

**Low seed viability in a rare plant, Desert yellowhead
(*Yermo xanthocephalus*), in central Wyoming**



Joy Handley and Lusha Tronstad

Wyoming Natural Diversity Database, University of Wyoming, Laramie, Wyoming

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ABSTRACT

The reproductive ecology of rare plants is often unknown, yet the persistence of most plant populations depend on successful mutualisms with pollinators and favorable environmental conditions. Sexual reproduction maintains genetic diversity within plant populations using pollinators to transport pollen grains from other plants and producing seeds. We investigated the reproduction of Desert yellowhead, a perennial endemic plant in central Wyoming that grows in two locations within 8 km of each other. Our objectives were to evaluate a method for monitoring changes in the populations as well as assess the pollination and seed-set of Desert yellowhead. We inventoried four monitoring locations, partially replicating past work to evaluate trends along each transect. We conducted seed-set experiments in both populations to measure self-pollination and estimate if pollinating insects limited seed production. We used vane traps and bee bowls to capture pollinators, and we examined pollen carried on bees. The number of plants along transects declined since 2013. Desert yellowhead can self-pollinate, but seed viability was extremely low. The number of seeds produced in flowers pollinated by insects did not differ from hand-pollinated blooms with excess pollen indicating that pollinators did not limit seed production in 2018. Pollen from Desert yellowhead was carried by nine bee genera, suggesting that no one specific insect pollinates this plant. Only 12% of seeds were viable in the main population and 0% were viable in the other population suggesting that something beyond pollinators limit seed-set (e.g., precipitation). We recommend regular monitoring and periodic censuses, as well as continued seed-set research to address what is limiting seed production to advance the knowledge and management of this declining plant population.

INTRODUCTION

Rare plant species provide vital services in the habitats and ecosystem in which they live (Xu et al. 2020), but little is known about these unique species. Rare plants can be the dominant species in the habitats where they occur and other rare plants may have lower densities (Rabinowitz 1981). Regardless of their density, rare species often have a distinct combination of traits and are more likely to support the more vulnerable ecological functions in their communities (Mouillot et al. 2013; Leitão et al. 2016). Therefore, rare species not only increase biodiversity, they also increase the diversity of ecosystem functions (Mouillot et al. 2013; Leitão et al. 2016). Despite their critical roles, little is typically known about the ecology of rare plant species (Burmeier and Jensen 2008; Miller et al. 2019) leaving managers with little information upon which to base decisions. Investigating vital life history characteristics of rare plants, such as their life cycle, reproduction, pollination and seed production, are critical pieces of information about their ecology and essential for making informed management decisions (USDI 2001, 2010).

Knowledge of a plant's reproduction is critical to understand a species current trajectory and their ability to persist (Willemse 2009). Plants can reproduce through two main ways, vegetative and sexual reproduction. Vegetative reproduction occurs by producing ramets, which are genetically identical to the parent plant through rhizomes or similar structures. Sexual reproduction occurs through seed production where pollen is usually transferred from another plant resulting in increased genetic diversity (Jabis et al. 2011). Plants that can reproduce both vegetatively and sexually may be more likely to be self-incompatible (pollen and ovule from same plant cannot produce seeds) to maintain genetic diversity (Aigner 2004). For example, Aigner (2004) found that clonal diversity within 10 m² had a strong positive relationship to seed-set in Beach spectacle pod (*Dithyrea maritima*), a rhizomatous member of Brassicaceae (Mustard family) endemic to the coastal sand dunes in southern California. Similarly, Wolf et al. (2000) also found a strong relationship between seed-set and the availability of pollen from a genetically different individuals in Coast Range false bindweed (*Calystegia collina*), a clonal member of Convolvulaceae (Morning-glory family) endemic to serpentine outcrops in northern California, indicating the pollen from nearby vegetatively produced clones is incompatible with the neighboring genetically identical plants. Nearly 88% of plants globally rely on animals to transport pollen among plants to maintain genetic diversity (Ollerton et al. 2011). Most insects visit flowers to drink the nectar and pollen sticks to their bodies while they drink. In addition to drinking nectar, bees actively collect pollen to feed their young making bees the most prolific pollinators in most ecosystems (Larson et al. 2018). When we observe a bee visiting a flower, that bee may be getting a drink, collecting pollen or both. The pollen loads on bees must be examined to understand which plants bees actively collect pollen at (Popic et al. 2013). Examining the pollen on pollinators collected in the vicinity of a specific plant is a valuable method of estimating what insects transport their pollen.

Pollinators, especially bees and butterflies, are declining precipitously throughout the world (Abrol 2012; Wagner 2020; Potts et al. 2010; Goulson and Nicholls 2016; Rhodes 2018) and their decline will likely decrease the production of viable seeds resulting from pollen transported by insects (Cameron et al. 2011; Potts et al. 2010). Additionally, emerging pollinators may not coincide with flowers blooming as spring advances due to warming temperatures which may further exacerbate pollination. Models predict drier conditions in most areas which may hamper plant reproduction and blooming. Fewer pollinators alone could translate to less genetic diversity in plant populations and plants may produce fewer viable seeds (Aigner 2004; Ramos-Jiliberto et al. 2020). Less genetic variation reduces biological fitness to confront changes in the environment (Agrawal 2001). Rare plants may be especially vulnerable to declining pollinators, because many rare species use specialist pollinators (Rasmussen et al. 2020). For example, Barneby's clover (*Trifolium barnebyi*) heavily relied on the uncommon mining bee (*Andrena*) for pollination (Handley and Tronstad 2021). Fewer insect pollinators have been connected to the decline of several rare plants (Biesmeijer et al. 2006).

We studied the reproduction of a Threatened plant to estimate the degree to which seed production was limited by pollinators. Desert yellowhead (*Yermo xanthocephalus*) is endemic to central Wyoming and a member of Asteraceae (Daisy family) that lives in soil developed from volcanic sandstone, tuff and conglomerate of the White River Formation (Van Houten 1964; Scott and Scott 2009; Heidel et al. 2011). Desert yellowhead is only known from a small area and blooms in early June in sparsely vegetated sagebrush (*Artemisia tridentata*), bunchgrass and cushion plant communities. Information about the reproductive ecology of Desert yellowhead is sorely lacking. We evaluated using transects to monitor population trends. We also measured seed-set in three treatments and assessed what pollinators transferred pollen grains. Our specific questions were: 1) Does a low-intensity monitoring method detect trends in Desert yellowhead populations over time? 2) Can Desert yellowhead self-pollinate? 3) What proportion of seeds produced by Desert yellowhead are viable and to what degree is seed production limited by pollinators? and 4) What pollinators carry the pollen from Desert yellowhead? Results will provide information to managers about what may limit the reproduction of this rare plant.

METHODS

Study Area and Background Information

Desert yellowhead is listed as Threatened under the US Endangered Species Act (USDI Fish and Wildlife Service 2002). This unique plant was discovered by Robert Dorn in 1990 at Sand Draw (Dorn 1991) and a second population, Cedar Rim, was found ~8 km downwind in 2010 (Heidel et al. 2011). These are the only known populations of Desert yellowhead in the world. The Sand Draw population is much larger (9,300-13,250 plants based on extensive grid censuses between 1995-2004; Scott and Scott 2009) than the Cedar Rim population (~750 plants in 2016; Freeland, personal communication). Both populations of Desert yellowhead occur on Beaver Rim in Fremont County, Wyoming (Figure 1). Desert yellowhead grows in soils derived from the White River Formation north of the Sweetwater River. All known Desert yellowhead populations occur on lands managed by the Bureau of Land Management (BLM) Lander Field Office. Most Desert yellowhead grow in sparsely vegetated sagebrush steppe and cushion plant communities (Fertig 1995, Scott and Scott 2009, Heidel et al. 2011). The area receives an average of 25 cm of precipitation annually (Jeffrey City weather station; Western Regional Climate Center 2021).

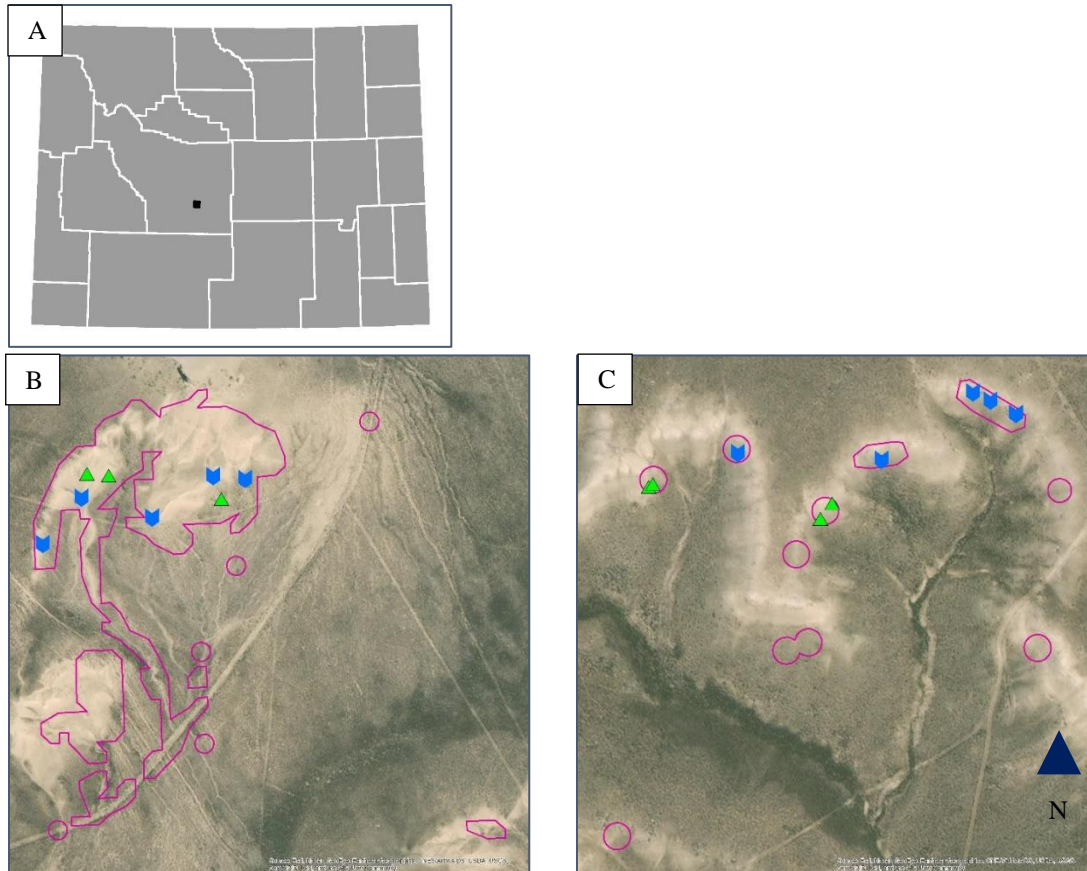


Figure 1. A.) Desert yellowhead occurs in central Wyoming. The location of monitoring transects (green triangles) and pollinator sampling stations (blue chevrons) in the Desert yellowhead populations (pink outlines) at B.) Sand Draw (ca 0.5 km east to west) and Cedar Rim (ca 0.35 km east to west) (ESRI 2011).

Monitoring

The BLM and US Fish and Wildlife Service (USFWS) are discussing procedures to continue monitoring Desert yellowhead populations using quantitative methods that can be done annually. Desert yellowhead has been monitored using two methods in the past making comparisons difficult. The first method censused all Desert yellowhead plants at Sand Draw and is labor and time intensive (~15 people days). Scott and Scott (2009) established this method and censused the Sand Draw population using a grid design from 1995 through 2004 (Figure 2). They set up permanent grids that are five (north to south) by six (east to west) hectares in the entire known population. The grid census in the primary population was repeated in 2013 as part of a population viability analysis (Dibner et al. 2019; Doak et al. 2016).

4W	4A	4B	4C	4D	4E
5W	5A	5B	5C	5D	5E
6W	6A	6B	6C	6D	6E
7W	7A	7B	7C	7D	7E
8W	8A	8B	8C	8D	8E

Figure 2. Sampling grid in the Sand Draw population established by Scott and Scott (2009). Occupied hectares in 1995 are yellow; additional hectares occupied in 2004 are light yellow; hectares never occupied by *Yermo* plants during the study are gold.

The second method monitored Desert yellowhead along transects in both populations between 2010 through 2013 and Sand Draw in 2014. This method was established by Dibner et al. (2019) and Doak et al. (2016) and is much less labor intensive because the entire population was not censused. The transects were one to two meters wide depending on the location and 2 to 25 meters long. They counted plants along 11 transects at Sand Draw and five transects at Cedar Rim (Doak et al. 2016).

We used a subset of Dibner et al. (2019) and Doak et al. (2016) transects to assess their usefulness in future monitoring. We counted the number of plants along two monitoring transects in both populations during 2018, 2019 and 2021 to assess the long-term trends in Desert yellowhead. We monitored when the plants were in bloom in early to mid-July. In the Sand Draw population, we monitored transect 2b-2c (25 meters long by two meters wide) and Ja-Jb (5 meters long by two meters wide; a new transect in a dry snowfield runoff with many plants). At Cedar Rim, we monitored transect 4a-4b (6 meters long by 2 meters wide), and 5a-5b (3 meters long by 1 meter wide; Dibner et al. 2019; Doak et al. 2016; Dibner personal communication). One person can inventory all four transects in a single day. Each transect consisted of a belt between 3 and 25 m in length where a 1 m² plot frame was placed on the ground along the belt to assess cover (Figure 3a). The frame was placed each meter along the belt transect so the total width of the transect was one or two meters below the belt (Dibner et al. 2019; Doak et al. 2016). We placed the frame on both sides of the belt at Sand Draw Ja-Jb. A photograph was taken of each plot frame along the belt, and we counted the number of vegetative and reproductive plants in each frame.

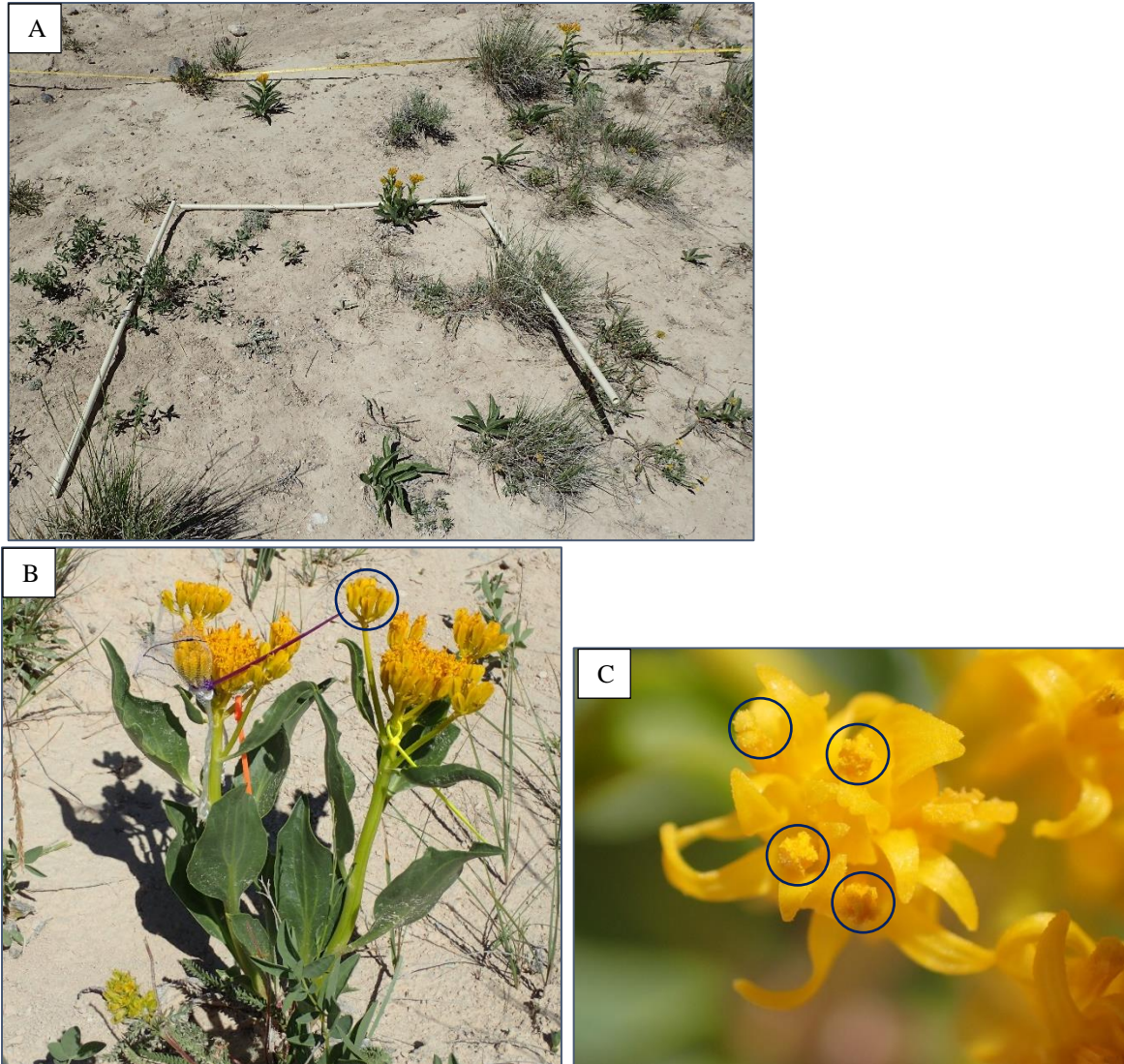


Figure 3. A) Monitoring plot with one reproductive plant in the upper right and two vegetative plants in the lower right. B) A Desert yellowhead plant with a mesh bag over the bagged treatment, and the hand-pollinated and open treatments marked with yellow and orange zip ties, respectively. Circled is one flowering branch. C) Close up of a Desert yellowhead flowerhead with four florets (circles).

Seed-set Experiments

We measured seed-set of bagged, open, and hand-pollinated flowerheads to estimate the relative importance of self- and cross-pollination. We selected 14 Desert yellowhead plants at the Cedar Rim population and 20 plants at the Sand Draw population to estimate the degree to which these plants were pollen-limited. Plants were selected before flowers bloomed on 12 and 13 June 2018. Three flower branches were selected on each plant to receive one of each treatment. Bagged treatments restricted pollinator access and measured the degree to which flowers can self-pollinate. Open treatments left blooms open for local pollinators to visit and measured the

number of seeds produced under ambient conditions. The hand-pollinated treatment added excess pollen in addition to local pollinators to measure seed production when pollen was not limiting. Pollen came from plants >50 m away and we delicately brushed collected anthers on the stigma of the treatment bloom. Pollen from Sand Draw was used in both populations due to few blooming flowers at Cedar Rim. Blooms were bagged with mesh bags before (bagged treatment) or after (open and hand-pollinated treatments) flowers bloomed to contain the developing seeds. We recorded the number of flowerheads pollinated and marked the flowerheads not ready for pollination in the hand-pollinated treatment so we only analyzed flowers that received excess pollen. We monitored treatments and collected fruits when flowerheads were ripe, 15 July through 4 September 2018. Flowerheads were placed in paper bags, returned to the laboratory and dried.

Each Desert yellowhead flowerhead had one to seven florets, which each had the potential to produce one seed (Figure 3c), and there were multiple flowerheads per flowering branch (Figure 3b). We cleaned, counted, and weighed all the seeds to estimate the degree to which Desert yellowhead self-pollinated or depended on pollinators. We counted the number of flowerheads per flowering branch and the number of seeds per flowerhead. We noted seeds that appeared viable (size and mass) and we used Tetrazolium staining to test for viability. Tetrazolium measures the germinative potential of seeds by turning red to indicate cellular respiration (Lindenbein 1964). We placed the seeds between moistened paper towels for 24 hours, cut them to expose the endosperm, and immersed them in tetrazolium solution for 24 hours. The endosperm of viable seeds turned pink or red and the endosperm of non-viable seeds remained white.

Pollinators

We collected pollinators at the same sites as the seed-set experiments to estimate which insects pollinated this Threatened plant. Pollinator stations were used to estimate the abundance and diversity of pollinators within the Desert yellowhead populations, and to assess which pollinators collect Desert yellowhead pollen. We deployed five pollinator stations in each population for 24-48 hours eight times between 12 June and 6 July 2018, and four times between 19 June and 17 July 2019. Pollinator stations consisted of one blue vane trap (vane trap hereafter) and three bee bowls (yellow, blue and white) filled with soapy water (Figure 4a). We recorded the location, dates and times we deployed and retrieved stations and only collected pollinators during fair weather. Pollinators collected with vane traps were used to assess pollen, because these traps were dry and minimally altered pollen loads. We also netted pollinators on Desert yellowhead blooms (Figure 4b). We removed the portion of the bee's body with scopa that carried pollen which was typically a hind leg except Megachilidae who carry pollen on the underside of the abdomen. We performed acetolysis on the removed portion to strip pollen grains of lipids, proteins, and carbohydrates, making pollen easier to identify, and stained the pollen with Safranin O (Jones 2014). Resulting pollen was slide mounted and identified under a compound

microscope at 200x. Pollen was compared to a library made from plant pollen collected in the area. Pollen can generally be identified to genus. We counted the number of pollen grains of each plant taxa on individual bees to estimate pollen loads and which species collected pollen from Desert yellowhead (Figure 4c).

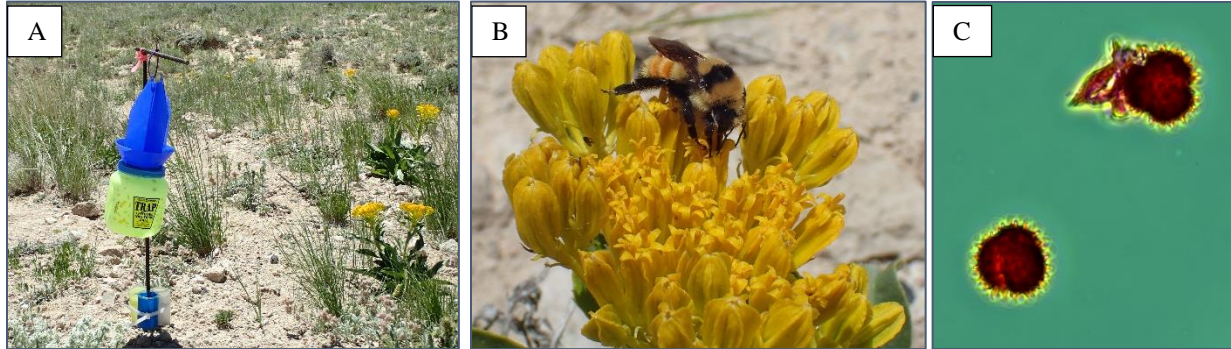


Figure 4. A.) We collected pollinators using pollinator sampling stations with vane traps (top) and bee bowls (bottom). B.) *Bombus huntii* visiting a Desert yellowhead bloom and a C.) stained Desert yellowhead pollen grains.

Analyses

We estimate how precipitation, air temperature, and the number, mass and viability of seeds differed through time or between populations to address our questions. Precipitation, air temperatures and the number of plants in a transect were autocorrelated through time (Durbin-Watson Test), so we used the Prais-Winsten timeseries analysis (Mohr 2019). We estimated how precipitation and air temperature varied among years and months. We estimated how the number of plants along each transect varied through time and we investigated if spring precipitation or mean spring air temperature explain any trends in the number of plants. We measured how seed characteristics differed between populations and among treatments using general linear models (glm). The number of seeds meet statistical assumptions and we analyzed using a normal distribution. Seed mass and viability had non-constant variance and transforming the mass and viability of seeds did not improve the fit, so we used the non-parametric Kruskal-Wallis test. We used the Wilcox test to assess differences among treatments. We also used glm to assess differences in pollinator population between sites. Data analysis occurred in Program R (R Core Team 2017) and we used the plyr package (Wickham 2011) to sort and compute data.

RESULTS

Weather

Precipitation did not vary among years (prais, $t = 0.27$, $p = 0.78$), but the amount that fell varied among some months. April, May, June and October received more precipitation than most other months (prais, $t > 2.6$, $p < 0.001$). When we considered only spring precipitation (April – June), May received the most moisture (prais, $t = 5.0$, $p < 0.0001$) but spring precipitation did not vary

among years (prais, $t = 0.4$, $p = 0.69$; Figure 5a). Mean temperature varied among years (prais, $t = 2.8$, $p = 0.006$), and mean temperature differed among most months (prais, $t > 8.5$, $p < 0.001$). Spring mean temperatures varied among years (prais, $t = 2.8$, $p = 0.006$; Figure 5b) and among months with June having the highest temperatures (prais, $t > 9.7$, $p < 0.0001$).

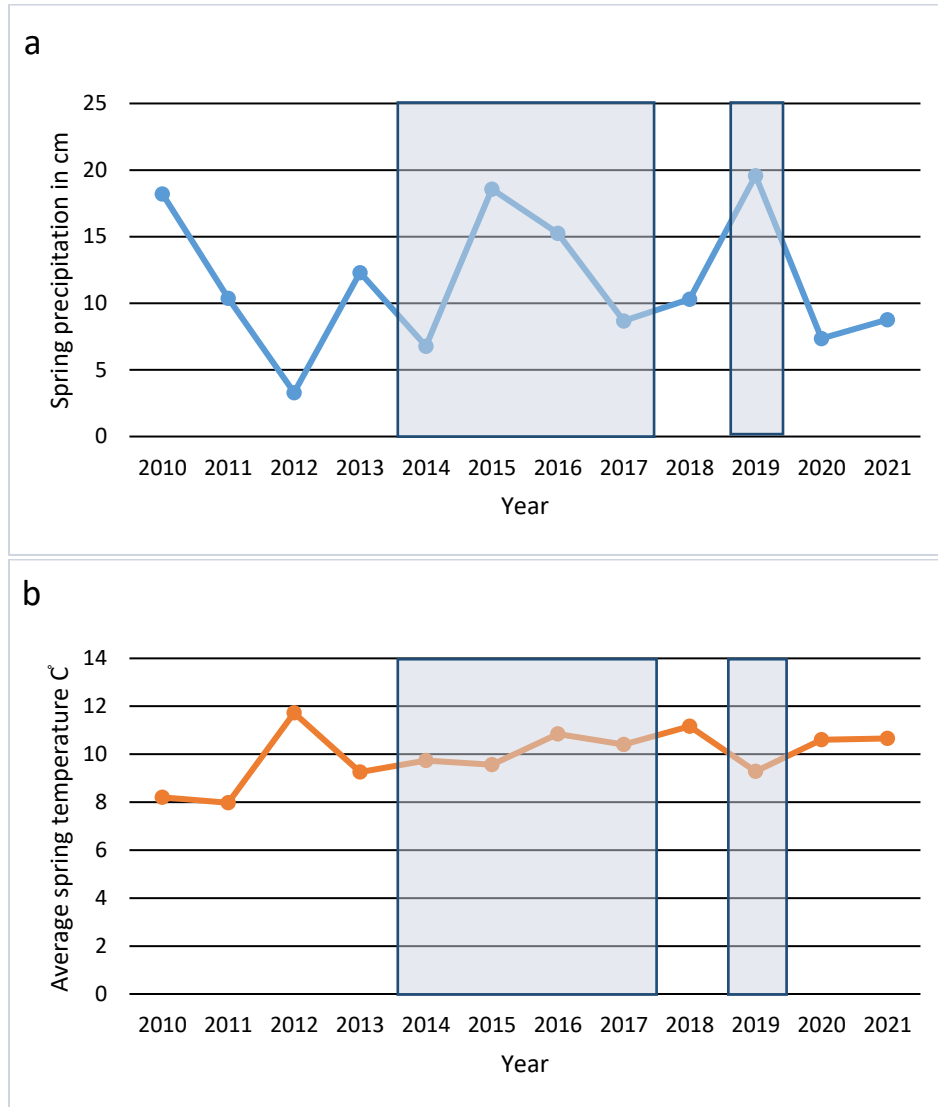


Figure 5. A.) Total precipitation (cm) at the Jeffrey City weather station during spring (April-June) from 2010 to 2021. B.) Average temperature at the Jeffrey City weather station during spring between 2010 and 2021. Boxed areas are years without monitoring.

Monitoring

The number of plants observed varied among years (prais, $t = 9.1$, $p < 0.0001$) and transect (prais, $t > 2.3$, $p < 0.03$). The number of plants decreased through time (prais, slope = -3.2). Cedar Rim transect 5a-5b had the fewest plants (prais, $t = 9.2$, $p < 0.0001$) and Sand Draw 2b-2c had the most plants (prais, $t = 39$, $p < 0.0001$). Transect 2b-2c at Sand Draw had fewer plants

between 2018 and 2021 compared to previous estimates. The new transect at Sand Draw, Ja-Jb, declined from 2018 to 2019 and increased by 10 plants in 2021. Both transects in the Cedar Rim population had fewer plants than any of the years prior to 2018, with the exception of 5a-5b in 2013 (Figure 7; Appendix 1). Neither spring precipitation (prais, $t = 0.55$, $p = 0.58$; Figure 8a) nor spring mean temperature (prais, $t = 1.5$, $p = 0.13$; Figure 8b) significantly affected the number of plants along transects. If the trend continues, spring mean temperature may have a significant negative relationship with the number of plants over time.

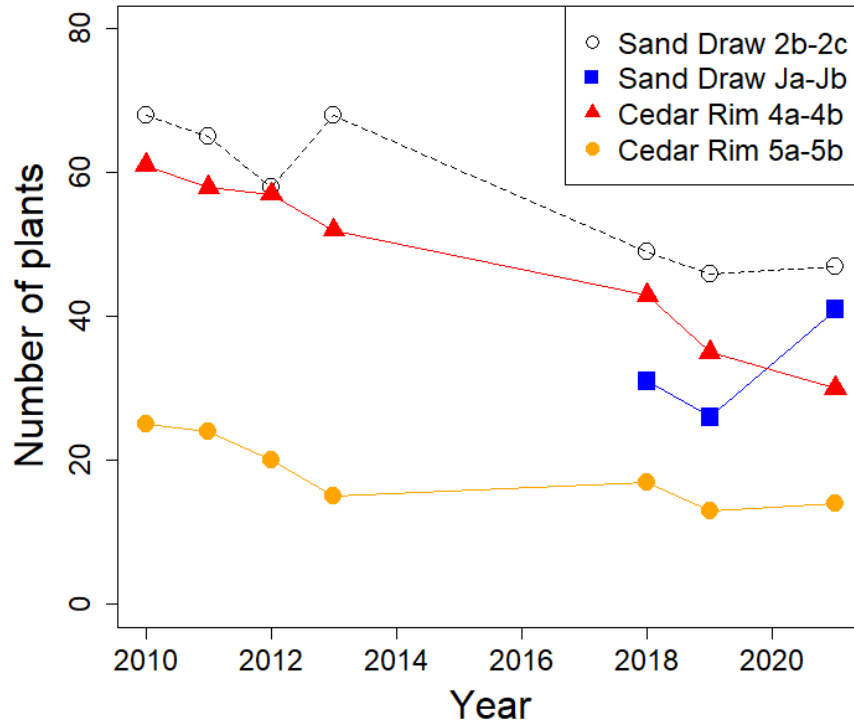


Figure 7. Number of Desert yellowhead plants counted in transects in 2010 to 2013 and 2018 to 2021. Data collected before 2018 were from Doak et al. (2016) and Dibner et al. (2019). We established Sand Draw Ja-Jb in 2018.

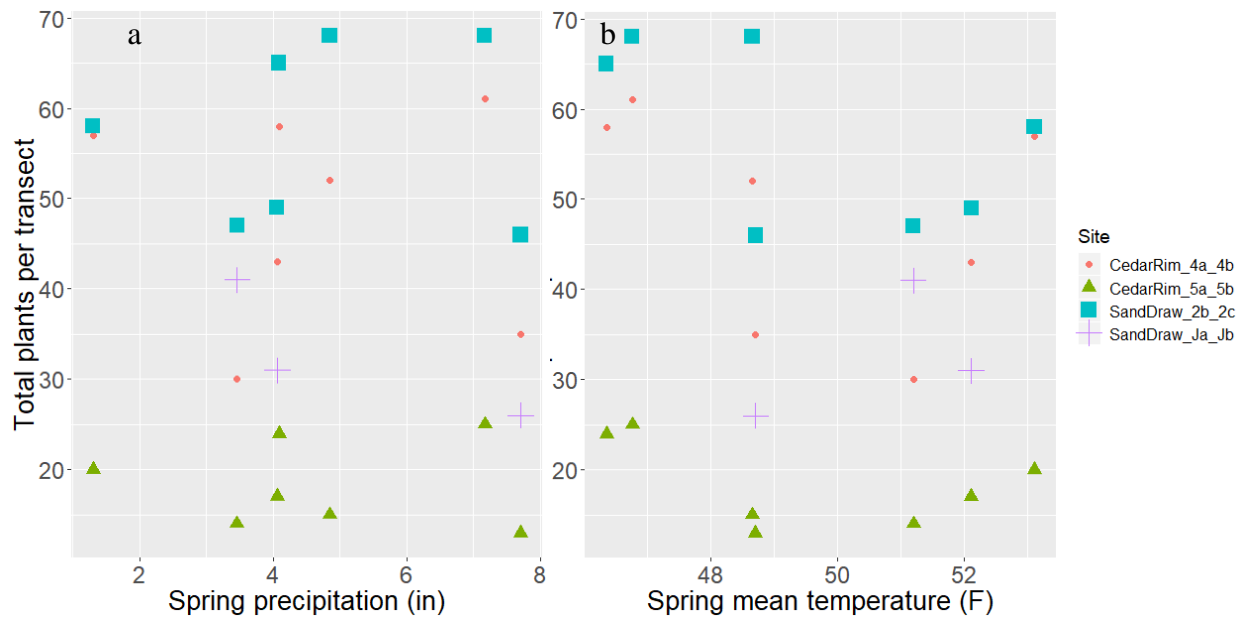


Figure 8. The number of Desert yellowhead plants in transects among years did not vary with a.) spring precipitation or b.) mean spring temperatures. We used past data from Doak et al. (2016) and Dibner et al. (2019) and new data we measured in our current study.

Seed-set Experiments

Desert yellowhead produced nearly 5 seeds per flowerhead on average (range 2-7) and the number of seeds per flowerhead did not differ between populations (glm, $t = 1.3$, $p = 0.21$) or among treatments ($t < 1.1$, $p > 0.26$). Seed mass averaged 2.4 mg and ranged between 0.05 (non-viable) and 7.8 mg (viable). Seed mass was higher at Sand Draw than Cedar Rim (Kruskal-Wallis, $X^2 = 48$, $df = 1$, $p < 0.0001$; Figure 10). Seed mass did not vary among treatments at Cedar Rim ($X^2 = 0.22$, $df = 2$, $p = 0.90$), but seed mass was lower in the bagged treatment at Sand Draw compared to the hand-pollinated and open treatments ($X^2 = 10$, $df = 2$, $p = 0.006$; Pairwise Wilcox Test, $p < 0.03$) indicating that Desert yellowhead produced few seeds through self-pollination. The Cedar Rim population produced zero viable seeds and the Sand Draw population produced 12% viable seeds ($X^2 = 4.5$, $df = 1$, $p = 0.03$). At Sand Draw, only 6% of seeds in the bagged treatment were viable compared to 15% in the hand-pollinated and open treatments ($X^2 = 8.3$, $df = 2$, $p = 0.016$; Pairwise Wilcox Test, $p < 0.10$).

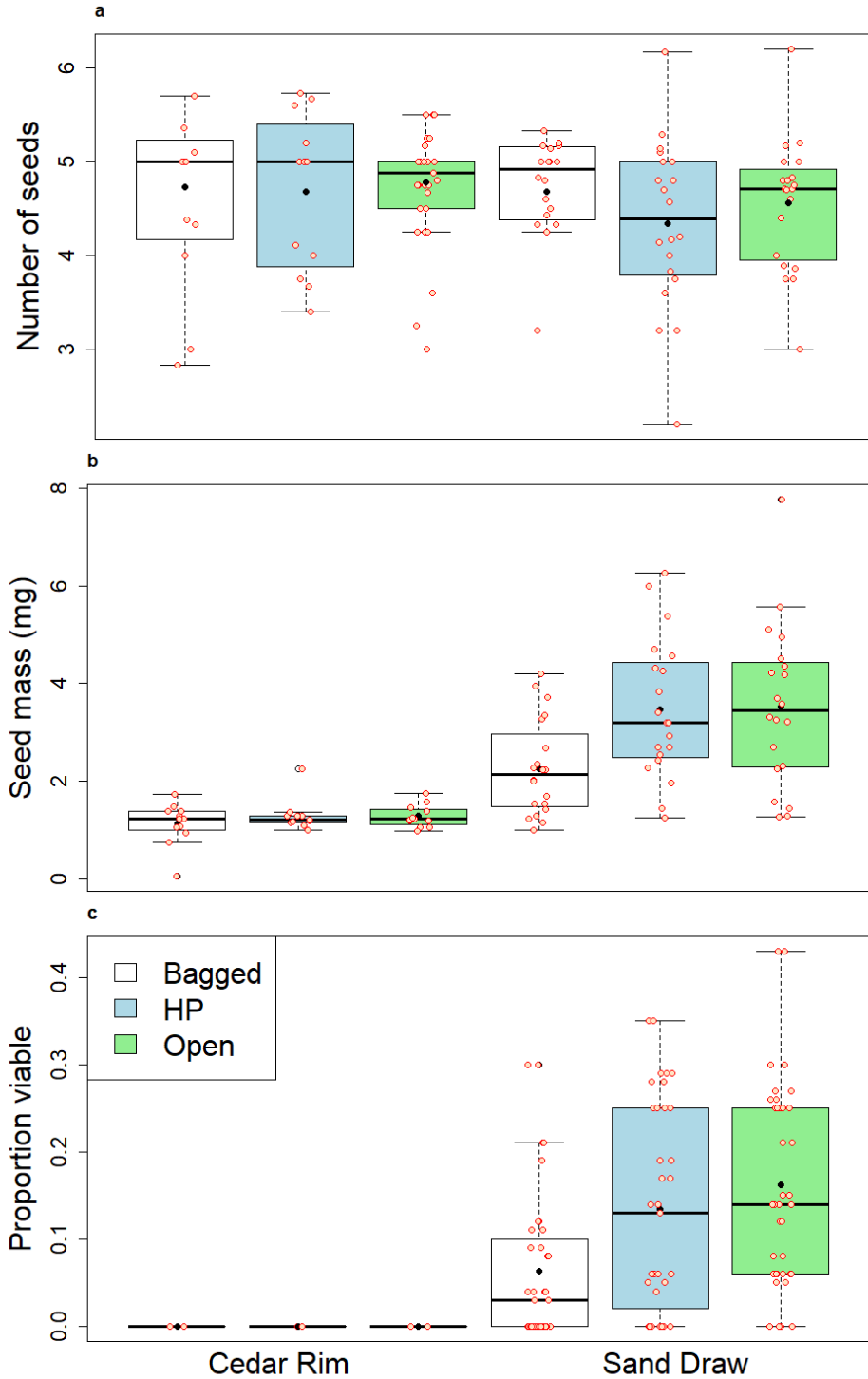


Figure 10. A.) The number, B.) mass and C.) viability of seeds produced by Desert yellowhead in the Cedar Rim and Sand Draw populations in three treatments. The bagged treatment tested the plant's ability to self-pollinate, the hand-pollinated (HP) treatment measured seed production with excess pollen and the open treatment estimated seed production under ambient conditions. The bold line is the median, the black circle is the mean, the lower and upper limits of the box is the 25th and 75th percentile, whiskers are the upper and lower limits of the data excluding outliers and the open circles are data points.

Pollinators

We captured 5 orders of insects including 22 genera of bees and 6 genera of butterflies when Desert yellowhead was blooming (Table 3). The bee genera *Agapostemon* and *Lasioglossum* were the most abundant followed by *Ceratina*, *Osmia* and *Halictus*. We captured 32% more bees at Sand Draw than Cedar Rim (glm, $t = 2.2$, $p = 0.03$), but the number of bees captured did not differ between traps (glm, $t < 1.5$, $p > 0.15$). We captured 43% more bee genera at Sand Draw compared to Cedar Rim (glm, $t = 2.0$, $p = 0.046$), and we caught near twice as many genera in vane traps than bee bowls (glm, $t = 2.4$, $p = 0.02$).

Table 3. We captured 5 order of insects when Desert yellowhead was blooming including 22 genera of bees and 6 genera of butterflies.

Order	Common name	Genus	Subgenus	Species	Number	Pollen
Coleoptera	Beetle	<i>Coccinellidae</i>			4	
Coleoptera	Beetle				990	
Diptera	Fly				264	
Hemiptera	True bug				35	
Hymenoptera	Ant				27	
Hymenoptera	Bee	<i>Agapostemon</i>		<i>angelicus/texanus</i>	281	Yes
Hymenoptera	Bee	<i>Agapostemon</i>		<i>sericeus/obliquus/femoratus</i>	58	Yes
Hymenoptera	Bee	<i>Agapostemon</i>		<i>virescens</i>	1	Yes
Hymenoptera	Bee	<i>Andrena</i>			5	Yes
Hymenoptera	Bee	<i>Anthidium</i>			8	
Hymenoptera	Bee	<i>Anthophora</i>		<i>bomboides</i>	2	
Hymenoptera	Bee	<i>Anthophora</i>			6	Yes
Hymenoptera	Bee	<i>Ashmeadiella</i>			3	
Hymenoptera	Bee	<i>Atoposmia</i>			3	
Hymenoptera	Bee	<i>Bombus</i>		<i>californicus</i>	1	
Hymenoptera	Bee	<i>Bombus</i>		<i>huntii</i>	8	Yes
Hymenoptera	Bee	<i>Bombus</i>		<i>fervidus/californicus</i>	3	Yes
Hymenoptera	Bee	<i>Ceratina</i>			35	Yes
Hymenoptera	Bee	<i>Diadasia</i>			11	
Hymenoptera	Bee	<i>Dufourea</i>			1	Yes
Hymenoptera	Bee	<i>Eucera</i>			1	
Hymenoptera	Bee	<i>Halictus</i>		<i>farinosus</i>	13	
Hymenoptera	Bee	<i>Halictus</i>		<i>parallelus</i>	2	
Hymenoptera	Bee	<i>Halictus</i>		<i>rubicundus</i>	2	
Hymenoptera	Bee	<i>Hoplitis</i>			15	Yes
Hymenoptera	Bee	<i>Lasioglossium</i>	<i>Dialictus</i>		71	
Hymenoptera	Bee	<i>Lasioglossium</i>	<i>Evyllaesus</i>		2	
Hymenoptera	Bee	<i>Lasioglossium</i>	<i>Lasioglossum</i>		77	Yes
Hymenoptera	Bee	<i>Lasioglossium</i>	<i>Sphecodogastra</i>		1	
Hymenoptera	Bee	<i>Megachile</i>			1	
Hymenoptera	Bee	<i>Osmia</i>			30	Yes
Hymenoptera	Bee	<i>Perdita</i>			2	
Hymenoptera	Bee	<i>Sphecodes</i>			3	
Hymenoptera	Wasp	<i>Chrysididae</i>			38	
Hymenoptera	Wasp	<i>Crabronidae</i>			2	
Hymenoptera	Wasp	<i>Pseudomasaris</i>		<i>vespoides</i>	1	
Hymenoptera	Wasp	<i>Sphecidae</i>			40	
Hymenoptera	Wasp	<i>Vespidae</i>	<i>Eumeninae</i>		20	

Order	Common name	Genus	Subgenus	Species	Number	Pollen
Hymenoptera	Wasp	<i>Vespidae</i>			2	
Hymenoptera	Wasp	Wasp			1296	
Lepidoptera	Butterfly	<i>Cercyonis</i>		<i>oetus</i>	2	
Lepidoptera	Butterfly	<i>Cercyonis</i>		<i>sthenele</i>	3	
Lepidoptera	Butterfly	<i>Chlosyne</i>			1	
Lepidoptera	Butterfly	<i>Colias</i>		<i>christina</i>	1	
Lepidoptera	Butterfly	<i>Hesperia</i>			2	
Lepidoptera	Butterfly	<i>Plebejus</i>		<i>icariodes</i>	1	
Lepidoptera	Butterfly	<i>Plebejus</i>			4	
Lepidoptera	Butterfly	<i>Speyeria</i>			1	
Lepidoptera	Moth	<i>Geometridae</i>			1	
Lepidoptera	Moth	Micromoths			269	

Pollen analysis

The pollen from Desert yellowhead is spherical. Each grain has three deep longitudinal grooves and the surface has spikes. Each pollen grain is 6.9 to 11.9 μm in diameter and the spike varied between 0.7 and 1.4 μm long (Figure 4c). Although several species of Asteraceae have similarly shaped pollen, Desert yellowhead pollen is usually larger than most other species, the spike length varied among species and the flowering phenologies differed.

Nine genera of bees carried pollen grains from Desert yellowhead on their scopa indicating that they actively collected the pollen (Figure 11a, Table 3). Of all the Desert yellowhead pollen we encountered, a mason bees (*Hoplitis*) carried the most pollen (25%; mean per individual) followed by a mining bee (*Andrena*; 17%), bumble bees (*Bombus*; 14%), a sweat bee (*Agapostemon*; 13%), another sweat bee (*Lasioglossum*; 11%), a digger bee (*Anthophora*; 8%), another sweat bee (*Dufourea*; 7%), a mason bee (*Osmia*; 4%) and small carpenter bees (*Ceratina*; 1%; Figure 11a). Of all the pollen grains identified on individual bees, pollen from Desert yellowhead made up 33% of pollen loads for *Hoplitis*, 26% for *Lasioglossum*, 19% for *Bombus* and *Agapostemon*, 16% for *Ceratina* and *Andrena*, 11% for *Anthophora*, 10% for *Osmia* and 9% for *Dufourea* (Figure 11b). The most pollen from Desert yellowhead was found on bees in early July (Figure 12a). Between 42 and 100% of individuals in each bee genus were carrying pollen from Desert yellowhead, suggesting that this rare plant is used by many bees (Figure 12b).

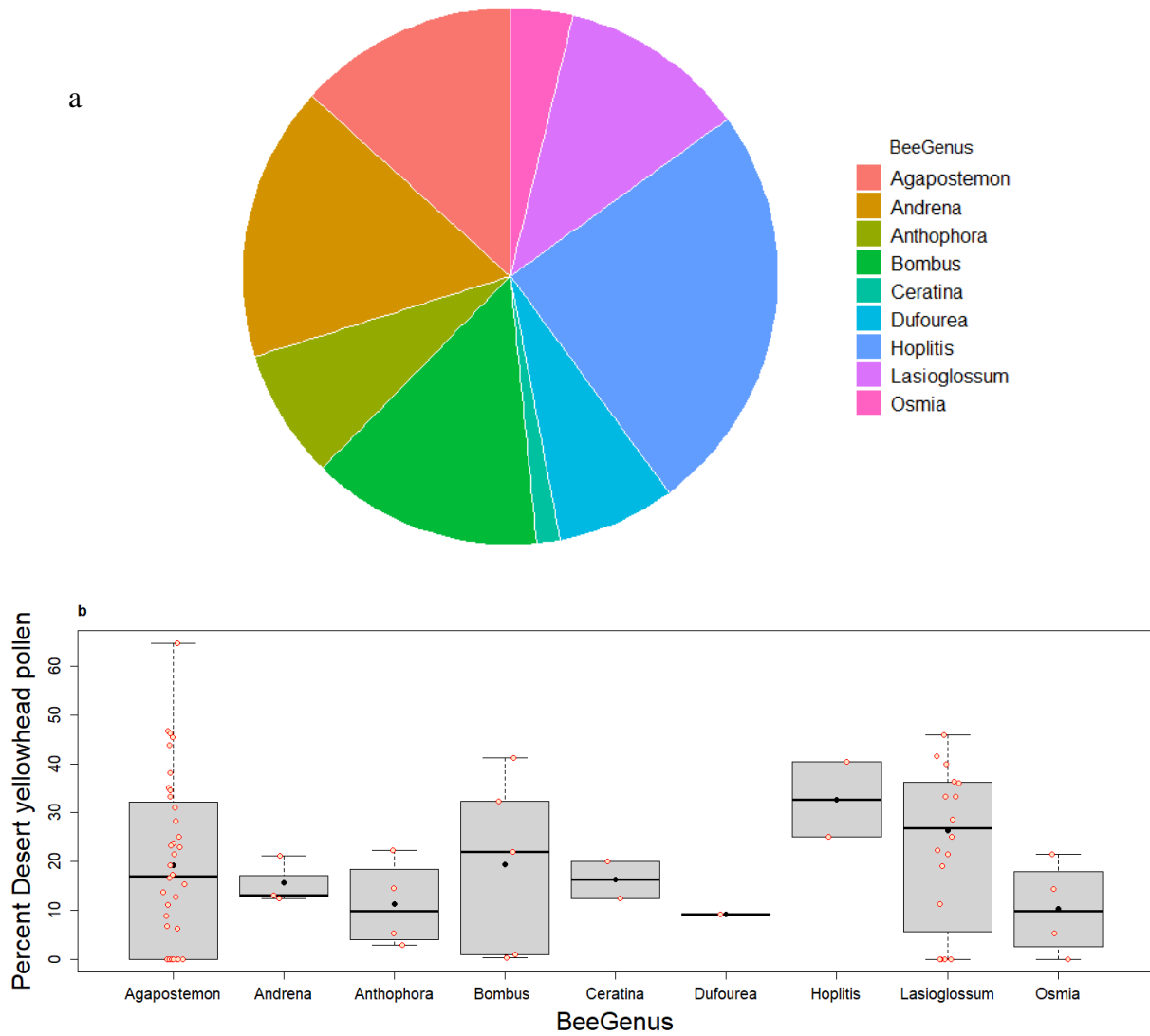


Figure 11. a) Mean percent of pollen grains from Desert yellowhead carried by nine bee genera. The mason bee, *Hoplitis*, carried the most Desert yellowhead pollen. b) The percent of pollen grains from Desert yellowhead carried on individual bees by genus compared to all pollen grains counted on a bee. The bold line is the median, the black circle is the mean, the lower and upper limits of the box are the 25th and 75th percentiles, whiskers are the upper and lower limits of the data excluding outliers and the open circles are data points.

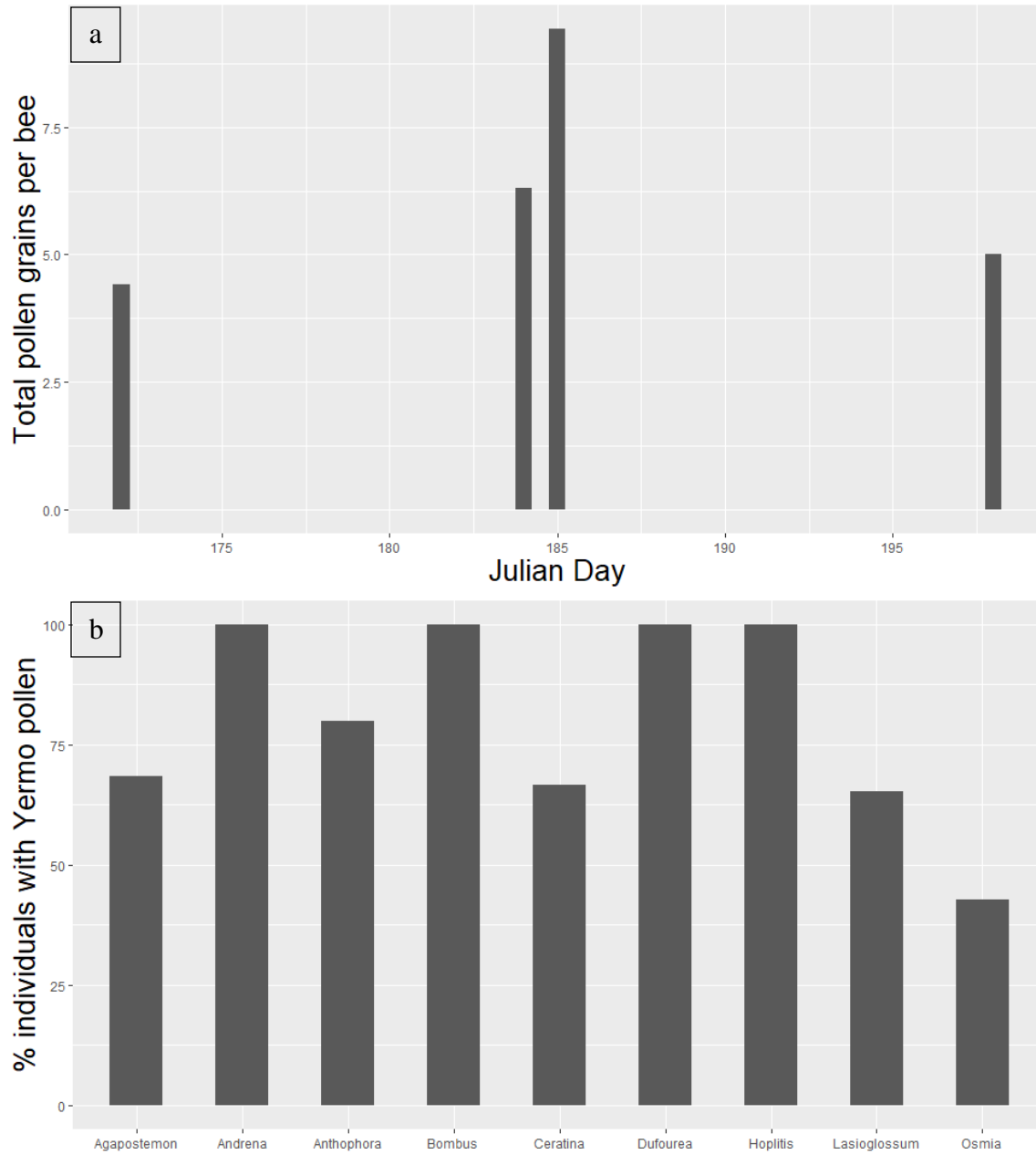


Figure 12. a) Bees carried the most pollen from Desert yellowhead in early July. b.) A high percentage of individuals in each bee genus carried pollen from Desert yellowhead indicating that many bees collected pollen from this plant while it was blooming.

DISCUSSION

Desert yellowhead can self-pollinate; however, the number of viable seeds produced through this process was extremely low. Overall, the number of viable seeds produced in all treatments was very low, much lower than reported for other rare species in Asteraceae (Colling et al. 2004; Diamond et al. 2006). Colling et al. (2004) found germination rates of 0-97% in seeds from four populations of Viper's grass (*Scorzonera humilis*), while Diamond et al. (2006) found Eared coneflower (*Rudbeckia auriculata*) produced up to 31.4% viable seeds in five populations. Both of these species are also rare members of Asteraceae, although Viper's grass grows in moist meadows, and Eared coneflower occurs in wet meadows and bogs, rather than the steppe habitat of Desert yellowhead. Two species of the Patagonian steppe, Solbrig's snakeweed (*Gutierrezia solbrigii*) and Slender ragwort (*Senecio subulatus*), produced 82% and 88% viable seeds, respectively (Masini et al. 2016). None of our plants at Cedar Rim produced viable seeds, which is concerning from a genetic diversity standpoint and for the perpetuation of the species. Fifteen percent of seeds in the open and hand-pollinated treatments at Sand Draw were viable, which is more encouraging than the other site, but still low compared to other rare plants. Pollinators did not limit seed production in Desert yellowhead because the number of viable seeds produced in the open and hand-pollinated treatments did not differ; therefore, we hypothesized that other factors (e.g., precipitation, air temperature) are limiting seed production. Seed production can vary with nutrients, temperature (Young et al. 2004) and water availability (Pol et al. 2010). We predict that seed production for Desert yellowhead may be higher during wetter years, but this idea has not been tested. We measured seed production in 2018 which had below average precipitation. Seeds may be transferred from the Sand Draw population to the Cedar Rim population via wind or animal transport, but we do not know how often this may occur. The longevity of individual Desert yellowhead plants is unknown as well as the average number of years a plant produces an inflorescence. Therefore, we do not have an estimate of how many viable seeds might be produced over the lifespan of a mature Desert yellowhead plant. Closely monitoring individual plants over time could disentangle these questions.

Monitoring is crucial to detect changes over time and estimate the mechanisms causing the change. Intensive census surveys count all plants in a population and are the best method to track changes in populations, but they take days to complete and may only be done every few years because of time and money constraints. Conversely, transects take less time and can be done every year, but they subsample the population. We monitored Desert yellowhead using a modified transect method for three years to estimate trends over a 12 year period (Dibner et al. 2019; Doak et al. 2016). Our modified transect method detected declining trends over that time period and appeared to collect valuable data. A dual method of monitoring may be used where transects are monitored annually and a census of Desert yellowhead is done every 3-5 years as time and funding allow.

The number of Desert yellowhead plants along transects did not vary with mean spring temperature or spring precipitation. Dibner et al. (2019) suggested that both drier and wetter years caused declines in Desert yellowhead populations. Our monitoring and previous data

(Dibner et al. 2019; Doak et al. 2016) did not show a relationship between spring precipitation or temperature at our monitoring transects. We would expect to see a rounded curve peaking at intermediate precipitation if Desert yellowhead did best under such conditions; however, the slope of the line is flat and not significant. Doak et al. (2016) placed more emphasis on dry years, and we did not find a positive relationship between spring precipitation and the number of plants in each transect. We do see a negative relationship between the number of plants in each transect and spring mean temperature, and we expect the relationship will be significant with more data if the relationship is real. This suggests that Desert yellowhead may produce more plants in years with cooler springs, but more data are needed to validate this hypothesis.

Desert yellowhead pollen was found on nine genera of the bees we collected, and the amount of Desert yellowhead pollen carried coincided with flowering time. Desert yellowhead lives in a sparsely vegetated area with a limited number insect-pollinated flowering plants. The bees we collected usually carried pollen from several species including Desert yellowhead, indicating that bees use as much of the available pollen as possible. Blooming flowers in mid-summer are likely scarce, especially during hot and dry years, making Desert yellowhead a welcome site for many pollinators. Flowers provide both nectar to fuel adult activities and pollen to feed young. Rare plants often have specialized pollinators; however, we did not discover any bee taxa that appeared to specialize on Desert yellowhead. Instead, Desert yellowhead is an oasis in the semi-arid, unique habitat that this plant calls home.

On-site data loggers recording site conditions would provide a much more accurate portrayal of the weather to estimate how temperature and precipitation alter Desert yellowhead. We used data from the Jeffrey City weather station which is ~30 km from the sites and 200 m lower in elevation. Dibner et al. (2019) and Doak et al. (2016) used weather data from Riverton which is ~50 km away and ~600 m lower in elevation. Precipitation is often extremely localized and using data from the closest source may not accurately reflect conditions. Loggers on-site could measure other variables beyond air temperature and precipitation, such as soil temperature and soil moisture. We suggest measuring abiotic conditions within the Desert yellowhead populations to better understand how climate may alter the number of plants, and sexual and asexual reproduction.

The ecology of Desert yellowhead still has many mysteries to uncover. The low seed viability is worrying and deserves more research to tease apart potential reasons. The lack of viable seeds produced in the Cedar Rim population is especially disturbing and we wonder how much this population depends on Sand Draw. Pollinators did not limit the number of viable seeds produced by Desert yellowhead at Sand Draw in 2018. This indicated that some other factors are limiting seed production. Few viable seeds may constrain the perpetuation of the species and the continued decline in plant numbers along transects showed that the number of plants are decreasing over a 12 year period. We recommend annually monitoring transects and periodic census to estimate the condition of the Desert yellowhead population. Notes on demographics, growth and flowering status of select individuals would be valuable to gauge reproduction and learn more about this plant's life cycle. Both seed production and monitoring should be

compared to weather data, including soil temperature and soil moisture, to ascertain the effects of climate on Desert yellowhead reproduction.

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Appendix A. The number of Desert yellowhead plants along transects at Sand Draw through time. Transects were began by Doak et al. (2016).

Year	Total plants
Transect 2b-2c	
2010	68
2011	65
2012	58
2013	68
2014	66
2018	49
2019	46
2021	47
New Transect Ja-Jb	
2018	31
2019	26
2021	41

Table 1. The number of Desert yellowhead plants along transects at Cedar Rim through time. Transects were began by Doak et al. (2016).

Year	Total plants
Transect 4a-4b	
2010	61
2011	58
2012	57
2013	52
2018	43
2019	35
2021	30
Transect 5a-5b	
2010	25
2011	24
2012	20
2013	15
2018	17
2019	13
2021	14