Effect of Style Age, Resource Allocation, and Pulse Pollination on seed set of *E. angustifolia*

Ashley R. Barto

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I. Project description

Introduction

In this experiment, I will test how style persistence duration (style age) affects successful pollination and seed set as measured by style shriveling and x-ray, respectively. Further, my research will explain what prevents seed development, whether it be prior resource allocation within a head or pollen limitation. Studying pulse pollination may inform conservationists about successful species repopulation after disturbance. Additionally, understanding what limits seed development will clarify what effect low floral resources in isolated populations may have on seed production.

Background

My study species is *Echinacea angustifolia. E. angustifolia*, a Minnesotan native, is self-incompatible, which means pollen from one floret cannot successfully pollinate a style on a head on the same plant (Wagenius 2004). This means in my experiment, I will be able to control exactly when compatible pollen strikes each of the styles. Wagenius (2004) found that styles persist in the absence of pollen, so I know that by excluding pollen, I will be able to control when styles receive compatible pollen and thus know the age of the styles when I pollinate them.

Nice Island, a remnant *E. angustifolia* population, is an ideal location my study. It is located near the research base, so I can walk to it daily, and there are many Echinacea I can use as experimental plants and pollen sires. Additionally, because the site is between two agricultural fields, I may include plants affected by herbicide in my study. While I will not know which plants are affected, those some without major deformities but in range of herbicide spray will be included and broaden the applicability of my findings.

Based on the findings of Wagenius (2010), *E. angustifolia* reproduction is limited by pollen and not pollinators, so my conclusions will be useful to inform conservationists on the relative importance of saving isolated patches of prairie where pollen limitation may influence the capacity of a population to reproduce.

Goals of research

My primary question for my pollen exclusion experiment: How does style age affect successful pollination and seed set development? If style age influences shrivel rate and seed set, I hypothesize style shrivel rate and seed set will decrease in older styles. This means I anticipate younger styles to be more successful. I also ask how

prior resource allocation affects shrivel rate and seed set development. If prior resource allocation impacts shrivel rate and seed set, I anticipate Row 7 in the pulse pollination treatment to have a higher shrivel rate and larger seed set than the Row 7 of the steady pollination treatment because Row 7 of the steady pollination treatment will have six rows previously pollinated below it. Finally, I ask if there is a difference in shrivel rate and seed set size between pulse pollinated and steady pollinated plants. If there is a difference between the two treatments, I anticipate shrivel rate and seed set to be higher in steady pollinated species because steady pollination emulates regularly available pollen.

Research plan

Objectives

Overall, my objective is to examine the effect of pulse pollination and style age on successful pollination and seed set development. Additionally, I aim to examine the effect of prior resource allocation on successful pollination and seed set development.

Methods and Procedures

Initially, I will need to identify suitable *Echinacea angustifolia* at Nice Island. Suitable plants will have minimal deformities—minimally tilted receptacles or bent stems—and will not have yet flowered. I will assign suitable Echinacea randomly to the steady or pulse pollination treatments. I anticipate I will need 10 to 15 heads of each treatment to have a large enough sample size, but 10 in each group would be acceptable. In total, I will need 20 heads minimum. Once I identify the eligible plants based on conspicuous deformities and developmental stage, I will need to put a pollen exclusion net over each plant's head. To expedite pollination, I will place a labeled flag by each plant.

Each day, I will collect pollen from *E. angustifolia* from at least 3 heads. The sires' locations may vary from day to day. The general timetable for a given head:

- **Day 1:** First head display anthers. Paint every bract with an anther pink in the first row for both Treatment 1 (steady) and Treatment 2 (pulse).
- **Day 2:** First styles present. Count styles present Row 1 for Treatment 1. If in Treatment 1 (steady), collect pollen from donors. Pollinate present styles on the first row. If in Treatment 2 (pulse) wait until Day 7.
- **Day 3:** Second row styles present. Count styles present in Row 2 for Treatment 1. If in Treatment 1 (steady), count number of withered styles in Row 1. Collect pollen like in Day 1 and pollinate Styles in the second row. Paint bracts in Row 3 with anthers white for both Treatment 1 (steady) and 2 (pulse).
- **Day 4:** Third row styles present. Count styles present in Row 3 for Treatment 1. If in Treatment 1 (steady), count number of withered styles in Rows 1 and 2. For Row 1, the total number of shriveled styles today is total % shriveled. Collect pollen like in Days 1 and 2, and pollinate Row 3.
- **Day 5:** Fourth row styles present. Count styles present in Row 4 for Treatment 1. If in Treatment 1 (steady), count number of withered styles in Rows 2 and 3. For Row 2, the total number of shriveled styles today is total % shriveled. Collect pollen like in previous

days, and pollinate Row 4. Paint bracts in Row 5 with anthers turquoise for both Treatment 1 (steady) and 2 (pulse).

- **Day 6:** Fifth row styles present. Count styles present in Row 5 for Treatment 1. If in Treatment 1 (steady), count number of withered styles in Rows 3 and 4. For Row 3, the total number of shriveled styles today is total % shriveled. Collect pollen like in previous days, and pollinate Row 5.
- **Day 7:** Sixth row styles present. Count styles present in Row 6 for Treatment 1. If in Treatment 1 (steady), count number of withered styles in Rows 4 and 5. For Row 5, the total number of shriveled styles today is the total % shriveled. Collect pollen like in previous days, and pollinate Row 6. If in Treatment 2 (pulse), count the number of styles in each row. Pollinate all rows at once. Paint bracts in Row 7 with anthers green for both Treatment 1 (steady) and 2 (pulse).
- **Day 8:** Seventh row styles present. Count styles present in Row 7 for Treatment 1. If in Treatment 1 (steady), count number of withered styles in Rows 5 and 6. For Row 6, the total number of shriveled styles today is the total % shriveled. Collect pollen like in previous days, and pollinate Row 7. If in Treatment 2 (pulse), count the number of styles in each row. Pollinate all rows at once.
- **Day 9:** Observe Rows 6 and 7 in Treatment 1. All styles shriveled today in Row 6 of Treatment 1 is total % shriveled. Observe all rows in Treatment 2, and count how many styles are shriveled in each row.
  Day 10: Observe Row 7 in Treatment 1. All styles shriveled today in this row is the total % shriveled. Observe all rows in Treatment 2, and count how many styles are shriveled in each row. This is the total % shriveled in these rows.

Following pollination, I will make daily observations on the styles' condition. If the style shrivels, I will infer the plant was successfully pollinated.

To observe successful seed production, x-ray technology at the Chicago Botanic Garden will be used.

For analysis, I hope to use a Generalized Linear Model to test what which variables affect style shriveling and seed set. Additionally, a simple test is needed to identify which pollination treatment, pulse or steady, produces a larger seed set under pollen exclusion stress. Finally, an analysis to see which variables interacted will inform me of the ideal condition for *E. angustifolia* pollination.

Projected Outcomes

I anticipate I will have data that reflects the reproductive success of Echinacea under pollen exclusion and demonstrates how style age, resource availability, and pollination treatment affect seed set.

Significance

Intellectual Merit

This experiment will contribute to the general understanding of limits on plant reproduction. Further, understanding how pollen exclusion in particular affects seed development will be useful for conservationists who wish to restore communities.

Overall, this experiment will facilitate answering the question of whether it is worth it to preserve isolated patches with fewer floral resources that may not be pollinated. For the Echinacea Project, understanding how seed development is influenced by time elapsed will allow future members know when to complete their crosses in experiments for optimal seed production.

Broader Impacts (products and professional objectives)

I intend to create a poster from my research this summer. Besides learning how to create a professional presentation for poster sessions, I anticipate I will develop valuable skills in experimental design, data collection, and data analysis. Even more, I will learn how to complete artificial crosses. These skills will help me throughout graduate school and beyond.

II. References

Wagenius, S. & Lyon, S. P. (2010). Reproduction of *Echinacea angustifolia* in fragmented prairie is pollen-limited but not pollinator-limited. *Ecology*, *91*(3): 733-742.

Wagenius, S. (2004). Style persistence, pollen limitation, and seed set in the common prairie plant *Echinacea angustifolia* (Asteraceae). *International Journal of Plant Science*, *165*(4): 595-603.

III. Timeline for the proposed research

Optimally, I will start my research by Monday, July 11. Identification of ideal plants and set-up will take only a couple hours. For each head, I have to observe florets for 9 days, so if all of them flower on the same day, I will be done by July 20. If flowering happens over 2 weeks, meaning the last flowers starts to flower on July 25, I will be done observing flowers by August 3.

IV. Data management plan

To collect data, I will visit each plant daily and record its status. If flowering, I will note the date of its first anther, and the date at which each row's styles emerge and are pollinated. From that I will be able to calculate style age at pollination. As I pollinate the styles, I will count the number in each row. I will count the same row's shriveled anthers the next day to calculate percent shriveled.

Data can be easily managed in an Excel file and exported as a .csv file. R will be a valuable tool to run GLM analysis as discussed above. To use R, I will need guidance from an experienced member of Team Echinacea.

I intend to present my project at poster sessions. One potential session is the Junior Academy of Sciences in Arkansas and other sessions may include the Spring National Honors Collegiate Meeting and the South Eastern or Midwestern Ecology and Evolution Conference.

V. Environmental impacts (of the proposed research)

While field observations can be made with few negative implications for prairie communities, trampling of surrounding plants is inevitable. Additionally, to collect seed set data, Echinacea heads will be removed; therefore, seed availability in local prairie populations will be reduced. Finally, pollinators will lose floral resources, as I use pollen in my experiment and exclude pollinators from accessing Echinacea.