Sky Islands Pollination Protocol 2021

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ABSTRACT

Goals:
1. Measure seed set on 7 focal plants per meadow
a) a single visit from pollinator (SV, red)
b) over an entire reproductive period from open pollination (OP treatment, yellow)
c) pollinator exclusion (PE treatment, white)
2. Measure how much pollen a pollinator visitor is carrying
3. Record visitation for single visit and all visits across a day

Timing: You want to complete the experiment two times at each site. Steps 1-6 should occur early in the morning.

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Supplies

1 In the field pollination monitoring:
1. **Pollination datasheet**
2. **Fruit monitoring datasheet**
3. **Specimen datasheet**
4. Nylon bag covers
5. 5 colored strings/hoops (yellow, green, blue, red, purple)
6. White pin flags
7. Pre numbered stickers
8. Sterile screw-top vials
9. Transect tape

10. GPS
11. PVC quadrants
12. Video camera (gropro-like camera)

In the lab: fruit collection

1. **Fruit processing datasheet**
2. Ziplock bags
3. Sterile screw-top vials
4. Dissecting blade
5. Tweezers
6. Tally counters

Focal plants

2

1. *Campanula rotundifolia (CAMROT)*
2. *Erigeron spp. (ERISPP)*
3. *Gentiana affinis (GENAFF)*
4. *Erysimum capitatum(ERYCAP)*
5. *Penstamon strictus (PENSTR)*
6. *Hymenoxys hoopesii(HYMHOO)*
7. *Potentilla hippiana (POTHIP)*

Gentiana affinis

Erysimum capitatum

Penstemon strictus

Hymenoxys hoopesii

Campanula rotundifolia

Potentilla hippiana

Erigeron spp.

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Set up

3 Set up should occur in the morning before pollinator sampling, or in the evening the day you arrive at a site.

3.1 You want to find up to 10 plants from each of the 7 focal plant species at a site (sites will not have all species). Prioritize plants that are outside of the area where bees are being collected. Prioritize plants that look healthy and have only a few neighbors. Demarcate the plants with flags/field tape that are labeled with unique numbers, pre-printed numeric labels. In the pollination datasheet label each plant by the individual number (1-2000), the site name, plant abbreviation (above). For example, if you are at site JC, and find your first Gentiana, it will be labeled 0001_JC_GENAFF

3.2 Using your GPS, walk a path between each plant. Start a pathway of waypoints for each plant. Label each location on the GPS as the site, plant abbreviation, and the number of the plant. (e.g. 0001_JC_GENAFF)

3.3 For each plant, measure the distance to its nearest neighbor using the transect tape, and identify that neighbor if possible.

3.4 Place a 1x1 m quadrant with the selected plant at the center of the quadrant. Take a bird's eye view picture of the entire quadrat and note what number the picture is on the camera roll.

3.5 For each plant, record the following data in the pollination datasheet: number of closed buds, number of open buds/flowers, number of fruit (finished flowers with an enlarged endosperm), plant height to the highest tip (cm), plant width at the widest point (cm). We are measuring width and height in order to estimate plant volume. For the number of flowers, count inflorescences for ERISPP and HYMHOO as 1 flower.

Penstemon fruit example

Erigeron seed pod

Gentina pod (opened)

Hymenoxys mature seeds

Penstemon seed pods

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Potentilla seed pods

3.6 Bag every bud on the plant or unopened inflorescence using the nylon bags. You want to do this in the morning before sampling while the pollinators are still sleepy. Pick buds that look about to open. Note the date and time each bag is bagged on the datasheet. If the plant has inflorescences, you want to bag the entire inflorescence. However, use red string to mark out a single bud within the inflorescence that you want to focus on and take data on the number of open flowers and buds on that infloresence on the datasheet.

Experiment

4 To conduct the following steps, you want to use buds that have recently opened (within 12 hours) because this is when the plant is receptive to pollinators. Do not use buds if you are unsure of when they opened, as buds should not be open for more than a full day.

Keep checking on your buds periodically. When the bagged buds have opened (either in the afternoon or the next day) you will do the following steps (If bud hasn't opened, keep bag on until it does):

4.1 For these newly open buds, randomly assign each bag into three different treatments using different strings/hoops of different colors at the base of the flower. Each plant should have all three treatments. Markdown the date you do this as the “experiment date” on the datasheet and the single treatment assigned to each plant.

NOTE: For flowers with spikes, ideally each bud of a different treatment would be on a different spike.

a. red string (SV, single visit)
b. yellow string (OP, open pollination)
c. blue string (PE, pollinators excluded)

For each plant, there should only be one set of treatments.

4.2 **Open pollination treatment (OP):**

- Unbag all OP buds (leaving the yellow string on them). For other bagged buds on the OP plant, leave them bagged.
- Set up the video camera on a tripod and start recording. Start eh video by displaying the flag with the plant number.
- After 8 hours have passed, re-bag all OP buds on all plants and add a green hoop (leave the yellow)

4.3 **Single-visit treatment (SV):**

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- Unbag all SV buds (leaving the red-colored strings/hoop).

- Watch as a pollinator visit, allow it to visit. Right as it leaves capture the pollinator (very carefully, without touching the flower!) and place into a pre-sterilzed tube.

- Label the flower visitor tube with the following:

1. plant name (e.g. 0001_JC_GENAFF)
2. treatment (SV)
3. a general id for the insect if you know it (ex: syrphid, bee, wasp, Apis, Bombus).

- Place specimen in a tube then into a ziplock bag (labeled Poll exp) to put in your fanny. At the end of the day, process these specimens the same way as netted specimens: add a label, put into a freezer box, then into cooler, fill out specimen datasheet.

- Re-bag the visited SV bud with the nylon fabric bag. Label the bud with a **green** hoop or string (leave the red there as well) so you know this bud received a visitor.

- If your SV bud does not receive a visitor in the time that you're observing, add a green string/hoop and mark on that pollination sheet that it was never visited. Start the process over with a new unopened bud on that same plant. As you do the experiment over days, randomize the order you monitor plants. Do not spend more than one hour observing a plant for a single visit.

NOTE: make sure to complete your OP treatment after doing your single visit monitoring.

4.4 For one plant of each species at each site (7 plants per site max), mark an open or enclosed bud to monitor for fruit growth with a purple string. Record dates on “fruit monitoring datasheet”. Include drawings and detailed notes. We want to harvest the fruit and pods off each time at the same time for each plant species. Re-visit your plant each sampling round. We are currently unsure of how long it will take the different species to mature.

4.5
- When fruit/seed is mature/almost mature (pick a stage for each plant and stick with it), remove the bags and collect the fruit into a ziplock labeled by the plant label and treatment (e.g. 0001_JC_GENAFF_SV).

- Additionally, for each plant, record the following data in the pollination datasheet: number of closed buds, number of open buds/flowers, number of finishing/finished flowers, number of fruit

Fruit processing

5 After harvesting fruits from the meadows, bring them back to the lab.

5.1 Open each fruit and count all healthy-looking, fully formed seeds. Record the number of seeds on the **fruit processing datasheet.**

5.2
- Place all seeds into sterile, screw-top vials, which you will label with the plant label and treatment (0001_JC_GENAFF_SV)

5.3 Put in freezer boxes and store seeds in the -80.

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