Flower Preparation Protocol

Materials:

- Fine tip forceps
- Shovel-end forceps or Scoopula
- 1 mL Glass tubes
- Parafilm
- Gloves
- Water
- Permanent marker
- Flower material
- Micropipette (200-1000 µl)
- Test tube racks
- 5 mL Travel tubes with modified lids (see Figure 1)
- Paper towels
- Protocol checklist
- Large magnifying glass with light
- Glacial acetic acid
- Glass micropipette tips

Note 1: There should be two sets of 24 tubes for each batch. Both sets will be prepared identically. After processing, one set will be used in mounting for light microscopy and the other set will be used for SEM photography. This will allow for ample pollen for each method.

Note 2: The flower material may need to be broken up in order to fit ideally into the tube. If so, try to break the material as little as possible to minimize debris. Also, if it is necessary to break up the material, try to do so within the tube to capture any pollen that may come loose from the flowers.

Note 3: If possible, only use a portion of the flower material in the packet. Any remaining material can be used for second preparation, if necessary.

Note 4: Prior to the first preparation of the day, be sure to thoroughly wash all tools to ensure that they are clean before the preparation begins. If there is any doubt about the cleanliness of the tools or workstation, thoroughly re-clean everything.

Tube Preparation:

1. Label the two sets of 24 plastic tubes with the processing code number with a permanent market. Each tube should be labeled with the batch number and the tube number. For example Batch 75, Tube 3 would be labeled with code 75-3. This method should be used for labeling all of the tubes in the batch (see Figure 1).

Figure 1: Left: Example of numbered tube, Right: Test tube rack set up

2. Place a lid with a corresponding number for that sample onto each tube. Lids are numbered in the general format of 1 through 24 to allow for reuse. There should be 24 samples per batch, prepared as two sets.
3. Place a clean 1 mL glass tube upside down into the numbered tube.
4. Put each set of tubes (numbered 1 through 24) into the test tube rack, leaving a vacant space in between each tube (see Figure 1).

Flower Preparation:

1. Be sure the workstation is clean and free of open air flow.
2. Gather a group of 24 samples to process, typically categorized by herbarium, family and then genus. Try to use samples from the same family. Work through an entire family before starting the next. If there are not enough samples from a single family, use multiple families. Try to keep all of the samples from a single family together throughout processing.
3. Write the batch "prep" number, the family, as well as the genus and species for each sample on the 'POLLEN PREPARATION PROTOCOL' sheet.
4. Select the first sample and label the outside of the envelope with the appropriate code number. Write the genus and species of the sample onto the first available space on the protocol sheet. Wear gloves from this step forward.
5. Place a clean paper towel on the counter of the workstation. Adjust the magnifying glass to the appropriate level for viewing. Place both of the corresponding numbered tubes onto the paper towel for ease of access (see Figure 2).
6. Open the envelope containing the flower material and dump the contents onto the clean paper towel.

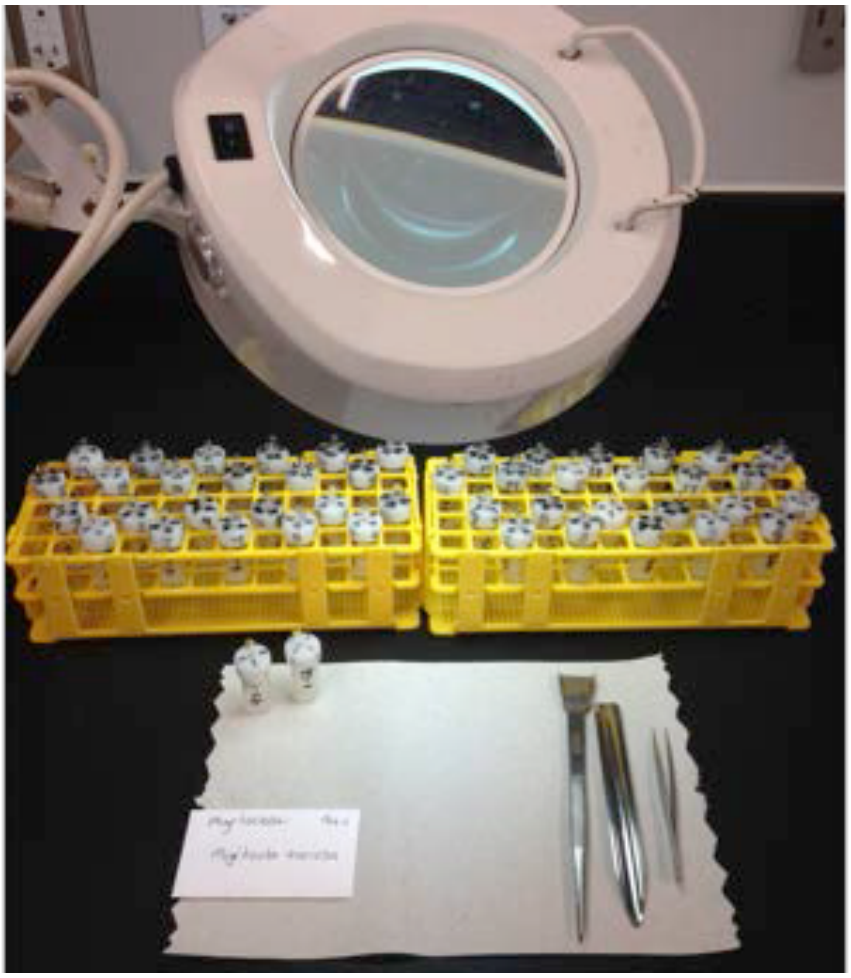

Figure 2: Workstation set up

7. Examine the contents. Search for anthers or flower material that is most likely to contain pollen. If the flowers are very small, the entire flower can be placed into the tube. If the flowers are large, place anthers and small portions of plant material into the tubes. If whole flowers or anthers are not present, use any plant material in the packet that may have pollen on it.
8. Gather the desired material with the metal tools and place into the glass tube. Equal amounts should be placed into each of the two tubes. Each tube should be filled no more than 1/2 full. An ideal tube is approximately 1/3 full.
9. Place the tube with plant material back into the test tube rack.
10. Cover the tube with small piece of parafilm or a clean paper towel to prevent contamination.
11. Place the remaining flower material back into the envelope and reseal it with the use of labels or tape. Set the envelope aside to store with the samples that have been processed.

12. Throw away the contaminated paper towel.
13. Wash all tools and gloves. Dry thoroughly.
14. Repeat this processing for the remaining samples in the batch.
15. Place the stack of plant material envelopes into the proper storage container for future use.
16. Wipe down the entire workstation with damp paper towels to remove any plant material that may have contaminated the area.

Adding the Glacial Acetic Acid:

1. Transport the prepared tubes to the fume hood, keeping the tubes covered to prevent contamination.
2. Uncover the tubes.
3. Using a micropipette, place 400 µl of 100% glacial acetic acid into each tube. Be careful not to let the glass tip of the micropipette touch the inside or outside of the glass tube. If the tip touches the tube, replace it with a clean tip to prevent contamination.
4. Thoroughly wrap each tube with parafilm.
5. Allow the samples to sit overnight before processing.