Using *Zea mays* pollen to introduce undergraduate students to the dynamic development of pollen tubes within an Introductory Biology laboratory curriculum

-- Wing, Kieslana M; Hamilton, Brooke L; Kayes, Lori J; Fowler, John E
Oregon State University
CORVALLIS, OREGON

Rationale

We developed a lab activity (a portion of a larger lab on plant anatomy and life cycles) in our institution's Introductory Biology series to investigate maize pollen germination *in vitro*. The objectives of this activity are: to provide students with continued practice using microscopes; to provide experience with the process of science (collecting and summarizing quantitative data, drawing conclusions from data, and developing hypotheses based on observations); to explore how structures of flowers relate to plant reproduction; to demonstrate how pollen and pollen tubes are active biologically; and finally, to link the research and teaching missions at an R1 institution. Additionally, the pollen tube activity fits within the broader set of labs in the course that compare plant life cycles and anatomical structures, and ties to the evolutionary history of organisms in general. For example, students look at the structures of fungal hyphae and moss protonema to determine the similarities with pollen tubes. The lab also provides a very brief introduction to plant developmental biology, with emphasis on two stages of pollen development: the dormant grain stage and the active tube stage. These stages have contributed to the success of seed plants on land (a major theme in the evolution of plants). Finally, we suggest that this activity could be adapted to a more advanced course (e.g., cell biology), as inhibitor studies (e.g., affecting the cytoskeleton, translation, etc.) are straightforward in liquid culture, and effects are visible within a short time period (e.g., Gibbon et al. 1999).

This lab activity always falls during late October, when native plants are no longer producing pollen, necessitating the use of pollen from fresh flowers purchased from the florist (e.g., *Antirrhinum majus* (snapdragon), *Alstroemeria* sp. (Peruvian lily), or *Delphinium* sp. (larkspur)). However, students have had inconsistent and limited success at actual germination of this pollen, quite likely due to the pollen source. We thought that the maize pollen germination protocol developed for research purposes in the Fowler lab could be adapted for the undergraduate lab setting, and had promise to be more successful than our previous attempts. Because Dr. Fowler is also the instructor of this portion of the course, using maize additionally offered an opportunity to more clearly demonstrate the linkage between the institution's research and the teaching missions. We piloted the protocol in a subset of five labs with two graduate teaching assistants (GTAs) and forty students in each lab.

Aspects of maize pollen and genetics relevant to the activity

A number of features of maize pollen make it a particularly useful subject for an undergraduate lab activity: pollen grains are large (~100 µm in diameter); pollen tube germination occurs within 15 minutes of exposure to liquid medium; and pollen tube growth is rapid in liquid media (~200 µm in length after 30 min). Due to the large size and speed of growth, with higher resolution optics, organelles and cytoplasmic streaming are easily discernible in the growing tube. The required amounts of maize pollen can be easily collected from a few plants each day using

paper bags, and pollen can be handled easily when fresh and kept dry. Although germination rates in liquid media can be somewhat inconsistent, we have found rates of ~40-80% using a media recipe (below) adapted from Schreiber and Dresselhaus (2003). One major hindrance to the use of maize as a pollen source in the past was the need to grow the plants to flowering (~3 months) in a large greenhouse. However, the recent development of Fast-Flowering Mini-Maize (FFMM) (McCaw et al. 2016), which is small in stature (~80-90 cm) and reaches anthesis in ~5 weeks, provides an feasible alternative which is more suitable to the resources available to an Intro Biology curriculum. Because FFMM tassels are relatively small and pollen shed can be limited, the Fowler lab has adopted a W22/FFMM hybrid (stature: ~100-150 cm; time-to- anthesis: ~9 weeks) for its research needs, as these plants generate larger tassels which produce significant amounts of pollen for ~5 consecutive days. Pollen from this hybrid was used for the undergraduate activity; small amounts of hybrid seed are available upon request (fowlerj@science.oregonstate.edu).

Experiences in the teaching lab setting

GTAs with previous experience in running pollination lab activities piloted this new protocol, and reported much higher percentages of germination than in a previous experiment utilizing Snapdragon or Peruvian Lily pollen. Groups using maize pollen reported pollination percentages ranging from 15%-80% (possibly dependent on the freshness of the collected pollen), whereas other pollen types often experience 0-5% pollination at best. In previous years, showing a video of germinating pollen during this exercise was highly recommended since so few students could observe germination under the microscope. Using maize pollen, all GTAs reported that the use of the video was unneeded due to the high germination percentage and overall large size of the pollen tube, as compared to other species used in previous years or other sections. The maize pollen also germinated more quickly than other types of pollen, creating observable growth within about 15 minutes at room temperature, as compared to longer incubation times under heat lamps when using other pollen.

This work was partially supported by NSF grant IOS-1832186 to JEF.

REFERENCES

Gibbon, B.C., Kovar, D.R. and Staiger, C.J. (1999) Latrunculin B has different effects on pollen germination and tube growth. *The Plant Cell*, 11, 2349–2363.

Schreiber, D. and Dresselhaus, T. (2003) *In vitro* pollen germination and transient transformation of *Zea mays* and other plant species. *Plant Molecular Biology Reporter*, 21, 31-41.

McCaw, M.E., Wallace, J.G., Albert, P.S., Buckler, E.S. and Birchler, J.A. (2016) Fast-Flowering Mini-Maize: Seed to Seed in 60 Days. *Genetics*, 204, 35–42.

Instructor Protocol

This lab activity will take approximately 30-45 minutes if the instructor harvests the pollen. If students harvest the pollen, it will take longer. We designed this activity to be delivered to ~1000 students in approximately 28 different laboratory sections, and can be scaled as appropriate.

Prior to the set-up of the actual lab, hybrid 'Fast-Flowering Mini-Maize' (FFMM) seeds will need to be planted in a greenhouse timed to reach anthesis (anther emergence and pollen shed) during the week of the activity. To ensure that pollen will be shedding from the W22/FFMM plants when needed: 1) five pots should be planted with seeds (obtained from the Fowler lab) nine weeks in advance of the start of the lab week, and 2) five more pots should be planted a week later. We use SunGro Sunshine Mix #4 for potting, adding 1-2 tablespoons of 14-14-14 slow-release fertilizer (e.g., Osmocote) per pot; pot size is either 0.7 or 2.5 gallon. Plant 2-3 seeds/pot approximately 2.5 cm below the soil surface, and after 2-3 weeks thin to 1 plant/pot. Alternatively, seeds can be started in small peat pots, with a ⅔ potting mix and ⅓ vermiculite mixture, followed by transplanting seedlings into larger pots after two weeks.

Anthesis is recognizable by the exsertion of a few anthers from the tassel at the top of the plant (these look a bit like grains of rice dangling off the branches); a brief shake of the tassel should release some pollen. If there is no pollen shedding at the start of the lab week, alternatives will need to be considered. Prior to the lab, make the pollen growth medium (PGM) using the recipe below. PGM is only good for 1 week, so a new batch must be made before the week of lab. Five ml of 1X PGM, stored in the fridge, should be enough for approximately 40 students.

Fresh pollen needs to be collected prior to the start of each lab period since maize pollen viability decreases significantly a few hours after release. To collect pollen, place a small paper bag (plastic ziplock bags can work, too) over the tassel of a shedding plant, and gently bend the plant stem so that any shedding pollen will fall into the bag. Tap the bag on both sides of the tassel to shake pollen loose. Remove the bag from the tassel carefully without losing the pollen or breaking the stem. Repeat for each shedding plant; the collected pollen can be pooled. (An informative video about maize pollination methods is at https://www.youtube.com/watch?v=ZjWk9AM30Qo). Once you have pollen collected, transfer equal amounts of it onto weigh paper sheets, one for each lab group, and remove any anthers from the pollen. Set the PGM (stored in the fridge) on the lab bench just before starting the lab, as PGM works best at room temperature. Be sure to return PGM in the fridge once all groups have their pollen germinating.

Student materials for lab (in addition to the pollen): compound microscope, microscope slide (preferably depression slide), cover slip, disposable pipette, weigh paper and PGM.

Students complete a pre-lab assignment on our Learning Management System prior to coming to lab (see below) and germinate the pollen during the lab itself. The pre-lab and in-lab activities as delivered to our students are provided below the PGM recipe.

Recipe for 400 mL of 1X PGM (pollen growth medium) for maize pollen

Adapted from Schreiber, D. and Dresselhaus, T. (2003) *In vitro* pollen germination and transient transformation of *Zea mays* and other plant species. *Plant Molecular Biology Reporter*, 21, 31-41.

Materials:

- Sucrose - 20 g
- $CaCl_2$ - 1.6 mL of a 2.5 M stock
- PEG 4000 (polyethylene glycol 4000) - 24 g
- H_3BO_3 (boric acid) - 200 ml of a 1% stock
- KH_2PO_4 - 20 ml of a 1 M stock
- DI Water

Steps:

1. Add about 150 mL DI Water to a 500 mL beaker with a stir bar on a hot plate.
2. Add the sucrose, $CaCl_2$, PEG 4000, boric acid, and KH_2PO_4 to the beaker.
3. Top off beaker with DI Water to get it to 200 mL.
4. Heat at 70° C (give or take 10°) for 10 minutes while stirring.
5. After mixture has cooled a bit, add another 200 mL of DI water and thoroughly mix.

Student Pre-lab assignment:

Read the article at http://www.aganytime.com/Corn/Pages/Article.aspx?name=A-Closer-Look-at-Corn-Pollination&fields=article&article=910 (downloadable as a PDF)

Answer the following questions**:**

1) Label the following maize (corn) plant parts with the correct term:

Tassel	A: Male flower
Ear	A: Female flower
Silk	A: Structure that functions as both stigma and style of the female flower
Kernel	A: Produced by successfully fertilized ovules

2) Which of the following can result in poor fertilization for corn plants? (Select all that apply)

Desiccation (Yes) Heavy
Rain (Yes) Cloudy
Conditions (Yes)
Variable Flowering Dates (Yes)
Pollen competition for silk access (No)

3) What time of day does most flowering occur?

Morning (Answer)
Noon
Afternoon
Evening
Night

Student Protocol, In-lab: Phylum Anthophyta: Pollen Tube Germination

1. Pollen, once deposited on the stigma, will germinate and produce a pollen tube that grows down through the style toward the ovules within the ovary. Today, we will be using pollen from maize (species name: *Zea mays*, and more commonly called 'corn' in the USA) to examine the process of germination.

a. Using a disposable pipette, aliquot a small amount (no more than 1 mL) of PGM for your group into a 5mL beaker.

b. Take one sheet of weigh paper containing pollen back to your lab bench.

c. Using your disposable pipette, put just a few drops of PGM onto the middle of a depression slide until the liquid covers the depressed section.

d. Take your sheet of weigh paper with pollen, and ***very gently*** tap one side of it over the slide until a small amount of pollen falls on to the PGM.

*** You want to be able to see individual pollen grains with your microscope – if too much pollen is on the slide, this becomes very difficult. In addition, very high pollen density in liquid media can inhibit successful pollen tube germination.

e. ***Gently*** stir pollen and PGM together on the slide with the tip of your disposable pipette until pollen is evenly distributed throughout the PGM.

f. Wait 10-15 minutes for pollen tubes to form before viewing slide under the microscope. Use this time to answer the following questions:

Maize pollen is released from the parent plant at different rates throughout the day; moreover, the rate of pollen tube germination can also vary throughout the day.

· At what time of day do you think maize pollen tubes would be most likely to germinate and why? Why do you think it would be advantageous for maize pollen to be released at specific times of the day?

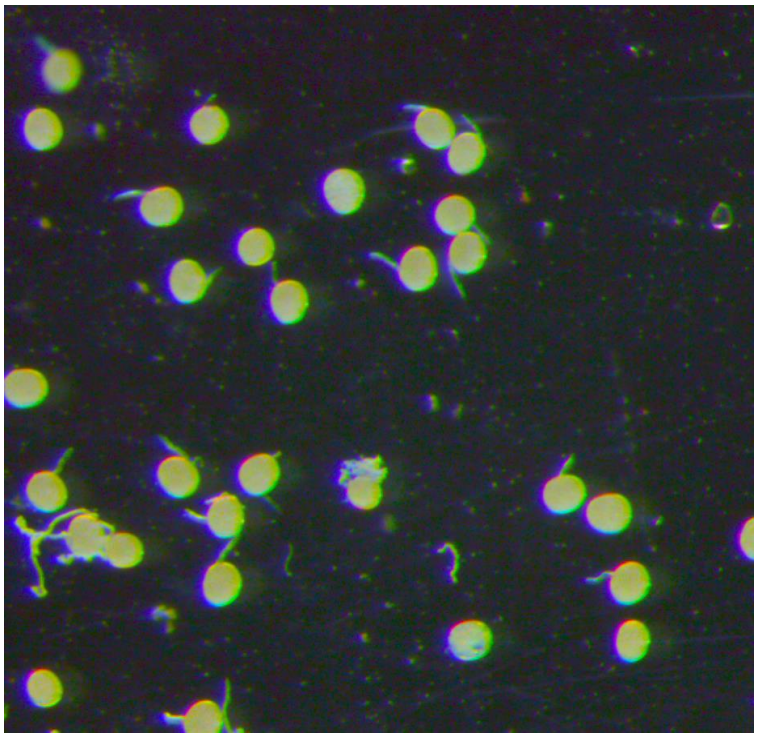

Figure 1. Shown above are examples of corn pollen which is germinated (top circle), burst (middle circle), and ungerminated (lower circle).

2. Once your pollen is ready to view in the liquid media, place it under the microscope and bring the pollen into focus with the 10x lens. You will be counting the numbers of ungerminated, burst (i.e., dead due to cell rupture), and germinated pollen (see Figure 1) in **three** different fields of view. Your TA will collect the data from your groups and the results will be shared in the main lecture. **You will need to close the iris diaphragm all the way to see the tubes.**

	# Ungerminated	**# Germinated**	**# Burst**
Area 1			
Area 2			
Area 3			

3. Answer the following questions:

· What percentage of your pollen successfully germinated? What percentage burst? Didn't germinate?

· Why is it useful for us to understand how and when maize pollen is most likely to germinate? Come up with at least **two** ideas.

· A single maize plant can produce several milliliters (volume) of pollen. Compare this to the amount of pollen released from the other flowers in lab (e.g., lily, snapdragon). Why might there be a difference between these species? (Hint: consider how pollination works in the different species.)

· Maize pollen tubes are among the fastest growing plant cells known (up to 10 mm - i.e., 100x the size of the original pollen grain - per hour). What hypothesis can you come up with to explain why this rapid growth rate could provide an adaptive advantage?