Pollen Growth

Introduction

Why don't roses and tulips cross fertilize and produce rolips? A simple question with a very complex answer! One aspect of species specific biology will be explored in this laboratory—conditions required for pollen growth.

Concepts

- Pollen germination
- Pollen tube growth
- Plant reproductive cycles

Materials

Mature anthers from flowers
Sugar solutions (2%, 5%, 10%, 20%)
Coverslips
Depression slides
Dissecting needle
Forceps
Microscope
Petroleum jelly
Toothpicks

Safety Precautions

The materials in this laboratory exercise are not considered hazardous but normal safe laboratory procedures should be employed throughout the activity. Please review current Safety Data Sheets for additional safety, handling, and disposal information.

Pre-Lab

Mix various concentrations of sucrose solutions prior to the laboratory (2%, 5%, 10%, and 20%). Be sure to do this lab when good ripe pollen is available either by collecting in nature or from a florist. See the Tips section for flower selection.

Procedure

1. Place a drop of sugar solution in the well of a depression slide. Record the concentration of the sugar solution.
2. Get a flower stamen—being careful not to knock the pollen off of the end of the anthers. (This may involve dissecting a flower provided by your instructor.) Record the species of the flower. With a dissecting needle carefully knock some ripe pollen off the anther and into the sugar solution on the depression slide. Be sure the pollen lands in the sugar solution.
3. Use a toothpick to form a ring of petroleum jelly around the lip of the depression well. Carefully place a coverslip over the well and on top of the petroleum jelly. Be sure the petroleum jelly makes a complete seal under the coverslip. This will prevent evaporation (see Figure 1).

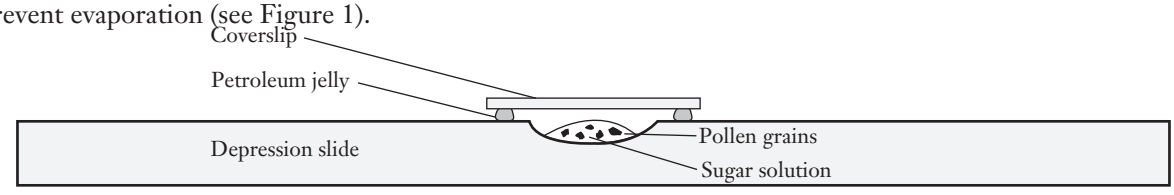


Figure 1. Pollen Growth Depression Slide

4. Observe the slide under the high power of a microscope. The curved depression in the middle of the depression slide allows for a very thick drop of solution compared to a regular flat slide and coverslip. Practice focusing up and down through the entire depth of the depression well. Be careful not to break the coverslip. Find several pollen grains and make a sketch of them on your record sheet.
5. Observe the pollen grains for the rest of this initial lab period. In about ten minutes some of the pollen grains should start to grow pollen tubes. They grow at a rapid rate and can literally grow off your field of view as you watch. Make a

Publication No. 10128
061616

sketch of a growing pollen tube on your record sheet. Show as much detail as you can observe. Can you see any structures in the pollen tube? Observe the pollen tubes with reduced light on your microscope.

6. Label the slide and place in a safe, warm storage place as directed by your instructor.
7. Examine the pollen slide after 24 hours. How do the pollen tubes compare in length to 24 hours ago? What percentage of the pollen grains would you estimate have germinated? Why didn't they all grow a pollen tube? Record all results.

Going Further

If different lab teams used different flower species and different sugar concentrations there was probably quite a difference in pollen tube growth. Some botanists have suggested that different species of plants require specific sugar types, concentrations, and other conditions for their pollen to grow. These variations of germination conditions are species specific and might help explain, in part, why more cross species fertilization does not occur. It is further thought that some pistils might secrete some attracting or stimulating substances that are likewise species specific. All these mechanisms might help to prevent the germination of "foreign" pollen on a pistil. Design at least one experiment and test at least one variable to see what differences you can create for pollen growth. Consider: sugar types, sugar concentrations, other nutrients, plant growth hormones, plant growth inhibitors, pollen type, pollen age, light conditions, pH conditions (acid rain?), and any other variables you might hypothesize contribute to this interesting phenomenon. Write a report describing your experiment and summarize your findings.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. The leftover sugar solutions may be disposed of by disposing of down the drain with plenty of excess water according to Flinn Suggested Disposal Method #26b.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12
Evolution and equilibrium
Form and function
Content Standards: Grades 5–8
Content Standard A: Science as Inquiry
Content Standard C: Life Science, structure and function in living systems, reproduction and heredity, diversity and adaptations of organisms
Content Standards: Grades 9–12
Content Standard A: Science as Inquiry
Content Standard C: Life Science, biological evolution, matter, energy, and organization in living systems

Tips

- Some of the students may never have examined a flower closely. It is recommended that a flower dissection be performed prior to this lab. Flower anatomy, with an emphasis on structure/function relationships, should be discussed before this lab.
- If flowers are blooming outdoors in your locality, it would be economical to gather flowers yourself. Try to select flowers of rather simple structure; avoid double blossoms, composites, and flowers in which the stamens adhere to the pistil. Gladioli and sweet peas may be available from a florist. Pollen from flowers of the Lily and Amaryllis families germinate especially easily. Others that have been reported as easy to germinate include Impatiens, Tradescantia, and Viola.
- Do this lab at a time in the course when students are experienced enough to design and conduct their own experiment. It is a lab well suited for student's original experimental work. It is possible for students to prepare multiple deep-well slide preparations and test several variables, all within one class period.

Discussion

The growth of the pollen tube, its movement down through the pistil to the ovule, followed by the discharge of the sperm into the ovule is an evolutionary achievement for the male gametophytes of angiosperms and gymnosperms. The complex union of the male and female gametes in the ovary ends the short monoploid stage of the flowering plant. The resulting diploid cell from fertilization develops into an embryo. The other nuclei and materials in this complex process develop into the rest of the seed we associate with flowering plants.

This amazing plant reproductive process is occurring in plants all around us all spring and summer. Most of us do not notice the flowers on a maple tree much less think about pollen tubes in the flowers! (Some people are well aware of pollen due to allergies but are unaware of what it looks like and why it is such an irritant.) Microscopic examination of pollen and watching pollen tube growth can make these abstract concepts take on a more real-life meaning. The use of rapidly germinating pollen facilitates direct observation of an aspect of the plant life cycle usually not witnessed. In addition to being responsive to temperature, pollen growth is influenced by chemical substances in the germination medium. This might also include pollutants. The sensitivity of pollen to adverse environmental conditions might account for the sensitivity (even disappearance) of the sporophyte of some species.

The media that works best for germinating pollen is somewhat species specific. You might want to experiment with sugar type and sugar concentration for the specific pollen you collect before students do the laboratory exercise. A 10% sucrose solution is the best starting point for the species of flowers suggested earlier. Some botanists have suggested adding 0.01 g of boric acid, H_3BO_3, and a 0.03 g calcium nitrate, $CaNO_3$, (for 100 mL of sugar solution) to help stimulate pollen tube growth.

Pollen germinates poorly in a standard wet mount slide under a coverslip. The standard wet mount technique restricts diffusion of oxygen from the air to the pollen and thus restricts pollen viability. The depression slide technique greatly increases the chance of success and the resulting student excitement of seeing streaming protoplasm in the growing tube.

Germination is often enhanced with greater concentration of pollen in the media, probably because substances diffusing out of the pollen grains promote germination (another survival benefit). Therefore, you will likely see a greater germination rate where there are clumps of pollen in the slide well. Pollen tubes are typically evident within 10 to 15 minutes after being placed in the sugar solution and are well developed in 30 to 45 minutes.

Materials for *Pollen Growth* are available from Flinn Scientific, Inc.

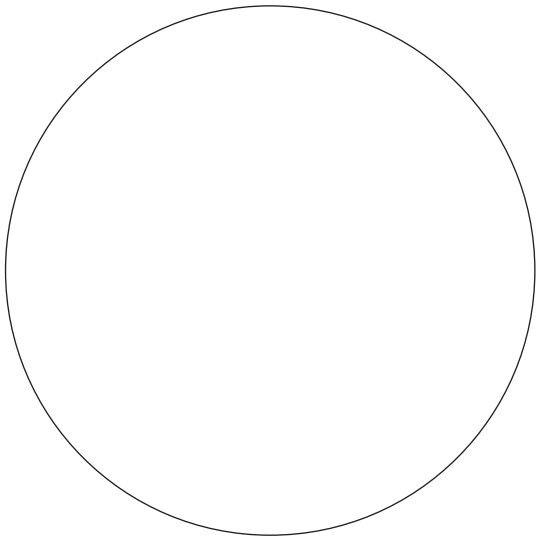
Catalog No.	Description
ML1378	Depression Slides
ML1382	Coverslips
AP2016	Toothpicks
S0134	Sucrose
P0230	Petroleum Jelly

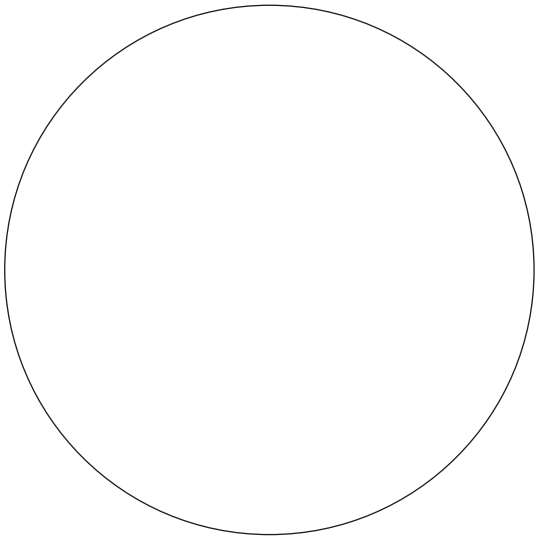
Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.

Name: _____

Record Sheet

Flower Type _____ **Sugar Concentration** _____





Pollen Grain Before Germination

Magnification: _____