EFFECTS OF POLLEN SOURCE AND ABUNDANCE ON FITNESS OF A CARNIVOROUS PLANT

by

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EFFECTS OF POLLEN SOURCE AND ABUNDANCE

ON FITNESS OF A CARNIVOROUS PLANT

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ABSTRACT

Plants with fragmented populations and those living in threatened habitats may require repatriation efforts to maintain healthy populations. Populations of *Sarracenia alata,* the pale pitcher plant, are severely fragmented, and the species is near threatened. A complete understanding of its reproduction will be crucial in establishing and maintaining healthy populations. The goals of this study were to determine 1) if *S. alata* is capable of selfing (reproducing with pollen from the same individual) and autogamy (selfing without pollen transfer by an outside source); 2) whether fitness differs between selfed and outcrossed plants; and 3) whether pollen load affects reproductive success. We used seed set to measure individual fitness. Thus, it was necessary to determine a reliable method of counting seeds. Two methods (DotDotGoose and Image J) were examined, and these gave statistically similar results. We found that while *S. alata* is capable of selfing, it is not autogamous. Seed set was significantly higher in outcrossed individuals than in selfed individuals. In 2019, supplemental pollen resulted in increased seed production compared to control and pollen restricted groups. During 2021, there was no significant difference between the number of seeds produced by control plants and those receiving supplemental pollen. This study demonstrates the important role of pollinators in maintaining healthy populations in this system.

INTRODUCTION

Numerous plant species have been negatively affected by human encroachment and land-use changes. Disruption of habitats has led to fragmented communities that struggle to maintain healthy population numbers. With heavy fragmentation of populations and small population sizes, these plants are more susceptible to the negative effects of genetic drift (random loss of alleles) and inbreeding (reproduction with close relatives, which can lead to the expression of deleterious recessive alleles). Understanding plant reproduction is essential to understanding how to mitigate these problems (Cross et al. 2020). A key step in plant reproduction is pollination.

Pollination plays a significant role in determining the fitness of an individual. During pollination, pollen is transferred from the male reproductive organ (anther) to the receptive surface of the female reproductive organ (stigma). This transfer can be facilitated by abiotic factors like wind, or biotic factors like moths, beetles, and bees (Yang 2012). The quantity and source of pollen transferred to the stigmatic surface can determine the number and genetic quality of offspring produced (Aizen & Harder 2007). This information is useful when constructing strategies to repatriate populations of plants.

The wetlands of Mississippi, Alabama, Louisiana, and Texas have been fragmented or destroyed due to land use changes and ecological degradation. Among the plant populations that inhabit these wetlands are carnivorous plants. Carnivorous plants are considered markers of ecological degradation due to their specialization for oligotrophic (nutrient-poor) wetland habitats. As these habitats are destroyed due to human-caused disturbances carnivorous plants will be among the first to disappear (Sheridan & Karowe 2000). Furthermore, small, fragmented populations are susceptible to the negative effects of genetic drift, which could exacerbate population shrinkage (Barrett 1991). *Sarracenia alata,* the pale pitcher plant, is among the species living in these habitats (Schnell 2002). Repatriation efforts may be necessary in the future to ensure survival of the remaining populations of *S. alata*.

Selfing, autogamy, and outcrossing have been extensively studied in other species. Self-compatibility and autogamy (self-pollination without a pollen vector) are common in plants, reducing dependence on scarce pollen vectors (Larson & Barrett 2000). Typically, inbreeding depression from self pollen or pollen from a close relative results in reduced seed production compared to outcrossed pollen (Charlesworth and Charlesworth 1987). Self-compatibility has been observed in *Sarracenia flava,* while *Sarracenia purpurea* exhibits both self-compatibility and autogamy (Sheridan & Karowe 2000, Schnell 1983, Ne'eman et al. 2006). The self-compatibility of *Sarracenia flava* comes at the cost of reduced seed production compared to outcrossed groups (Sheridan and Karowe 2000).

Pollen limitation has not been well studied in carnivorous plants. Pollen limitation typically reduces seed production in flowering plants (Larson & Barrett 2000), and current studies on the effects of pollen limitation indicate that 63% of species exhibit some level of pollen limitation (Knight et al 2005). *Sarracenia purpurea*, a species of carnivorous plant, is frequently pollen limited (Ne'eman et al. 2006). Kang (2020) examined pollen limitation in *S. alata*, but sample sizes were small due to loss of samples from uncontrollable factors (Kang 2020).

The purpose of this study was to determine if *S. alata* is capable of self-pollination (selfing), and if self-pollination occurs without intervention by a pollinator (autogamy). A second goal was to examine the reproductive outcomes of selfed vs. outcrossed individuals to determine if there is an effect of selfing on fitness. Finally, we examined whether pollen limitation influences seed set in *S. alata*. Ultimately, these results will contribute to the understanding of the reproductive process in *S. alata.*

METHODS

Study Species

Sarracenia alata is a carnivorous plant found in nutrient-poor bogs across Texas, Mississippi, Louisiana, and Alabama (Schnell 2002). Individuals usually produce one flower, with petals that interdigitate with a stylar umbrella. This floral design forces pollinators to crawl through gaps in the interdigitation between the petals and style to access the anthers and pollen located inside the flower. As pollinators pass through this gap, they brush along the stigmatic surface, allowing for pollination.

Study Site

The study was conducted in a private plot of land in Leon County, Texas. The surrounding area is a post oak savannah, with loblolly pine (*Pinus taeda*) and post oak (*Quercus stellata*) populations. The bog is populated with willows (*Salix spp*.), maples (*Acer spp*.), grasses, and non-carnivorous flowering plants. Two other carnivorous genera, sundews (*Drocera spp*.) and bladderworts (*Utricularia spp*.), occur alongside the study species *Sarracenia alata*.

Self-compatibility vs Autogamy

We examined the ability of *Sarracenia alata* to self-fertilize autogamously, and when hand-pollinated with self pollen. Individuals were randomly assigned to the hand-pollination and autogamous groups. Each group contained 25 individuals. Flowers in both treatments were protected from pollination by covering their flowers with pollinator-excluding bags on 8 April, 2019, before flowers opened. Flowers selected in the hand-pollination group were un-bagged, loaded with excess self pollen on the stigmatic surface using a cotton swab, then re-bagged. Replicates 1-20 of the hand-pollinated group received treatment on 14 April, 2019. Replicates 21-25 of the hand-pollinated group received treatment on 19 April, 2019. Flowers in the autogamous group remained covered for the duration of the experiment. Both groups were protected from herbivory by cutting a straw lengthwise, placing it around the peduncle, and covering the straw with Tanglefoot ™, a sticky pesticide. All samples were collected on 29 September 2019.

Selfing vs. Outcrossing

To compare the fitness of selfing to outcrossing, a random selection of six selfed flowers from the previous experiment was compared to an outcrossed group. Outcrossed flowers (n = 35) were selected and treated on 12 and 19 April, 2019 and hand pollinated with a mixture of pollen from ten donor flowers. The samples were collected on 29 September, 2019. Because of losses due to floral herbivores, large animals (deer and feral hogs), and heavy rainfall events, only six outcrossed pollen recipients were recovered.

Pollen Limitation

We randomly selected 20 flowers for each of three treatments (supplemented pollen, control, and restricted pollen). Pollen from 10 randomly selected donors (none of which was also a pollen recipient) was collected and mixed. Excess pollen was loaded on the stigmatic surface of flowers in the supplemented treatment. Pollen was delivered dry via cotton swab. These supplemented individuals were treated shortly after flower opening and were left open for eight weeks before flowers were covered in a pollinator excluding bag. Flowers in the control group were open to pollinators for eight weeks and received no supplemental pollen. Eight weeks after opening, control flowers were covered with pollinator exclusion bags to protect them from herbivory. Individuals in the restricted group were covered in a pollinator excluding bag approximately one week after opening. This bag remained in place for the duration of the experiment.

This experiment was performed twice, once in 2019 and once in 2021. In 2019, dehiscence of anthers began on 8 April (replicates 1-25) and 14 April (replicates 26-35). The supplemented group of 2019 received pollen on 14 April, 2019 (replicates 1-25) and 19 April, 2019 (replicates 26-35). This group was bagged on 13 May, 2019. The control group was also bagged on 13 May, 2019. Restricted flowers were bagged on 14 April (replicates 1-25) and 19 April (replicates 26-35). Mature ovaries from all samples were collected on 29 September.

During the 2021 season, individuals in the supplemented group received pollen from 10 random non-participating flowers on 18 April and were bagged on 27 May. Control flowers were bagged on 27 May. Flowers in the restricted group were bagged on 3 April. Bags on flowers in the restricted pollen group were removed on 11 April and replaced on 18 April. Mature ovaries from all treatments were collected on 9 October.

Dissection and seed counting

Sample ovaries were removed from the peduncle, and petals and sepals were removed. Height, diameter, and mass of the ovaries were measured. Ovaries are segmented into five sections (locules), that can be taken apart individually. Each locule was examined for evidence of herbivory damage before processing. The number of intact and damaged locules was recorded for each ovary. The contents of undamaged locules were collected; contents of damaged locules were excluded. The walls of intact locules were removed, and seeds and ovules were collected and sorted by hand and by 500-µm sieve. The total mass of seeds was recorded. Seeds and ovules for each sample were scanned and counted.

A correction factor was incorporated into the seed mass and seed and ovule counts for each sample, depending on how many locules were damaged (Multiplier = $\frac{5}{\#\ \text{Intact Locules}}$).

Using scans of seeds (n = 12) and ovules (n = 12) from previous experiments, we compared the accuracy of an automated count (using the software ImageJ) to the results of computer-assisted hand counting (using the software DotDotGoose). Images counted using imageJ used the following parameters:

Size:
- Seeds: 0.6-infinity (mm)
- Ovules: 0.02-0.4 (mm)

Circularity:
- Seeds: 0.4-0.9
- Ovules: 0.5-0.98

Data Analysis

A Wilcoxon signed-rank test was used to compare seed and ovule counts collected from imageJ and DotDotGoose. Seed count and the proportion of ovules fertilized for the self-compatibility vs. autogamy and the selfed vs. outcrossed experiments were compared using two-sample t-tests assuming equal variances with an alpha level of 0.05. Seed count and proportion of ovules fertilized of restricted, control, and supplemented pollen groups were compared using single factor ANOVA tests with an alpha level of 0.05.

RESULTS

Counting Methods

There was no statistically significant difference between the counts obtained by ImageJ and DotDotGoose for either seeds (Figure 1) or ovules (Figure 2).

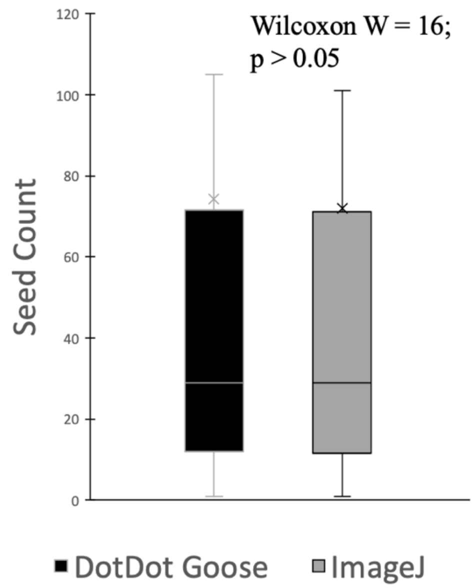

Figure 1: Comparison of seed counting methods (n = 12). Bars represent minimum and maximum values. Box represents 1st to 4th quartile range, horizontal lines represent medians, "x" represents mean. One outlier excluded.

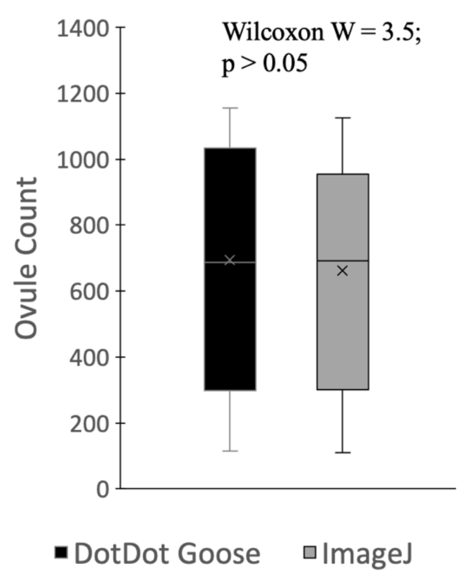

Figure 2: Comparison of ovule counting methods (n = 12). Bars represent minimum and maximum values. Box represents 1st to 4th quartile range, horizontal lines represent medians, "x" represents mean. One outlier excluded.

Self-compatibility vs. Autogamy

Hand-pollinated selfing yielded a significantly higher seed count compared to autogamously self-pollinating individuals (Figure 3).

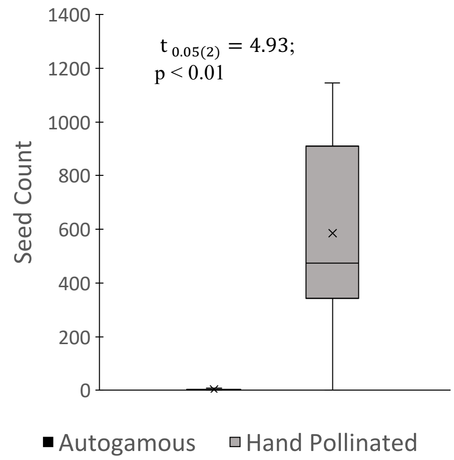

Figure 3: Total seed count in flowers with pollinators excluded and not receiving supplemental hand pollination (n = 13) and flowers with pollinators excluded but receiving supplemental hand pollination with self pollen (n = 14). One outlier was excluded in autogamous group. Bars represent minimum and maximum values. Box represents 1st to 4th quartile range, lines represent medians, "x" represents mean.

Selfing vs. Outcrossing

Flowers receiving excess outcrossed pollen yielded significantly higher seed production compared to flowers receiving excess self pollen (Figure 4).

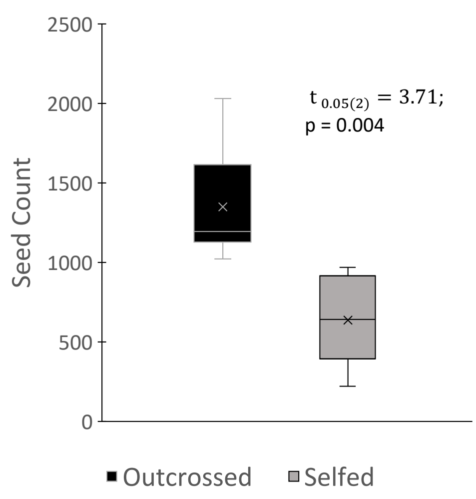

Figure 4: Seed production of flowers receiving outcrossed pollen (n = 6) and self pollen (n = 6). Bars represent minimum and maximum values. Box represents 1st to 4th quartile range, lines represent medians, "x" represents mean.

Pollen Limitation

Flowers in the restricted and control groups produced significantly fewer seeds than those in the supplemented group in 2019 (Figure 5). In 2021, there was no significant difference in the number of seeds produced by flowers in the three groups (Figure 6).

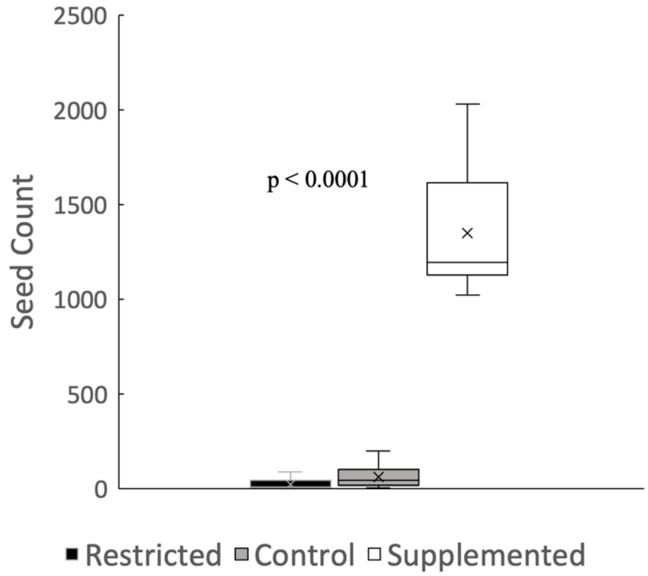

Figure 5: Seed production of restricted (n = 16), control (n = 9), and supplemented (n = 6) flowers in 2019. Bars represent minimum and maximum values. Box represents 1st to 4th quartile range, lines represent medians, "x" represents mean.

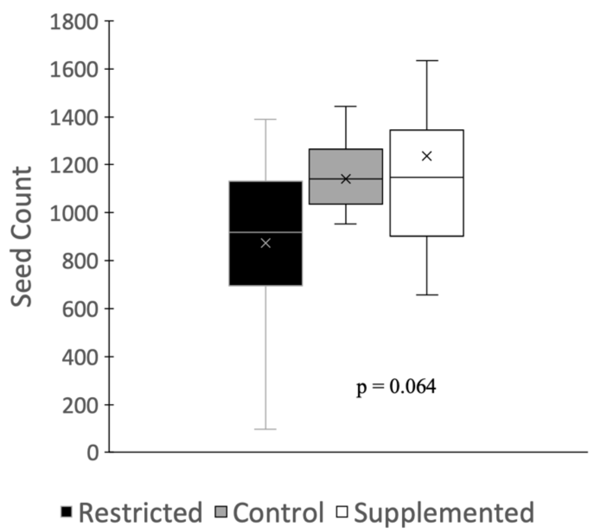

Figure 6: Seed production of restricted (n = 14), control (n = 16), and supplemented (n = 14) flowers in 2021. Bars represent minimum and maximum values. Box represents 1st to 4th quartile range, lines represent medians, "x" represents mean.

DISCUSSION

Populations of *Sarracenia alata* have become fragmented, raising concern about their future (Horner et al. 2014, Cross et al. 2020). Small, fragmented populations are susceptible to genetic drift, which may further reduce genetic variation and thereby fitness (Barrett 1991). Rapid decline of population size may also lead to inbreeding depression, which results in lower seed production (Lande & Shemske 1985). These negative effects may be compounded by alarming reduction in pollinator numbers, which can lead to pollen limitation (Rhodes 2018). Numerous plant species experience pollen limitation, and this effect has the potential to reduce seed set (Ashman 2004). Populations of *S. alata* will be threatened in the future if the habitat destruction of their native bogs continues. It is likely that ecologists and conservationists will require a complete understanding of the sexual reproduction of *S. alata* if rehabilitation efforts are needed. Thus, we examined the fitness of selfed and outcrossed flowers and pollen limitation of *S. alata*.

Seed and ovule production was used to quantify fitness, so a reliable method of quantifying seeds and ovules was established. Two methods to quantify seeds and ovules were compared. DotDotGoose, software that assists hand counting, was compared to ImageJ, software that automatically counts seeds or ovules. While ImageJ can work automatically, parameters for roundness, size, and color saturation must be established. Additionally, correction factors for clumps of ovules or seeds must be applied. Despite being more labor intensive, DotDotGoose does not require the establishment and checking of parameters that allow recognition of seeds and ovules. DotDotGoose ultimately provided faster counting, and the programs produced results with no statistically significant difference. Thus, either method can be used to return accurate counts.

Sarracenia alata flowers that were hand pollinated with their own pollen produced seeds. Therefore, *S. alata* appears to be self-compatible. However, selfing does not occur in the absence of biotic pollen vectors. Numerous plant species are known to be self-compatible and autogamous. While self-compatibility has been observed in both *S. purpurea* and *S. flava* (Sheridan & Karowe 2000, Schnell 1983, Ne'eman et al. 2006), only *S. purpurea* has been observed to have some degree of autogamy (Mandossian 1965, Schnell 1983). Selfed individuals produce fewer seeds than outcrossed individuals, indicating that selfing leads to a lower fitness compared to outcrossing. This effect has been also observed in *S. flava* (Sheridan and Karowe 2000).

Pollen limitation is capable of influencing seed output as well. In 2019, flowers in the supplemented group produced significantly more seeds than those in the control and restricted groups. Therefore, there was clear evidence for pollen limitation. Conversely, in 2021 there was no significant difference in seed production among groups and therefore no indication of pollen limitation in restricted and control groups. This variability in pollen limitation may be attributed to a change in the pollinator activity at the study site, potentially caused by a local change in pesticide or chemical use.

Future directions for study include the extent of pollen abundance and outcrossing required for maximal seed production. An over-abundance of pollen may potentially lead to a decrease in seed production. Populations of plants that are infrequently pollen limited may experience stigma or anther clogging when supplied with excess pollen, leading to a reduction in seed production (Holland & Chamberlain 2007). Under some conditions, outcrossing with individuals from great distances can lead to outbreeding depression due to differences in local adaptation (Frankham 2011). While the results of this study suggest that outcrossing and pollen supplementation during low pollinator years optimize seed production, further experiments may reveal if there is a limit to the distance and amount of pollen required.

CONCLUSION

The source and abundance of pollen can affect the reproductive output of a flower. While self-compatibility, autogamy, and the comparison of selfing to outcrossing have been studied in other *Sarracenia* species, they have not been studied in *Sarracenia alata*. The effects of pollen limitation have previously been addressed, but the study was plagued by small sample sizes. Flowers of *Sarracenia alata* were found to be self-compatible, but no seed production occurred when flowers were protected from pollinators and left undisturbed. Selfed flowers had lower fitness than outcrossed flowers. Flowers of *S. alata* experienced pollen limitation during 2019 but not during 2021.

This study examined factors that influence reproductive success in *S. alata*. This information could be used during rehabilitation of these fragmented populations by optimizing reproductive output. Some questions remain before the effects of pollen source and abundance on seed production is fully understood, namely if, and at what distance, outbreeding depression occurs, and if pollen over-supplementation can lead to a reduction in fitness.

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