia nova* (black sagebrush) and *Artemisia tridentata* ssp. *wyomingensis* communities. The habitats capable of supporting *A. simplex* occur from open plains to lower montane settings.

## Methods

### *Artemisia simplex* cover

We measured the coverage of *A. simplex* populations by measuring transects while the plant was in bloom. Transect location was chosen based on land ownership, accessibility, and the presence of *A. simplex* populations. We marked the end of transects with flagged rebar and we recorded the coordinates with a GPS unit. We laid a reel tape measure on the ground and recorded the presence or absence of *A. simplex* in each of 16 cells (7.6 x 7.6 cm) within a 30.5 x 30.5 cm quadrat. We placed the quadrat above and below the tape measure, and recorded the percent cover for ~10 m length.

### Seed production

We performed seed-set experiments on five *A. simplex* populations using four treatments (Figure 2a). The open treatment left flowers exposed to ambient levels of insect and wind pollination (open treatment hereafter). The insect-secluded treatment isolated flowers from insect pollination but allowed wind pollination by placing mesh bags. In 2020, we used very fine mesh and in 2021 we used coarser mesh with 1 x 2 mm openings over blooms (mesh treatment hereafter). The wind-secluded treatment isolated flowers from wind and insect pollination, and tested for self-pollination by placing cloth bags over blooms (cloth treatment hereafter). The hand-pollinated treatment measured seed production when pollen was not limited by adding pollen from plants  $\geq 50$  m away when stigmas and anthers were visible in disk flowers. Before *A. simplex* bloomed, one flowerhead on each plant was marked with colored string that corresponded to one of the four treatments and bags were placed over flowerhead for the wind-secluded and insect-secluded treatments. We recorded the date that flowerheads bloomed in the open and hand-pollinated treatments. When *A. simplex* flowers started losing color and began dehiscence, bags were placed over the open and

hand-pollinated treatments to prevent seed loss. Plants were monitored throughout the flowering and dehiscence periods, and treatments were collected when disk flowers fell from a flowerhead when touched.

We counted, weighed, and performed Tetrazolium assays on *A. simplex* seeds. Floral parts were removed from each flowerhead and separated into developed seeds, underdeveloped seeds, and disk flowers. Floral components were counted and weighed by treatment. Individual mass was calculated by dividing the number of seeds by the total mass. Developed seeds were tested for viability by performing Tetrazolium assays (Lindenbein, 1965). Seeds were rehydrated for ~24 hours, cut in half to expose the embryo, emerged in a 1% triphenyl-tetrazolium chloride solution for ~24 hours, and examined for viability. Seeds were viable if the embryo turned red and non-viable if the embryo did not change color.

### ***Pollination mechanism***

The pollination mechanisms of *A. simplex* were estimated by examining the pollen carried on bees. We collected pollinating insects via blue vane trap (vane trap hereafter; Figure 2b; Stephen & Rao, 2005). Vane traps were attached to black rebar and consisted of a yellow basin with a blue vane and hung ~0.5 m above the ground. We deployed traps for ~48 hours throughout the summer after which insects were collected, labeled, and frozen until analysis.

We created a pollen library for pollen identification. Plant specimens of unique genera were collected throughout the summer at each site beginning in May. Collected plants were frozen, later identified, and a portion of their pollen was removed for analysis. Plants were identified to genus or species using Dorn (2001).

We prepared plants and insects for identification and pollen collection. Anthers and stamens from each plant specimen were harvested to create a pollen library. Insects were pinned, labeled, and identified. Bees were identified to genus (Michener et al., 1994) and all other insects were identified to family (Johnson & Triplehorn, 2004). We removed one hind leg from each bee and wasp because most Hymenoptera store pollen there, and we removed pollen from the abdomens of Megachilidae. Pollen was stored in a microcentrifuge tube until analysis.

Pollen from plants and insects were cleaned to enhance identification using acetolysis (Jones, 2014). We created a pollen library by making pollen slides from each plant genus. Up to 10 bee legs of the same genus collected on the same date and site were processed together. Pollen was stained with Safranin O after acetolysis, mounted on microscope slides, and labeled. The pollen library was used to identify pollen from insects to the plant genus. We subsampled each slide until ~100 pollen grains were identified. The slides were viewed under 400x magnification, and we recorded the number of fields viewed.

### ***Statistical methods***

We used generalized linear models (glm) and generalized mixed-effects models (glmer; Bates et al., 2015) to estimate differences among variables. We used generalized linear models to estimate the variance in *A. simplex* coverage, where site and year were predictor variables. To assess differences in the number, mass, and viability of seed-set data, we used generalized mixed-effect models where site was a random effect, and year, treatment, and an interaction between year and

treatment were fixed effects. The gamma distribution best fit our data; we added one to each value and viability of seeds to run models to better fit the distribution. When a predictor variable was significant, we used a pairwise comparison (emmeans; Lenth, 2020) to estimate which variables differed.

## Results

### *Artemisia simplex* cover

*Artemisia simplex* presence varied with site but not year. On average, *A. simplex* comprised 5.5% of a transect. The presence of *A. simplex* did not differ between 2020 to 2021 within a transect (Figure 3; glm,  $t = 1.95$ ,  $p > 0.06$ ), suggesting that the monitoring method was consistent between years. Overall, we observed the highest cover of *A. simplex* presence at Dodge (transects 1-2; glm,  $t = -1.0$ ,  $p < 0.05$ ) and the lowest cover at Sevenmile (transects 3-5; glm,  $t = -11.0$ ,  $p < 0.005$ ).

### Seed production

*Artemisia simplex* produced the most seeds when insects could pollinate flowers and fewer seeds when wind pollination was the only mechanism acting on blooms. Overall, the open (glmer,  $t = 26.7$ ,  $p < 0.005$ ) and hand-pollinated ( $t = 25.7$ ,  $p = 0.005$ ) treatments produced far more seeds than the cloth ( $t = 3.3$ ,  $p < 0.001$ ) and mesh treatments ( $t = 11.2$ ,  $p < 0.005$ ; emmeans,  $p < 0.001$ ; Figure 4a). *Artemisia simplex* produced more developed seeds in the hand-pollinated and open treatments in 2020 than in 2021 ( $t = -2.9$ ,  $p < 0.005$ ). In 2020, 48% of the seeds produced in the open treatment and 43% of seeds in the hand-pollinated treatment were developed, whereas only 34% of seeds in the open treatment and 30% of seeds in the hand-pollinated treatment were developed in 2021. Because the number of seeds produced by *A. simplex* under normal (open) and optimal conditions (hand-pollinated) did not differ, the plant was not pollen limited. In 2020, the cloth and mesh treatments produced  $<1$  developed seed per flower head, with the percent of developed seeds as low as 0.5% to 1.2%, suggesting that *A. simplex* does not readily self-pollinate flowers and needs pollen from an outside source. The mesh treatment produced 30 times more seeds in 2021 when we used larger mesh ( $t = 10.8$ ,  $p < 0.005$ ), suggesting that *A. simplex* is at least partially pollinated by wind. On average, 14% of the seeds in the mesh treatment were developed in 2021 (~2.4 times less than the open and hand pollinated treatments), suggesting that wind does not solely pollinate *A. simplex*.

*Artemisia simplex* produced developed seeds with similar mass and viability in all treatments. Overall, seed mass did not differ among treatments (glmer,  $t = -43.3 - 0.68$ ,  $p = 0.5 - 0.6$ ; emmeans,  $p > 0.05$ ) or between years ( $t = -0.7$ ,  $p = 0.44$ ; Figure 4b). The mass of an individual developed seed was 3.4 times heavier than an individual underdeveloped seed. Similarly, viability did not differ among treatments ( $t = -0.1 - 13.9$ ,  $p = 0.91 - 0.99$ ; emmeans,  $p > 0.05$ ) or years ( $t = 0.55$ ,  $p = 0.59$ ; Figure 4c).

### Pollination mechanism

We captured a variety of bees from 5 families and 23 genera (Table 1), and most bee genera were captured during both years. Overall, *Agapostemon* (37%) were the most abundant genera followed by *Lasioglossum* (15%), *Anthophora* (14%), and *Eucera* (14%), *Bombus* (7%), *Osmia* (5%), and *Andrena* (3%), and all other genera accounted for  $<1\%$  of the capture individually. *Agapostemon* were the most commonly collected bee in 2020 and *Eucera* were most abundant in 2021.

We observed *A. simplex* pollen on 9 bee genera from 3 families. *Artemisia simplex* pollen is trilobed, 8 – 10 microns wide, relatively rough, and has a pollenkitt (Figure 5a). *Artemisia simplex* pollen was observed on smaller, sparsely haired bees, (i.e., *Agapostemon*, *Lasioglossum*, *Andrena*, and *Halictus*) as well as larger, more densely haired bees (i.e., *Eucera*, *Anthophora*, *Osmia*, *Bombus*, and *Melecta*). *Artemisia simplex* pollen typically composed 0.5% of pollen loads on bees (Figure 5b), and *Halictus* carried the most *A. simplex* pollen (2.6%), followed by *Osmia* (1.6%), *Anthophora* (1.5%), and *Eucera* (1.4%). Our detection of *A. simplex* pollen on captured bees tracked flowering times. We observed the most *A. simplex* pollen, as well as the most bees carrying *A. simplex* pollen, from 25 May to 2 June when flowers were mature (Figure 5c). Detection of pollen was lowest before and after optimal flowering times.

## Discussion

The morphology of *A. simplex* flowerheads aligned with animal-pollinated traits (Table 2). *Artemisia simplex* has a showy perianth comprised of bisexual disk flowers, where the male flowers mature before female flowers. Each flower contained one branched stigma and several anthers that do not exceed the disk. An enlarged perianth, simple stigma, and reduced filament length is common among animal-pollinated flowers (Culley et al., 2002). Interestingly, other herbaceous sagebrush typically have minute flowers and long stigmas that extend past flower openings (Wetzstein et al., 2014); these traits largely contribute to the thought that most sagebrush species are wind-pollinated. Despite the obvious differences between the perianths of *A. simplex* and other sagebrush, all members of *Artemisia* have branched stigmas (Hitchcock & Cronquist, 2018). While feathered stigmas (commonly found in Poaceae) are best equipped to catch aerial pollen, simple stigmas with fine hairs (penicillate; present on some sagebrush species) may also catch aerial pollen.

*Artemisia simplex* pollen grains display wind and animal-pollinated characteristics (Table 2). Similar to other sagebrush species, *A. simplex* pollen is tri-lobed; however, *A. simplex* pollen is larger and rougher than woody (e.g., *A. tridentata* and *A. nova*) and herbaceous sagebrush (*A. campestris*) in our study areas. *Artemisia simplex* pollen is slightly smaller than most animal-pollinated plants (10 – 50 microns) and slightly larger than the pollen of most wind-pollinated plants. The small size of *A. simplex* pollen may aid in its ability to travel farther on air currents; however, the outer surface of *A. simplex* pollen is coated with a pollenkitt which is usually found on animal-pollinated pollen grains (Pacini & Hesse, 2005).

We observed *A. simplex* pollen grains on native bees, suggesting that pollinators at least visit and likely pollinate *A. simplex* plants. Overall, *A. simplex* pollen made up a small portion of pollen loads (<1%) perhaps because many plants were blooming at that time. *Artemisia simplex* flowers bloomed at the peak of flowering on the high plains which created many foraging opportunities for generalist bees. Most pollinating insects in our study appeared to be generalists because we observed many pollen genera on each bee genus. In the western United States, ~65% of the ~3,000 bee species are generalists (Fowler, 2020), indicating that many bees do not have narrow floral preferences, and therefore visit a variety of flowers for pollen collection and nectar consumption. The composition of our pollen loads may also be attributed to combining up to 10 bee legs per genus. One bee of a specific genus may have focused on one genus of plant, while another bee may have preferred a different plant genus; however, the presence of pollen on bees suggests that they are likely dispersing pollen (Popic et al., 2013). Therefore, bees likely transported pollen of



*A. simplex* among plants. Results from our open treatment further suggest that insects pollinated *A. simplex* flowers and produced viable seeds. We did not find *A. simplex* pollen on all captured bee genera because pollen load composition also depends on pollinator traits and preferences (Cullen et al., 2021).

*Artemisia simplex* is at least partially wind-pollinated. *Artemisia simplex* produced some developed seeds in the mesh seed-set treatment that allowed for wind to carry pollen grains to blooms, but excluded pollinators. Sagebrush steppe is ideal for wind pollination because this mechanism only occurs in open environments that receive continuous winds, low humidity, and low precipitation (Culley et al., 2002). Our study sites were semi-arid and received average wind speeds of 6-9 m/s, likely providing ample opportunities for pollen to be dispersed by wind. Despite ideal environmental conditions for wind pollination, we think that pollen transported by insects is needed to produce the most seeds. We attribute the intermediate seed production we observed in the mesh treatment to the lack of insect pollinators visiting flowers. Hand-pollinated and open treatments that were pollinated by insects and wind produced the most seeds. Unfortunately, we cannot test the seed production of flowers when pollen from only wind was excluded, but we predict that the number of seeds produced would be intermediate to high based. Some plants use a combination of wind and animal pollination, and the degree in which a species uses wind pollination may range from 8% to 93% (Culley et al., 2002). *Artemisia simplex* may be similar to other sagebrush in that it exhibits some wind pollination (~29% of viable seeds produced), but different in that pollinators also pollinated the plant (~61% of viable seeds produced).

Animal pollination is more widespread than wind pollination (Ollerton et al., 2011), but evidence is accumulating that many plants are pollinated along a gradient between animal and wind pollination (Culley et al., 2002; Walker, 2020b). A shift from animal to wind pollination may be facilitated by complete pollinator loss, habitat degradation that limits pollinator access, and temporal misalignment between pollinator emergence and floral phenology (Culley et al., 2002; Walker, 2020b). In the sagebrush steppe where *A. simplex* grows, habitat loss, fragmentation, and degradation (Knick et al., 2011) may attribute to shifts in pollination mechanisms. When plants transition from animal to wind pollination to compensate for changing environments, the plants display both pollination mechanisms. We observed that *A. simplex* shows more animal than wind pollination, which may suggest that this plant is in a transitional period and is adapting to a changing habitat; however, we are unaware of how long plants may remain in transitional periods.

*Artemisia simplex* required pollen out-crossing. We observed that *A. simplex* produced the fewest seeds in the cloth seed-set treatment, suggesting that this plant is largely self-incompatible. Most plants employ inbreeding avoidance strategies (Walker, 2020a) and *A. simplex* is likely a poor self-pollinator because male flowers mature before female flowers (protandrous; Handley & Heidel, 2010), thus limiting its ability to deposit fresh pollen grains onto mature stigmas (Walker, 2020a). The chance of self-pollination may be further decreased because most members of Asteraceae present pollen using the plunger mechanism, a mechanism in which a tightly sealed, immature stigma pushes pollen grains up and out of the way as it grows. As a result, *A. simplex* requires pollen from an outside source to produce seeds and new individuals that sustain populations.

Around half of the seeds produced by *A. simplex* flowerheads were developed and viable. Under ambient (open treatment) and optimal conditions (hand-pollinated treatment), *A. simplex* was not



pollen-limited and produced numerous seeds. The mass of developed seeds of *A. simplex* did not differ among treatments suggesting a potential genetic component controlling mass; however, the number of viable seeds varied among treatments. Typically, one disk flower produced one seed, and *A. simplex* flowerheads had many disk flowers. We suspect that the proportion of developed seeds per flowerhead is normal for this species, as the number of developed seeds a plant produced was largely dependent on flower morphology associated with a species (Lande et al., 2017). The development of seeds may further be affected by pollination success (Etcheverry et al., 2008; Haig & Westoby, 1988) and environment limitations such nutrients, temperature (Young et al., 2004), and water availability (Pol et al., 2010); therefore, *A. simplex* may have produced fewer viable seeds in 2021 than in 2020 because of decreased precipitation.

*Artemisia simplex* can be locally abundant within its range. Rare plants that have small geographical ranges typically have higher densities of individuals than plants with large geographical ranges (Lesica et al., 2006); however, more data is needed to estimate the density of *A. simplex* populations. In 2009, *A. simplex* was estimated to cover 2000 acres of land and consisted of 1.6 million plants in seven populations; however, these numbers were based on abundance estimations. Our monitoring methods may accurately track how *A. simplex* populations fluctuate over time at monitoring transects. We monitored *A. simplex* coverage instead of abundance because *A. simplex* grows in dense mats; therefore, it is difficult to distinguish one large plant from many small plants growing close together. Overall, *A. simplex* presence did not vary from 2020 to 2021 indicating that our method collects consistent results. The cover of *A. simplex* likely varied among sites because of different growing conditions, seed dispersal success, herbivory, precipitation, climate, or soil characteristics.

Understanding pollination mechanisms, flower morphology, and seed production is critical for protecting rare and endemic plants. These species contribute to ecosystem services (Dee et al., 2019), and conserving rare and endemic plants preserves biodiversity (Dee et al., 2017). Understanding the mechanisms by which plants are pollinated is essential to manage species, because the perpetuation of a species depends on this process. Without pollination, a plant cannot produce fertilize seeds, make fruit, disperse seeds or maintain genetic variation within a population (Walker, 2020a). Pollination also contributes to understanding plant adaptations and evolution, as well as species interactions (Armstrong, 1979). We demonstrated that assuming a plant is pollinated the same as other members of the genus can be misleading and we urge others to investigate the pollination of rare and endemic plants. The widespread decline of pollinating insects makes this need even more urgent (Cameron et al., 2011; Potts et al., 2010). We feel that measuring seed production is critical to understanding what may limit plant populations. For example, we measured that seed production was severely limited in *Yermo xanthocephalus* (desert yellowhead), a rare and endemic plant in central Wyoming, but this was likely due to climate instead of pollinators (Handley & Tronstad, 2021a). Conversely, a rare and endemic early blooming plant, *Trifolium barnebyi* (Barneby's clover), in central Wyoming was limited by pollinators at the beginning of the blooming period but not for individuals that bloomed later (Handley & Tronstad, 2021b). Measuring seed production and pollen carried on pollinators can identify limiting factors for plants to inform management decisions.

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## Tables

Table 1. The number of bee genera captured in 2020 and 2021 within *Artemisia simplex* populations. The presence of *A. simplex* pollen on bee genera is indicated in the pollen column.

Order	Family	Genus	Number	Pollen
Hymenoptera	Halictidae	<i>Agapostemon</i>	1019	Yes
Hymenoptera	Andrenidae	<i>Andrena</i>	92	Yes
Hymenoptera	Megachilidae	<i>Anthidium</i>	3	
Hymenoptera	Apidae	<i>Anthophora</i>	399	Yes
Hymenoptera	Apidae	<i>Bombus</i>	182	Yes
Hymenoptera	Apidae	<i>Ceratina</i>	1	
Hymenoptera	Colletidae	<i>Colletes</i>	7	
Hymenoptera	Apidae	<i>Diadasia</i>	1	
Hymenoptera	Apidae	<i>Eucera</i>	388	Yes
Hymenoptera	Apidae	<i>Habropoda</i>		
Hymenoptera	Halictidae	<i>Halictus</i>	17	Yes
Hymenoptera	Megachilidae	<i>Hoplitis</i>	26	
Hymenoptera	Halictidae	<i>Lasioglossum</i>	420	Yes
Hymenoptera	Megachilidae	<i>Megachile</i>	2	
Hymenoptera	Apidae	<i>Melecta</i>	28	Yes
Hymenoptera	Apidae	<i>Melissodes</i>	15	
Hymenoptera	Apidae	<i>Nomada</i>	1	
Hymenoptera	Megachilidae	<i>Osmia</i>	151	Yes
Hymenoptera	Halictidae	<i>Sphecodes</i>	23	
Hymenoptera	Megachilidae	<i>Stelis</i>	1	
Hymenoptera	Apidae	<i>Svastra</i>	1	
Hymenoptera	Apidae	<i>Xeromelecta</i>	2	

Table 2. A comparison of animal and wind pollination characteristics for the rare and endemic plant, *Artemisia simplex*.

Trait	Animal-pollinated plants	Wind-pollinated plants	<i>Artemisia simplex</i>	Animal or wind characteristic?
Perianth	Showy	Reduced	Showy	Animal
Filament length	Varies	Long	Does not exceed disk	Animal
Stigma	Simple	Feathered	Simple	Animal
Pollen grain structure	Elaborate	Smooth	Rough	Animal
Pollen grain size	Varies	10 – 50 microns	7 – 9 microns	Both
Pollenkitt	Present	Absent	Present	Animal



## Figures

Figure 1: a) *Artemisia simplex* only grows in southeastern Wyoming and we performed field work in the foothills of the Laramie Range. b) We worked at 6 sites in 2020 (all sites) and 4 sites in 2021 (South Laramie, Sybille, Dodge, and Sevenmile).

Figure 2: a) We collected pollinating insects in vane traps to examine pollen loads. b) We measured seed production at 5 populations in 2020 (South Laramie, Sybille, Dodge, Sevenmile, and Booteel) and 3 populations in 2021 (South Laramie, Sybille, and Sevenmile).

Figure 3: *Artemisia simplex* presence did not differ between 2020 to 2021, suggesting that our monitoring method collected consistent results.

Figure 4: The a) percentage of developed seeds, b) the percent of viable seeds, and c) the mass of seeds in 2020 and 2021 produced by *A. simplex*. The black line is the median, the top and bottom of the box are the 75<sup>th</sup> and 25<sup>th</sup> percentiles, and the whiskers represent the minimum and maximum values excluding outliers. Seed-set treatments measured seed production in the open (ambient pollination), hand-pollinated (excess pollen), mesh (excluded insect pollinators), and cloth (excluded wind and animal pollination) treatments.

Figure 5: a) *Artemisia simplex* pollen grain shown at 400x magnification. In 2020, b) 9 bee genera carried *A. simplex* pollen and c) pollen was most abundant when *A. simplex* flowers were fully mature.

Fig. 1

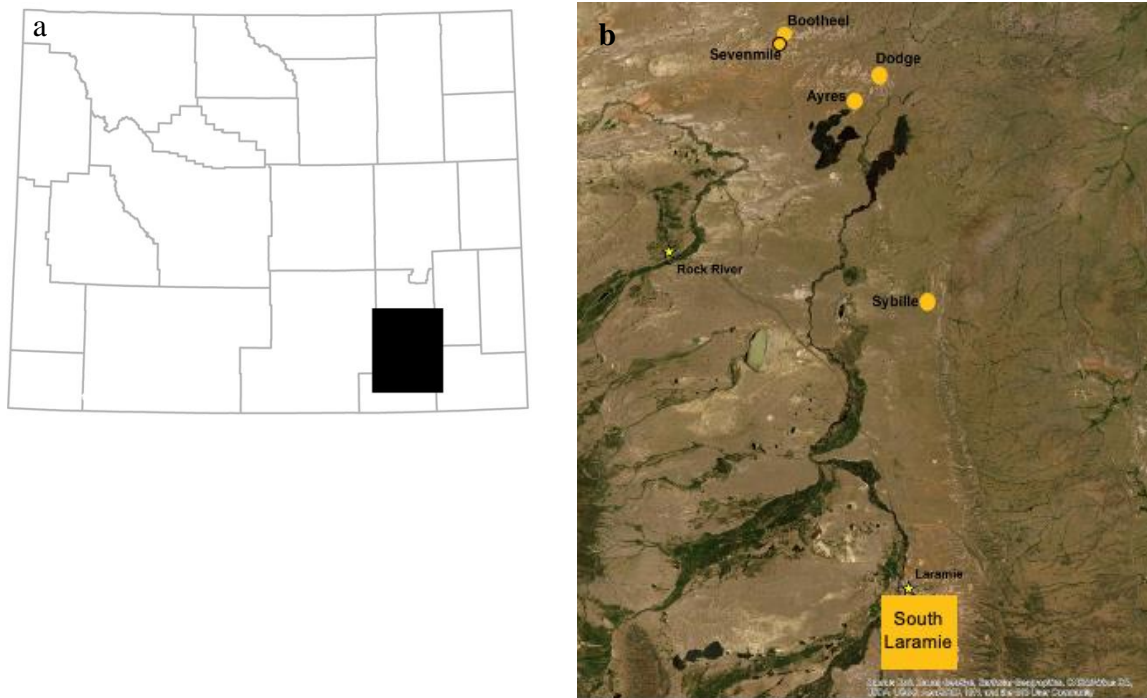


Fig. 2

a



b



Fig. 3

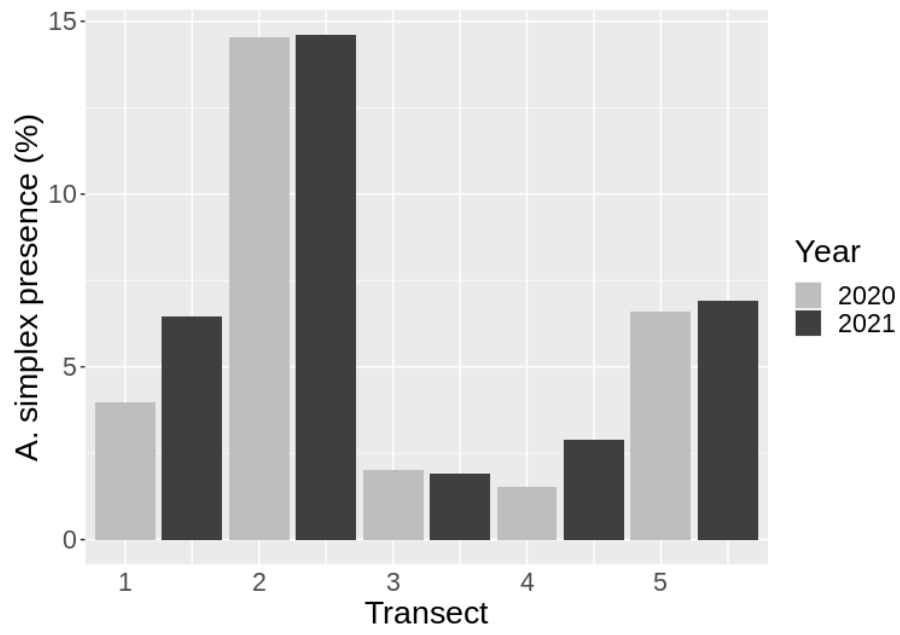
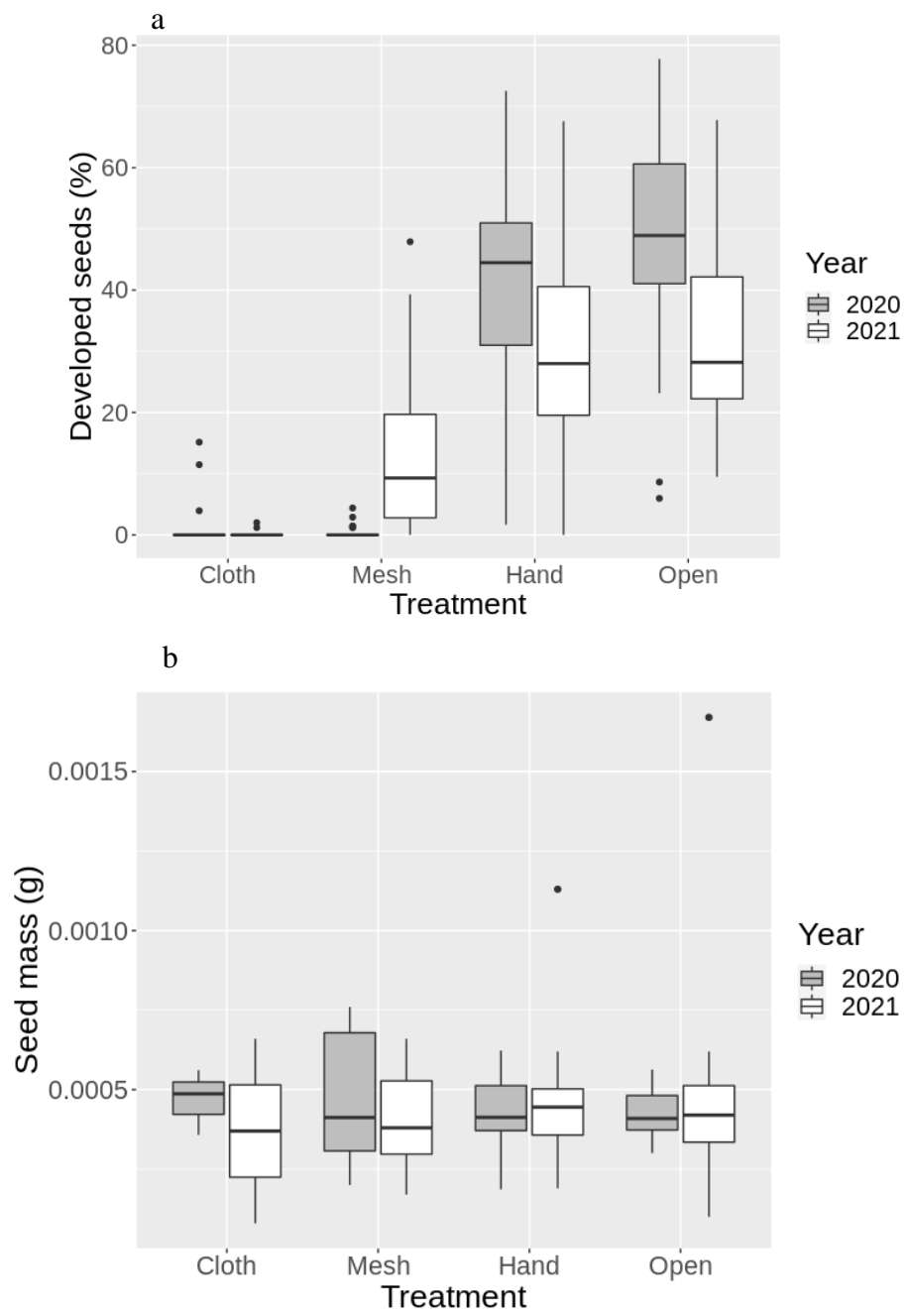


Fig. 4



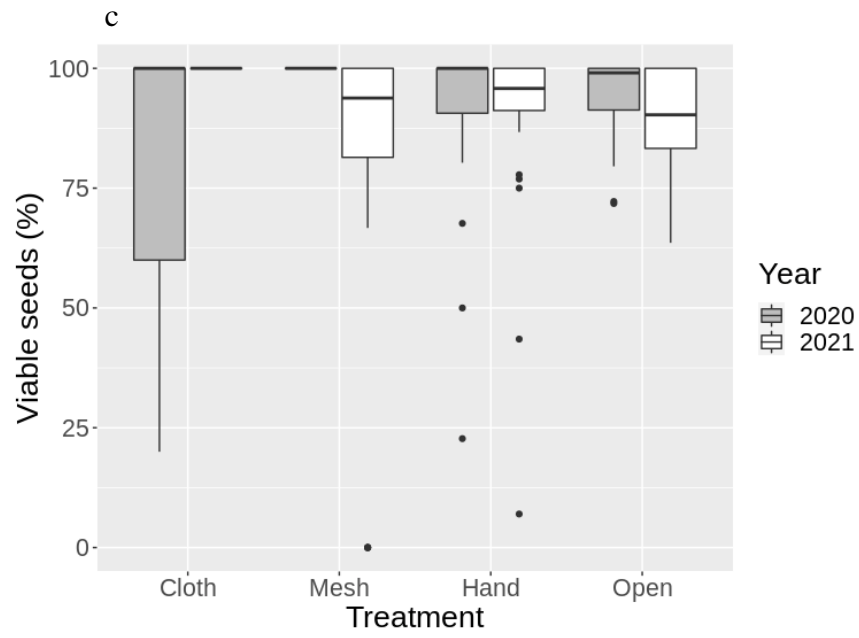


Fig. 5

