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Eugene W. Schupp, *Utah State University*



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Reproductive Ecology of the Endangered Utah Endemic *Hesperidanthus suffrutescens* with Implications for Conservation

MATTHEW B. LEWIS¹ AND EUGENE W. SCHUPP

Department of Wildland Resources and the Ecology Center, 5230 Old Main Hill, Utah State University, Logan 84322

ABSTRACT.—We investigated fruit set, seed set, and germination requirements of shrubby reed-mustard (*Hesperidanthus suffrutescens*), an endangered endemic shrub in the Uinta Basin of eastern Utah, U.S.A. To determine the degree of self-compatibility, 120 plants received four pollination treatments each for 2 y. Treatments included autogamy, geitonogamy, xenogamy, and an open control. Autogamy and geitonogamy produced substantially and significantly fewer fruits, seeds, and seeds per fruit than xenogamy, suggesting little self-compatibility. Additionally, the maternal reproductive success of outcrossed flowers was significantly greater than open control flowers suggesting pollen limitation, perhaps due to reduced pollinator abundance or activity. One study population showed significantly reduced reproduction during the second year, perhaps resulting from impacts of anthropogenic disturbance. Although both selfing treatments produced significantly fewer seeds, there were no significant differences in germination among treatments. Potential pollinators were observed and collected during three sampling efforts. A total of 77 flower visitors were collected during the study. Potential pollinators providing outcrossed pollen included several *Andrena* species and several *Lasioglossum* and *Halictus* species. Overall, these results indicate little self-compatibility while suggesting that the pollinators needed for successful reproduction may, in fact, be limiting. This study highlights the important role of native pollinator fauna in conservation while providing managers with the basic reproductive ecology needed to conserve this endemic desert shrub.

INTRODUCTION

According to the International Union for Conservation of Nature Redlist (IUCN Redlist), there are approximately 9100 threatened and endangered plant species worldwide (IUCN, 2011). In addition to small population sizes, rare and endangered plants face numerous threats including habitat fragmentation and loss, invasion of exotic species, and changes in pollinator abundances. While these factors may impact plant survival and reproduction independently, they often combine to present complex ecological problems for natural resource managers. Successful conservation of threatened and endangered plant species begins with an understanding of their reproductive ecology (Harper, 1979; Simberloff, 1988; Schemske *et al.*, 1994). Reproductive ecology studies typically include pollination treatments to determine the degree of self-compatibility (*e.g.*, autogamy vs. xenogamy), potential pollinator identification, and germination and establishment requirements (Diamond *et al.*, 2006; Lazaro and Traveset, 2006; Strong and Williamson, 2007; Tepedino *et al.*, 2007; Tepedino *et al.*, 2010; Watrous and Cane, 2011). The state of Utah has over 300 rare plants, 24 of which are federally listed as threatened or endangered (Utah Division of Wildlife Resources, 2011; Utah Native Plant Society, 2011). Unfortunately, many of Utah's imperiled plant species have received little scientific study beyond basic population estimates (Franklin, 2005) and many pollinators of rare plants have yet to be identified (Kearns *et al.*, 1998). Knowledge of reproductive ecology can assist managers faced with the task of conserving imperiled plant species.

¹ Corresponding author: e-mail: ma.le@aggiemail.usu.edu

Flowering plants have diverse mating systems that insure successful reproduction and many are hermaphroditic and self-compatible to some degree. In mixed mating systems, outcrossing provides opportunity for gene flow while selfing provides reproductive assurance, allowing plants to reproduce and maintain viable populations when pollinators or potential mates are scarce (Jain, 1976; Busch, 2011). Similarly, many predominantly selfing plants may also reproduce through outcrossing, maximizing reproductive output and gene flow. Self-compatibility can have negative consequences, including decreased fitness of populations via inbreeding depression (Waser, 1993) with a greater threat of extinction for demographically unstable populations (Cheptou, 2004). Additionally, self-compatible plants are more susceptible to the negative effects of small population size and therefore the effects of inbreeding depression may be amplified (Leimu *et al.*, 2006). Conversely, obligate outcrossing plants exhibit a low degree of self-compatibility and require pollen from another plant to successfully produce seeds. Therefore, obligate outcrossers may be more susceptible to impacts that affect pollinator abundance and habitat (Eckert *et al.*, 2009). Thus, it is important to determine the degree of self-compatibility and the underlying pollination mechanisms that maximize the reproductive output of imperiled plants, to help managers determine the extent to which they should emphasize management of pollinators and their habitat.

In addition to the perils of an obligate mating system, reproduction in rare plants may also be hindered by anthropogenic disturbances such as habitat fragmentation and loss. These impacts directly affect plant reproduction by decreasing and or altering habitat, killing plants, and disrupting or preventing dispersal. Anthropogenic disturbance may also negatively affect reproduction indirectly by altering pollinator communities and plant-pollinator interactions (Mustajärvi *et al.*, 2001; Cane *et al.*, 2006; Pauw, 2007) in ways that increase pollen limitation (Eckert *et al.*, 2010; Gomez *et al.*, 2010) and decrease plant fecundity (Andrieu *et al.*, 2008). Because of the potential impacts to reproduction, anthropogenic disturbance has important consequences for imperiled plants.

The federally endangered *Hesperidanthus suffrutescens* (Rollins) Al-Shehbaz (shrubby reed-mustard) is endemic to Utah's Uinta Basin, an area of rapid energy development. *H. suffrutescens* and its habitat are underlain by oil-shale and natural gas deposits and are threatened by further mineral extraction as all federal lands with known populations are leased for oil and gas development (U.S. Fish and Wildlife Service, 1994). Listed on 6 Oct. 1987, *H. suffrutescens* populations have declined in size and abundance since the species was described in 1935 with current population estimates of 2900 individuals in seven separate sub-populations (U.S. Fish and Wildlife Service, 1994, 2009). Unfortunately, little is known of the reproductive system or potential pollinators required by the recovery plan (U.S. Fish and Wildlife Service, 1994).

Here we experimentally determine the reproductive system of *H. suffrutescens*, identify potential pollinators, and discuss management implications. Specifically, we investigate three questions: (1) Does self pollination result in fruit and seed set, and if so, is it equivalent to that of outcrossed pollen? (2) Is reproduction pollen limited? (3) What are the potential pollinators? The data gathered here will provide managers with the reproductive ecology of *H. suffrutescens*, an essential step towards successful conservation.

METHODS

STUDY SPECIES

Hesperidanthus suffrutescens is perennial shrub in the mustard family (Brassicaceae). This species has several generic synonyms (*Shoenocrambe*, *Glaucocarpum*, and *Thelypodium*) but has

been placed in *Hesperidanthus* based on genetic analyses of sister taxa (Al-Shehbaz, 2005). *Hesperidanthus suffrutescens* is characterized by thick, glaucous leaves, and multiple yellow flowers on multiple stems emerging yearly from a woody taproot (Holmgren *et al.*, 2005). The hermaphroditic flowers have four petals, four sepals, and six stamens (Holmgren *et al.*, 2005). The flowers exhibit reverse herkogamy, with the stigma below the anthers. Flowers mature acropetally beginning in early Apr. and the siliques mature and release seeds three to four weeks after corolla loss. The deciduous leaves senesce in late fall and dead stems can remain for multiple years (M.L., pers. obs.).

Hesperidanthus suffrutescens inhabits cold desert shrub communities varying from site to site, but generally including the shrubs *Artemisia pygmae* (pygmy sagebrush), *A. nova* (black sagebrush), *Atriplex confertifolia* (shadscale), *Tetradymia spinosa* (shortspine horsebrush), and *Ephedra torreyana*. (Torrey's jointfir); the trees *Cercocarpus montanus* (true mountain-mahogany), *Pinus edulis* (twoneedle pinyon), and *Juniperus osteosperma* (Utah juniper); and the grasses *Achnatherum hymenoides* (Indian ricegrass), *Pseudoroegneria spicata* (bluebunch wheatgrass), and *Poa secunda* (Sandberg bluegrass) along with many native forbs (U.S. Fish and Wildlife Service, 1994). *Hesperidanthus suffrutescens* inhabits semi-barren, rocky outcrops of the Green River Formation described as the Evacuation Creek member in the Recovery Plan (U.S. Fish and Wildlife Service, 1994). Soils are typically shallow (10–20 cm) with many small fragments of white shale on the surface (M.L., pers. obs.).

Little is known about the pollinators of *H. suffrutescens*, although during a preliminary study, investigators observed the solitary bee species *Dialictus perdifficilis*, *D. sedi*, *Eurylaeus pulveris* and *Andrena walleyi* foraging on flowers (V. J. Tepedino, unpubl.). Of these, *A. walleyi* is thought to be a mustard specialist (Lanham, 1993) and may prove an important pollinator *H. suffrutescens* while the *Dialictus* species are generalists, likely important to the entire plant community (V. J. Tepedino, pers. comm.).

STUDY AREA

The Uinta Basin, located in eastern Utah on the northern Colorado Plateau (40°13'30"N, 109°32'32"W), has a mean annual precipitation ≈ 17.4 cm and a mean annual temperature ≈ 8.8 C (Utah Climate Center, 2006–2010). This study was conducted in the three largest populations (Fig. 1), ranging in elevation from 1400 to 2100 m, and located approximately 15 to 40 km apart. The Big Pack Mountain (Pack) population is heavily impacted by oil and gas development while the Bad Land Cliffs (Clif) and Johnson Draw (John) populations are less impacted by development.

REPRODUCTIVE SYSTEM EXPERIMENT

The reproductive system experiment included four pollination treatments: autogamy (A), geitonogamy (G), xenogamy (X), and open control (O). The autogamy treatment tests for spontaneous selfing (*i.e.*, pollination from the same flower without pollinators), the geitonogamy treatment tests for selfing (*i.e.*, pollen from a different flower on the same plant), the xenogamy treatment tests for outcrossing (pollen from a different plant), and the open control provides estimates of natural pollination levels. In both 2010 and 2011, 40 plants (of similar size) from each population were selected ($n = 120$) to receive treatments. On each plant, four stems were selected individually by blindly grabbing a stem; each received one of the four treatments randomly assigned by blindly grabbing a treatment tag ($n = 480$). On each stem, three flowers received the treatment ($n = 1440$). In 2011, both selfing treatments (A, G) were increased to five flowers per stem.

All open flowers were removed prior to treatment. Bud pedicels of treatment flowers were marked with a permanent marker prior to anthesis, and the stem was bagged with a 10 \times



FIG. 1.—Map of study area and the Bad Land Cliffs, Big Pack Mountain, and Johnson Draw populations

20 cm nylon tulle exclusion bag to prevent visitation. Bamboo skewers were placed in the soil next to the treatment stems and the exclusion bags were placed over stems and skewers to prevent losses in windy conditions. Bags were left on until anthesis, removed for manipulation, and replaced until fruit collection.

The “A” treatment stems were bagged and the flowers not manipulated. For the “G” treatment, the upper four anthers of treatment flowers were removed using forceps, exposing the stigma. Stamens from another flower on the same plant were used to pollinate the treatment flowers with a different stamen used for each treatment flower. Deposition of pollen was verified by visual examination and bags were replaced. “X” treatments were applied similarly, but donor stamens came from flowers on plants approximately 10 m away in an attempt to minimize both outbreeding and inbreeding depression (Waser, 1993). Due to the rarity of this species, occasionally optimal outcrossing was not possible and donor pollen came from individuals within, or farther than, 10 m. “O” treatment stems had three flowers marked but were not manipulated and bagged only after corolla loss to prevent the loss of fruits and seeds.

Exclusion bags were removed when fruits had matured but prior to dehiscence (approximately four weeks after treatment) and a small strip of transparent duct tape was wrapped around each fruit to prevent seed loss. Fruits were collected when they began to turn brown and taken back to the lab where seeds were removed, counted, and weighed to the nearest mg. Seeds not kept for germination trials were returned and distributed around the parent plant.

To determine if pollinators were limiting reproductive success, a simple pollen limitation index was calculated for each population in each year as $L = 1 - (P_o/P_s)$ where P_o is the percent open control flowers that set fruit and P_s is the percent supplemental outcrossed flowers that set fruit. The pollen limitation index ranges from zero to one where a zero indicates no pollen limitation (Larson and Barrett, 2000).

GERMINATION EXPERIMENT

Seeds for the germination experiment were selected from each treatment and population. For treatments that resulted in more than 100 seeds, a total of 100 seeds were selected systematically by selecting one seed from all fruits that produced at least one seed. Then, a second seed was selected from the remaining fruits that produced at least two seeds. This process was repeated until 100 seeds were collected from each treatment in each population. For treatments that did not produce 100 seeds (*A* and *G* in all populations in 2010 and 2011; and *O* in Bad Land Cliffs in 2010 and 2011; Johnson Draw in 2010; and Big Pack Mountain in 2011), all seeds were used. A total of 499 and 504 seeds were used in 2010 and 2011, respectively. Seeds were divided into as many replicates of 10 as possible. A petri dish was fitted with a filter paper soaked in a fungicide solution (0.2 g Hi-Yield Captan fungicide and 1 L distilled water). Seeds were placed on the filter paper and the lid was secured with a rubber band. Replicates were stacked and placed in quart-sized zipper plastic bags and then placed in large paper bags to minimize light exposure. Seeds were cold moist stratified in a refrigerator at 4 C to mimic natural overwintering conditions. Petri dishes were checked weekly and additional fungicide solution was applied when needed to maintain adequate moisture levels during stratification. If mold was detected, the filter paper was replaced and moldy seeds were discarded. Seeds began germinating abundantly in the refrigerator and were maintained there for 6 mo. As seeds germinated, germinants were planted individually into "cone-tainers" filled with cactus potting soil. In 2011, a 1:1 mixture by volume of cactus potting soil and native soil was used. Germinants were placed in a greenhouse and watered automatically once a day for 15 min for 2 wk or until the emergence of primary leaves. Seedlings were then moved to an adjacent greenhouse and watered automatically, once in the morning for 15 min every other day.

After 6 mo cold storage, the 2010 seeds that had not germinated were placed in a germination cabinet at cycles of 38 C for 14 hr and 10 C for 10 hr. After 1 mo seeds were returned to cold storage for an additional 6 mo. The 2011 seeds were in cold storage for approximately 3 mo and no germination cabinet was used. In Apr. of 2012, all remaining seeds were returned to the soil around parent plants.

POTENTIAL POLLINATORS

Insect specimens were collected from flowering individuals opportunistically, pinned in the field, and identified at the U.S.D.A. Bee Systematics Laboratory in Logan, Utah, U.S.A. Pollinator foraging behavior was observed during six 30 min periods (0900–0930; 1200–1230; 1800–1830) per population. Each observation period was conducted on a different plant ($n = 6$ plants per population). The observation periods were repeated once in each population on a different day during the year ($n = 36$ plants observed per year). However, due to inclement weather conditions in 2010, only 15 plants were observed. All 36 observation periods were completed in 2011. In 2012 an additional six plants were observed in each of the three populations ($n = 18$ plants). Pollinator foraging behavior was observed on a total of 69 plants over the course of the study.

STATISTICAL ANALYSES

Data were analyzed using SAS/STAT 9.2 software (SAS Institute, 2002). Generalized linear mixed-model Analysis of Variance (ANOVA) models were fitted using the GLIMMIX procedure to compare differences in fruit set, seed number, number of seeds per fruit, and mean seed weight (mg) between treatments, populations, and years. The factor '*population*' was a fixed effect while '*plant*', '*treatment*', and '*year*' were random effects. Interactions

examined were '*population*year*', '*population*treatment*', '*treatment*year*' and '*population*treatment*year*'. Denominator degrees of freedom were calculated using the Kenward-Roger method. The Tukey-Kramer method was used for comparisons between treatment effects and the Bonferroni adjustment method was used for interaction comparisons. Significance was set at the $\alpha = 0.05$ level. Fruit set was analyzed as a proportion, using an over dispersion parameter ($\#$ fruits/ $\#$ treated flowers) and a binomial distribution with the logit link function. When included in the fruit set model, the 3-way interaction term '*population*treatment*year*' led to model convergence issues and removal made for the most parsimonious model. For the seed number, seeds per fruit, and mean seed weight (mg) analyses, comparisons could only be made between the "X" and "O" treatments ($n = 250$) as the numerous zeros from the selfing treatments (A, G) led to model convergence errors. The most parsimonious seed set model used a negative binomial distribution with a log link function while the seeds per fruit and mean seed weight models used a Gaussian distribution with an identity link function.

Germination data were analyzed with the proportion of germinants (germinants/seeds) as the response, a binomial distribution with a logit link function, and weighted by $1/\#$ of seeds. Germination models included the effects '*year*', '*treatment*', '*population*', and a '*treatment*population*' interaction. Due to differences in trial length, the analysis was conducted on the first 3 mo from each trial.

A global chi-square (Fisher's exact) test that included all treatments was used to determine if '*treatment*' affected the number of seeds produced per fruit when a fruit is set (*i.e.*, all treatments where fruit set > 0). This test does not include nor account for any variations due to population or year.

The variables number of seeds per fruit and mean seed weight were transformed by taking the square root to meet the assumptions of homoscedasticity, symmetry, and for best model fit. The fruit set model included a binomial distribution and therefore no transformations were needed. Least squared means and standard errors were back-transformed for all figures unless otherwise noted.

RESULTS

REPRODUCTIVE SYSTEM

Results from the fruit set model indicate significant '*treatment*' and '*population*year*' effects (Table 1). The outcross (X) treatment produced significantly more fruits per treatment flower (0.47 ± 0.04) than the autogamy (A; 0.04 ± 0.01), geitonogamy (G; 0.02 ± 0.01), and open control (O; 0.24 ± 0.03) treatments. While the "A" and "G" treatments produced significantly fewer fruits than the "O" treatment, they did not differ significantly from each other. The significant '*population*year*' interaction is due to Big Pack Mountain producing significantly fewer fruits in 2011 than in 2010 while the other populations did not (Fig. 3).

The seed number model indicates significant '*treatment*', '*year*', and '*population*year*' effects (Table 1). The "X" treatment produced three times more seeds (6.52 ± 0.49) than the "O" treatment (2.30 ± 0.27) for all years combined. In 2010 Big Pack Mountain produced significantly more seeds (7.80 ± 1.00) than in 2011 (2.19 ± 0.45) and significantly more seeds than both Bad Land Cliffs in 2010 (3.96 ± 0.66) and 2011 (3.82 ± 0.66), and significantly more than Johnson Draw in 2010 (3.95 ± 0.65) and 2011 (4.71 ± 0.67). Interestingly, Big Pack Mountain produced fewer seeds (2.19 ± 0.45) than Johnson Draw (4.26 ± 0.66) in 2011. Across all populations and treatments, significantly fewer seeds were set in 2011 (2.81 ± 0.32) than in 2010 (4.30 ± 0.47).

TABLE 1.—Generalized linear mixed-model effects on reproductive system variables (significant P values at $\alpha = 0.05$ are in bold font)

Model	Effect	df (Num., Den.)	F	P
Fruit Set				
	<i>treatment</i>	3, 351	120.95	<0.0001
	<i>population</i>	2, 116	1.27	0.2844
	<i>year</i>	1, 116	0.73	0.3943
	<i>treatment*population</i>	6, 351	0.78	0.5869
	<i>treatment*year</i>	3, 300	1.81	0.1452
	<i>population*year</i>	2, 116	7.93	0.0006
Seed Set				
	<i>treatment</i>	1, 306	55.12	<0.0001
	<i>population</i>	2, 137	0.72	0.4866
	<i>year</i>	1, 306	7.35	0.0071
	<i>population*treatment</i>	2, 306	1.52	0.221
	<i>treatment*year</i>	1, 306	0.03	0.8645
	<i>population*year</i>	2, 306	10.96	<0.0001
	<i>population*treatment*year</i>	2, 306	2.97	0.0528
Seeds per Fruit				
	<i>treatment</i>	1, 326	58.51	<0.0001
	<i>population</i>	2, 117	0.33	0.7205
	<i>year</i>	1, 326	3.4	0.0660
	<i>population*treatment</i>	2, 326	2.07	0.1284
	<i>treatment*year</i>	1, 326	0.41	0.5220
	<i>population*year</i>	2, 326	13.32	<0.0001
	<i>population*treatment*year</i>	2, 326	2.38	0.0939
Mean Seed Weight				
	<i>treatment</i>	1, 119	0.18	0.6730
	<i>population</i>	2, 111	3.31	0.0402
	<i>year</i>	1, 119	0.67	0.4142
	<i>population*treatment</i>	2, 119	0.46	0.6295
	<i>treatment*year</i>	1, 119	2.08	0.1519
	<i>population*year</i>	2, 119	4.62	0.0117
	<i>population*treatment*year</i>	2, 119	0.89	0.4128
Percent Germination				
	<i>treatment</i>	3, 924	4.58	0.2060
	<i>population</i>	2, 924	4.25	0.1202
	<i>year</i>	1, 924	31.71	<0.0001
	<i>treatment*population</i>	4, 924	4.85	0.4351

The results of the seeds per fruit analysis indicate significant ‘*treatment*’ and ‘*population*year*’ effects (Table 1). The “X” treatment produced significantly more seeds per fruit (2.75 ± 0.18) than the “O” treatment (1.17 ± 0.12) across all years and populations. While Bad Land Cliffs and Johnson Draw did not differ between 2010 and 2011 in seeds per fruit, Big Pack Mountain produced substantially and significantly more seeds per fruit in 2010 than in 2011 (Fig. 4). In fact, in 2011 the number of seeds per fruit at Big Pack Mountain was significantly less than the other two populations. The chi-square analysis suggests that when fruits were set, the “X” treatment produced significantly more seeds per fruit (2.75 ± 0.18)

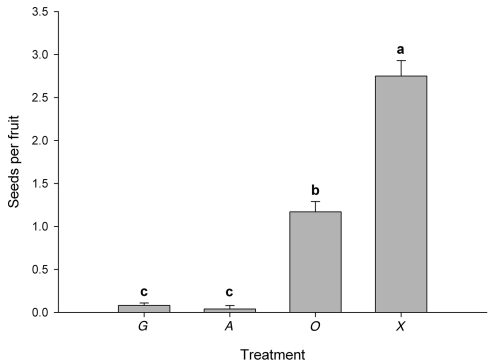


FIG. 2.—Mean number of seeds per fruit (+standard error) between four pollination treatments for all populations and years combined (different letters indicate significant differences)

than the “O” treatment (1.17 ± 0.12) and both the A (0.13 ± 0.04) and G (0.08 ± 0.03) selfing treatments ($\chi^2 = 37.39$, $P < 0.05$, Fig. 2). Additionally, the “O” treatment produced significantly more seeds per fruit than both selfing treatments.

While there was no significant difference in mean seed weight between the “X” and “O” treatments, there was a significant ‘population*year’ interaction (Table 1). When combined across treatments, in 2010 Big Pack Mountain produced lighter seeds (0.19 ± 0.03) than the Bad Land Cliffs (0.25 ± 0.03) and Johnson Draw (0.31 ± 0.04) populations. Interestingly, Bad Land Cliffs was the only population with a significant difference between years, producing lighter seeds in 2011 (0.25 ± 0.03) than in 2010 (0.31 ± 0.04). However, when combined across years, Big Pack Mountain produced significantly lighter seeds (0.61 ± 0.03) compared to the Johnson Draw and Bad Land Cliffs populations (0.69 ± 0.02 and 0.69 ± 0.03 , respectively). Table 2 summarizes the means and SE for each of the four reproductive measures by treatment.

During the second study year, pollen limitation increased in the Big Pack Mountain (0.19 to 0.73) and Badland Cliffs (0.33 to 0.45) populations, while decreasing in the Johnson Draw population (0.45 to 0.07, Table 3).

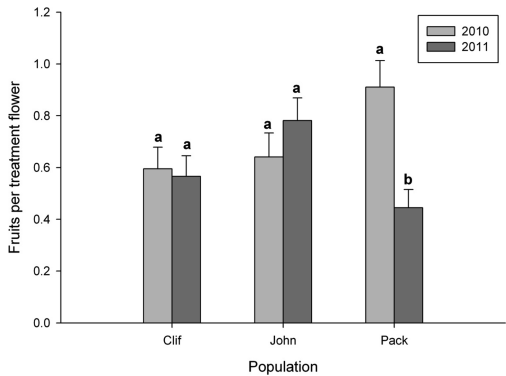


FIG. 3.—Mean number of fruits (+standard error) and the interaction between population and year (different letters indicate significant differences)

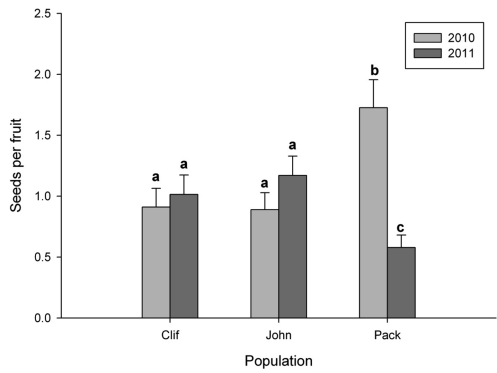


FIG. 4.—Mean number of seeds per fruit (+standard error) showing the interaction between population and year (different letters indicate significant differences)

GERMINATION RESULTS

Of the 494 seeds used in the 2010 germination trial, 23 were lost to mold. Of the 476 remaining seeds, 302 (63%) germinated across all treatments. All but four of the 2010 seedlings died in the greenhouse from what appeared to be damping off. In 2011, 46 of the 504 seeds were lost to mold and of the 458 remaining seeds, 181 (40%) germinated across all treatments. In 2011, a total of 18 seedlings survived.

Across all treatments and both years, 486 (52%) of the 934 seeds germinated during cold-moist stratification. No seeds from the 2010 trial germinated in the germination cabinet although one seed did germinate during the second cold storage trial. In the analysis of germination over 3 mo only ‘year’ was significant (Table 1), with less germination of 2011 seeds (0.40 ± 0.03) than 2010 seeds (0.59 ± 0.03). Table 4 summarizes the germination data across years, populations, and treatments.

POTENTIAL POLLINATORS

A total of 77 flower visiting insects were collected including several Lepidopteran species, Syphridae and Diptera species, and bees in the Andrenidae and Halictidae families (Table 5). The most common insect visitors were species of bees in Halictidae (*Lasioglossum* spp. and *Halictus rubicundus*). The Andrenidae and Halictidae species are potentially key pollinators of *H. suffrutescens* as their behavior put them in contact with both the pollen and stigma of flowers. Of the Halictidae, *Halictus rubicundus*, *H. tripartitus*, *Lasioglossum parforbessii*, and species of *Dialictus* and *Evylaeus* are likely important pollinators. Several species of Andrenidae collected (*Andrena prunorum*, *A. merriami*, and *A. walleyi*) also are likely

TABLE 2.—Mean number of fruits, seeds, mean seed weight (mg), and seeds per fruit by treatment for all populations and years combined

Treatment	Number of stems	Mean fruits	Mean seeds	Mean seed weight (mg)	Mean seeds per fruit
A	226	0.08 ± 0.03	0.19 ± 0.06	0.03 ± 0.01	0.13 ± 0.04
G	221	0.05 ± 0.03	0.13 ± 0.05	0.02 ± 0.01	0.08 ± 0.03
O	226	1.01 ± 0.07	2.30 ± 0.27	0.19 ± 0.02	1.17 ± 0.12
X	229	2.94 ± 0.02	6.52 ± 0.49	0.32 ± 0.02	2.75 ± 0.18

TABLE 3.—Pollen limitation index for three study populations and two study years, calculated as $L = 1 - (P_o/P_s)$ where P_o is the percent open control fruit set and P_s is the percent outcross fruit set

Year	Badland Cliffs	Johnson Draw	Big Pack Mountain
2010	0.33	0.45	0.19
2011	0.45	0.07	0.73

important pollinators of *H. suffrutescens*. Additionally, in both study years many treatment plants were observed with numerous small, unidentified beetles inside flowers.

Pollinator visitation and abundance appeared lower during the second study year as mean flower visitors observed per plant for each observation period was 6.4 in 2010, 4.1 in 2011, and 41.3 in 2012. No statistical analyses were conducted on these data.

DISCUSSION

REPRODUCTIVE SYSTEM

While a preliminary study suggested *H. suffrutescens* is capable of selfing (V. J. Tepedino, pers. comm.), our study clearly shows outcrossing is critical for successful reproduction.

TABLE 4.—Final seed counts for germination trials by population, treatment, and year

Year	Population	Treatment	Number of seeds	Germinants	Non-germinants	Number of seeds lost	Percent germination
2010	Pack	G	7	4	1	2	0.80
		A	8	6	1	1	0.86
		X	84	54	25	5	0.68
		O	88	54	32	2	0.63
	John	G	5	3	2	0	0.60
		A	0	0	0	0	0.00
		X	80	39	37	4	0.51
		O	53	21	28	4	0.43
	Clif	G	0	0	0	0	0.00
		A	11	8	3	0	0.73
		X	96	71	21	4	0.77
		O	67	42	24	1	0.64
2011	Pack	G	3	2	1	0	0.67
		A	3	3	0	0	1.00
		X	100	40	56	4	0.42
		O	27	12	15	0	0.44
	John	G	10	3	7	0	0.30
		A	18	6	7	5	0.46
		X	100	38	61	1	0.38
		O	100	50	45	5	0.53
	Clif	G	0	0	0	0	0.00
		A	3	1	1	1	0.50
		X	100	16	63	21	0.20
	Clif	O	40	10	21	9	0.32

TABLE 5.—Collection information for insects visiting flowers of *Hesperidanthus suffrutescens*

Family/Order	Genus (subgenus)	Species	Sex	Population	Year	Specimens collected by population	Accession number *
Andrenidae	<i>Andrena</i>	<i>merriami</i>	F	John, Pack	2012	1,1	BBSL817871
	<i>Andrena</i>	<i>prunorum</i>	F,M	Clif, Pack	2010, 2012	1,1	BBSL818219
	<i>Andrena</i>	<i>ualleyi</i>	F	John, Pack	2011, 2012	3,1	BBSL817868
Apidae	<i>Andrena</i> (<i>Micrandrena</i>)	<i>kristine</i>	M,F	John, Pack	2011, 2012	1,3	BBSL817875
	<i>Anthophora</i>	<i>urina</i>	F	Pack	2010	2	BBSL817864
Halictidae	<i>Agapostemon</i>	<i>texanus/angelicus</i>	F	Pack	2012	1	BBSL818217
	<i>Agapostemon</i>	<i>femoratus</i>	F	Pack	2010, 2011	2	BBSL818215
	<i>Lasiglossum</i> (<i>Dialictus</i>)	spp.	F	Clif, John	2010, 2011, 2012	11,5	BBSL817892
	<i>Lasiglossum</i> (<i>Erylaeus</i>)	spp.	F	Clif, John, Pack	2010, 2011, 2013	6,3,3	BBSL817876
	<i>Lasiglossum</i>	<i>paraforbessii</i>	F	Clif	2012	1	BBSL818218
Hymenoptera	<i>Halictus</i>	<i>rubicundis</i>	F	Pack	2010, 2012	8	BBSL818206
	<i>Halictus</i>	<i>tripartitus</i>	F	Pack	2012	1	BBSL818214
	<i>Odynerus</i>	spp.	—	Pack	2010	3	BBSL818228
Crabronidae	<i>Oxybelus</i>	sp.	—	Pack	2012	1	BBSL818227
Diptera	—	—	—	Pack	2010	6	BBSL818232
Syrphidae	—	—	—	Pack	2010, 2011, 2012	5	BBSL818223
Lepidoptera	—	—	—	Clif, John, Pack	2011, 2012	1,1,2	BBSL818238

* Each accession number represents a single voucher specimen from each taxon deposited in the National Pollinating Insects Collection at Utah State University, Logan, UT, USA

Both selfing treatments produced substantially and significantly fewer fruits and seeds per fruit than the outcross treatment, providing little evidence for self-compatibility. Although both selfing treatments produced a few fruits and seeds, these may have resulted from contamination from the small beetles observed inside a few exclusion bags during both study years. Collectively, these findings suggest that outcrossed pollen provided by pollinators is essential to maximize *H. suffrutescens* reproduction. Therefore, *H. suffrutescens* may prove susceptible to impacts on pollinator abundance, habitat, and foraging behavior.

Many studies have found pollen to limit the reproduction of rare, endemic, and endangered plants to some degree (Lazaro and Traveset, 2006; Strong and Williamson, 2007; Tepedino *et al.*, 2007; Tepedino *et al.*, 2010; Wagenius and Lyon, 2010; Becker *et al.*, 2011; Fernandez *et al.*, 2012). We found strong evidence for pollen limitation during both study years. Xenogamy produced nearly twice as many fruits than the open control (386 vs. 206). Additionally, the xenogamy treatment produced nearly three times as many seeds than the open control (1487 vs. 519). We observed few flower visitors during 2011 and low pollinator abundance may explain the difference in reproduction. Pollen limitation may also be attributed to pollinators preferring to visit other, more attractive flowers in the community. However, *H. suffrutescens* is one of the first species to flower in early spring (M.L., per. obs.). Pollinator foraging behavior may also result in substantial self pollen deposition, resulting in pollen (quality) limitation in self incompatible plants (Aizen and Harder, 2007). Resource allocation can lead to the appearance of pollen limitation as many plants allocate more resources to pollinated flowers and ovules than to those that have yet to be pollinated (Ashman *et al.*, 2004). As the outcrossed and open control flowers opened around the same time and position on stems, and the hand-pollinated flowers were a very small number of the total flowers per plant, this is not likely to be the case. Additionally, due to the sample size (outcrossed = 687, open = 675), we are confident in the determination of pollen limitation during the study.

Although fewer seeds were produced in open control flowers, there was no difference in the weight of the seeds or germinability when compared to seeds from the outcross flowers. This suggests that although pollinator abundance or visitation may be reduced and perhaps limiting the quantity of seeds, the quality of seeds produced is not affected. While Aizen and Harder (2007) suggest that quality limitation may have the greatest impact on overall survival of populations, we find no evidence of quality limitation in our study. However, quality limitation can also appear at seedling establishment or additional survival stages (Herrera, 2000), which we were unable to study due to low survival of seedlings.

Three measures of reproduction (number of fruits, seeds, and seeds per fruit) in the Big Pack Mountain population were significantly reduced in 2011 when compared to 2010 and seeds were significantly lighter than those from the other two populations in 2010. A number of factors could explain this pattern. This population is the most impacted by oil and gas development with five active well pads in the vicinity of plants and a heavily used unpaved road that surrounds most of the population. During the second study year, another well was installed and the increased activity may have affected pollinator habitat and foraging behavior. Trant *et al.* (2010) found that increased anthropogenic disturbance led to decreased visitation and flower handling times of an endangered gentian in Canada. Similarly, Gómez *et al.* (2010) found that decreased pollinator diversity led to increased pollen limitation. Interestingly, pollen limitation in Big Pack Mountain increased from 0.13 in 2010 to 0.77 in 2011 (Table 3). As many of the native bees are ground nesters, perhaps they were affected by the well installation thus decreasing flower visitation in 2011. However, the lack of a significant *treatment*population*year* interaction demonstrates the decreased

reproduction in Big Pack Mountain in 2011 was found in both the outcross and open treatments suggesting potential impacts independent of pollinators (*e.g.*, resource availability and/or allocation).

Seed weight can be directly related to the number of seeds produced as plants may invest in many small seeds, or few large seeds. In 2010, plants in Big Pack Mountain produced significantly more, and significantly lighter, seeds than the other two populations. The overall significant difference in mean seed weight of Big Pack Mountain seeds may be a reflection of the large number of smaller seeds produced in 2010. As with the reduction in fruits and seeds, differences between years were independent of treatment, suggesting the results are likely due to physiology, resource availability, or genetic incompatibility rather than pollinator changes.

GERMINATION REQUIREMENTS AND SURVIVAL

The germination study and field observations suggest that seeds overwinter in the soil and likely germinate in early spring when there is adequate soil moisture and temperatures are low. Our germination protocol appears quite successful as many seeds germinated in cold storage. While treatments did not differ in germination, the few seeds produced in the selfing treatments are perhaps a result of outcrossing (*i.e.*, contamination) from the beetles observed inside exclusion bags.

While overall survival was low, adding 50% by volume native soil appears to increase seedling growth and survival relative to using potting soil alone, although no statistical analyses were conducted. The native soil likely contains microbiota or nutrients essential for growth and survival of *H. suffrutescens*. A recent study on the soils of *H. suffrutescens* found, among other differences, significantly higher potassium levels in soils associated with plants than in adjacent soils (J. L. Boettinger and J. Baker, *in prep*). Perhaps potassium limits the survival and growth of containerized seedlings and further study is suggested.

POTENTIAL POLLINATORS

Many specimens that we collected are potential pollinators of *H. suffrutescens*. The Halictidae and Andrenidae bees likely provide outcross pollen, based on their foraging behavior. These species visit multiple flowers on a single plant before visiting other plants. The larger bees (*Halictus* spp. and *Andrena* spp.) contact the stigma with their head, thorax, and front legs while remaining in flowers for several seconds. The smaller “sweat bees” (*Dialictus* spp. and *Evyllaes* spp.) are small enough to completely enter flowers and thus many parts of their bodies contact the stigma. The small flies (Syrphidae, Diptera) observed are probably poor pollinators, as only the proboscises enter the flower, collecting only nectar. The unidentified beetles observed are likely poor outcrossers as they appear to remain on a single plant and likely have a limited dispersal range.

Few flower visitors were observed visiting *H. suffrutescens* flowers in 2011, likely due to climatic differences observed during the two study years. In 2012, flower visitation was greater than in 2011, and insect abundance appeared greater than observed in initial study year (2010).

IMPLICATIONS FOR CONSERVATION

Our study indicates that *H. suffrutescens* requires pollinators for successful reproduction while suggesting that pollinators limited reproduction during 2010 and 2011. Additionally, we found evidence of decreased reproduction in Big Pack Mountain during 2011, possibly due to increased anthropogenic activity (*i.e.*, a new well installation). Further study of the impacts these anthropogenic disturbances have on the plants in Big Pack Mountain is

needed. Just as importantly, investigation into the potential impacts of increased mineral extraction on *H. suffrutescens* pollinator abundance and behavior is suggested. As many of these potential pollinators nest in the ground, limiting any disturbance that greatly alters soil conditions (e.g., roads, oil pads) near populations of *H. suffrutescens* is suggested. Finally, additional efforts should identify and protect pollinator habitat from such disturbances.

The germination trials highlight potential for successful *ex situ* conservation efforts, as seeds are easily germinable in common greenhouse conditions. Additional studies are needed on seedling survival, although our preliminary observations indicate simple soil modifications may increase survival. Managers should use native soil whenever possible, or create soil mixes similar in composition to those found in *H. suffrutescens* habitat. With additional studies underway, managers may soon be able to create specific soils, potentially increasing greenhouse propagation success while providing healthy plants with which to begin successful conservation and restoration efforts.

Overall, our study highlights the importance of including native pollinators and their habitat in the conservation of this endangered shrub while suggesting potential impacts of energy development on successful reproduction. Successful conservation of endangered plants including *H. suffrutescens* not only requires protection and conservation of their habitat, but that of their pollinators as well.

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