



Double Fertilization and Post - Fertilization Events: Measuring

Concepts

In plants fertilization is the event in sexual reproduction which follows pollination. In higher plants, two sperm are involved in fertilization, reaching the ovule via a pollen tube from the germinating pollen grains. One sperm fertilizes the egg cell within the embryo sac to produce the zygote and begin the new generation. The other combines with the fusion nucleus to produce the *endosperm*, a special tissue that nourishes the developing embryo. Fertilization also stimulates the growth of the maternal tissue (seed pod or fruit) supporting the developing seed. In Fast Plants the fertilized egg cell develops through various stages over the next 20 days until it becomes a mature quiescent embryo, the seed.

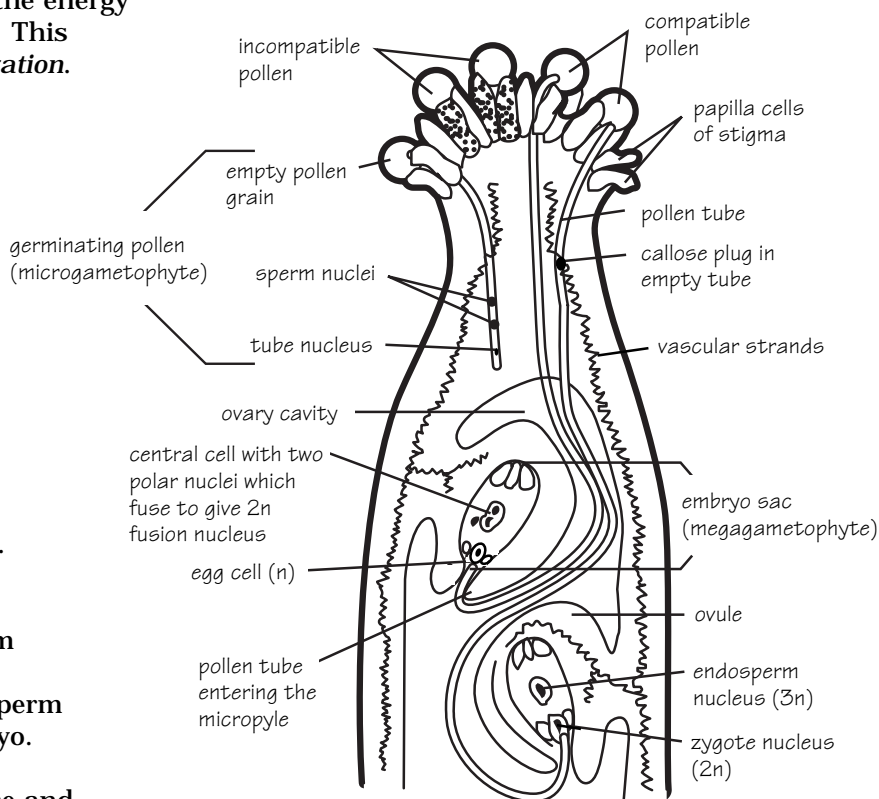
Background

What happens between fertilization and seed harvest? After pollination, each compatible pollen grain adhering to the stigma sends through the style a pollen tube which carries two male *gametes* (sperm) to the *ovule*, where the *egg* and other *cell nuclei* are housed in the *embryo sac*. One sperm unites with the egg cell (n) to produce a *zygote* ($2n$) which becomes the embryo. The second sperm (n) unites with the diploid fusion nucleus ($2n$) to form the triploid *endosperm* ($3n$), the energy source for the developing embryo. This process is known as *double fertilization*.

Within two to three days after fertilization, the pistil begins to elongate and swell to accommodate the enlarging ovules. The sepals and petals wither and drop off, having completed their functions.

Within the ovules, the embryos differentiate and enlarge through a series of developmental stages, known collectively as *embryogenesis*. Enlarging also within the ovule is the endosperm. In the latter stages of embryo development in brassicas, the nutrient reserves in the endosperm are used by the embryo and the space that was filled by the endosperm is occupied by the enlarging embryo.

In cereal crops, such as wheat, rice and corn, endosperm is not used by the enlarging embryo and remains a major portion of the seed as a starchy energy source for the germinating seedling.



Through the development of the seed, the plant has solved the problem of packaging its new generation to survive until favorable conditions for growth return. As the seed matures, the walls of each ovule develop into a protective seed coat and the entire ovary becomes a fruit (seed pod) known as silques. In Fast Plants, embryos mature into seeds in 20 days after successful pollination and fertilization.



Measuring Time on the Floral Clock

Introduction

Embryogenesis is a highly coordinated sequence of developmental events within the ovule and supporting maternal tissues that ultimately leads to the development of a viable seed.

Successful embryo and endosperm development is accompanied by rapid elongation and enlargement of the pistil which serves as supportive nourishing maternal tissue. There is a strong interdependency between the developing embryos, endosperm, ovule and maternal tissues of the pod: it is believed that hormones produced by the developing embryos and endosperm regulate the growth and expansion of maternal tissue (Stage G in the life cycle, see WFPID *The Life Cycle*). When either embryo or endosperm fails in its development, ovule development also ceases and pod enlargement may be slowed. Vigorous pod development supported by a healthy plant is also necessary for the developing embryo and endosperm in the ovule. Seed failure can result if the maternal plant comes under excessive environmental stress, as from excessive heat, water stress or nutrient deprivation.

Fertilization in sexually reproducing organisms represents the onset of the next generation. Following double fertilization in plants, the most visible event is the rapid wasting of all those flower parts which are no longer needed by the plant. Sepals, petals and stamens wither and fall. Nectaries dry up. At the same time, within a few hours of fertilization, the pistil with its complement of fertilized ovules begins rapid development.

This activity provides students with an opportunity to follow the results of student pollination by measuring the increasing length of the pistil at regular intervals until mature seeds are formed and harvested. Upon harvest, viability of the seeds will also be tested. In the WFPID *Embryogenesis*, students dissect pods at various stages of development and investigate in great detail the stages in embryogenesis.

Question: What are the indicators of normal fertilization and post-fertilization development that follow successful pollination?

Sample Hypothesis: Flower parts other than the pistil wither. The pistil enlarges to become a pod containing developing seed. At maturity the pod and seeds dry. Viable seeds germinate.

Design

- At specified intervals following pollination, measure the length of the pistil, observe developing embryos and endosperm and estimate the number of developing embryos. At maturity, harvest seed and verify viability with germination test. As a control follow the development of pistils of the unpollinated control plants.
- Students will record observations and measurements on the Floral Clock Student Data Sheet.

Time Frame

A period of 36 days from the sowing of seed is required for the growth of the Fast Plants and the completion of this activity. The time required each class period will vary according to the observations or measurements being made. Time will be required on seven separate days through the course of this activity, specified in the procedure.

Learning Objectives

In participating in this activity students will:

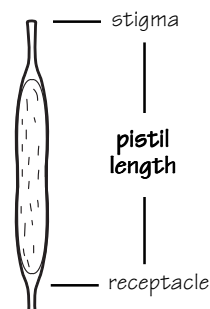
- learn to make observations and accurate measurements of pistil enlargement;
- understand the role of pollination and compatible pollen germination as a precursor to double fertilization (Stage F, Life Cycle);
- understand that following double fertilization a complex sequence of interdependent developmental events occurs over a period of 20 days that results in the production of viable seed for a new generation in the spiral of life (Stages F, G, H and I, Life Cycle);
- understand the unique nature of double fertilization in higher plants in which embryo and endosperm are interdependent specialized tissues within the ovule that function in normal seed development (Stage G, Life Cycle);
- understand the interdependent relationship of developing maternal tissue and developing fertilized ovules (Stage G, Life Cycle);
- understand that the development of a viable seed requires a healthy (environmentally non-stressed) plant during the period of growth in which the fertilized ovules are developing into seed.

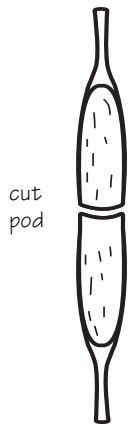
Materials

- flowering Fast Plants, post-pollination
- dissection strips (see *WFPID Dissection Strips*)
- fine scissors
- fine-tipped forceps and fine dissecting needles (e.g., tuberculin syringes with #23 or #25 needles)
- clear double stick tape
- 2 cm wide clear adhesive tape
- seed envelope
- glass microscope slide
- water and dropper
- sharp cutting blade

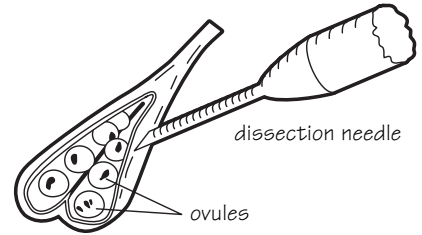
Procedure

1. At **3 dap** (days after pollination) students will notice that the flower parts that were important in pollination have withered and fallen from the plant.
 - Using a dissection strip without tape as a ruler, carefully measure the length of each expanding pistil, recording its length to the nearest millimeter in the appropriate column of the Floral Clock Student Data Sheet according to its position number. Remember the highest number (4) is the most apical (top) flower.
2. Repeat Step 1 at **6 dap** and **9 dap**.
3. Students will notice that the pods at **9 dap**, in addition to elongating, have swollen around what appears to be developing seeds or ovules (Stage H, Life Cycle).
 - Carefully hold the plants up to the light. Can you see the outlines of the developing ovules within the pods?
 - Estimate the number of developing seeds in each pod. Record the information on your Floral Clock Student Data Sheet. At harvesting you will be able to verify your estimations by counting the seed.
4. If students are **not** doing the activity on embryo dissection, each pair may sacrifice one pod from one of their plants in order to observe the developing embryos within the ovules at **9 dap**.

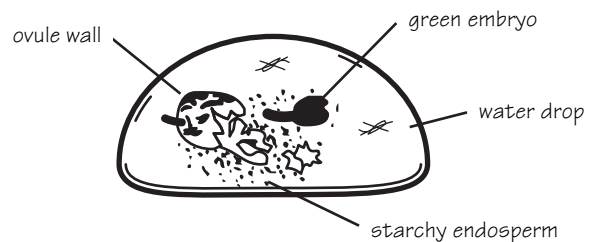
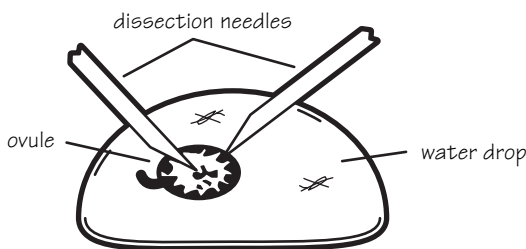




- Proceed by choosing a pod in which several ovules are visible. Carefully snip it off with a fine scissors.
- Cut the pod in half with a sharp blade and give one half to each student. Place each half on the sticky tape of another dissection strip.
- Holding one end of the half-pod, use a sharp needle to pry it open along its length to expose the ovules which look like tiny green grapes. With the needle or forceps, detach two or three ovules from the pod and place them in a cluster on the sticky tape of the dissection strip. Put the pod aside on wet paper toweling to stay fresh.



- With a dropper place a very small drop of water over the ovules on the dissection strip.
- With two needles, hold and squeeze the first ovule, cutting into it with one needle. A tiny green object should squirt out along with some cloudy material (the endosperm).



- With a hand lens or microscope, observe and draw the object to scale. Does it look like any of the illustrated stages in the WFPID *Embryogenesis*?
- If you didn't succeed with the first one, try another ovule.

5. At **12 dap**, repeat Step 1 and record the data on the Floral Clock Student Data Sheet.

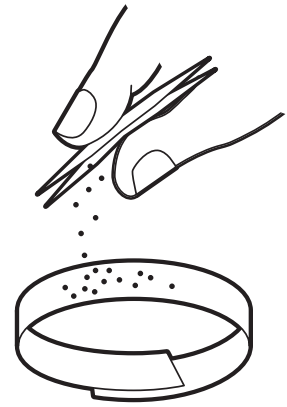
- By this time, the plant's lower leaves may be starting to turn yellow or even wither and dry. This is the normal succession in the life cycle of the plant, particularly if it is supporting a number of pods with developing seeds.
- If a plant fails to develop any seeds, it will frequently remain green and continue to produce flowers from side shoots developing in the axils of leaves. This should be the case in the unpollinated control plants, if a plant has been severely stressed so that embryogeny has failed or if it is genetically or phenotypically *sterile* (incapable of producing offspring).

6. At **21 dap**, plants are now approaching maturity. Normally Fast Plants embryos have fully developed by 18 to 20 dap, at which time stem and pods of the aging parent plant begin to turn yellow along with the leaves and the seed coat begin to turn brown (Stage I, Life Cycle). This is the time that water can be withheld from the plants to encourage seed ripening.

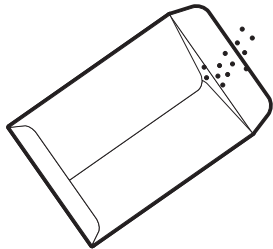
- At **21 dap**, students should take a final pistil length measurement.
- Empty the water from the reservoir and remove the capillary mats. Keep the plants under the lightbank and let them dry.

7. In **about a week**, or when the pods are dry, harvest the seeds from each of your pods onto separate loops of sticky tape. Record the plant and pod number on the tape.

- Count and record the number of seeds for each pod on the Floral Clock Student Data Sheet.
- Did the number of seeds in each pod agree with the estimates you made at 9 dap?
- As you harvest, notice any unusual seed. If these are of interest with you, tape them to a card where they can be examined or germinated later.



8. Take a sample of two seeds from each pod and test their viability in a bottle cap seed germinator according to the instructions in *WFPID Germination*.



- **Two days later**, record the seed viability on the Floral Clock Student Data Sheet. On the data sheet, "+" indicates a viable seed and "-" indicates a nonviable seed.
- Combine all the other seed in a bulk and store it in a seed or coin envelope within a sealed container with indicator silica gel, drying compound. Seed stored dry and cool in a refrigerator or freezer will stay viable for many years.

Concluding Activities and Questions

After setting the floral clock with the pollination activity, students have followed the course of reproductive development by observing and measuring pistil enlargement and seed production and completing the Floral Clock Student Data Sheet. Combine the student data into group data, and then into a class data summary using the Floral Clock Class Data Sheet.

If available, use data analysis software to create graphical and statistical summaries of class data. Notice how the various statistical notations (range, mean and standard deviation) change over time from pollination. Have students consider the following:

- From a class frequency histogram and statistical summary, does the measured plant character of pod length on a particular day exhibit a normal distribution within the class population?
- Do individual pod lengths for each day fall within one or two standard deviations of the class mean? Do students consider their post-fertilization events to be normal? Why or why not?
- Are there any "abnormal" seeds? They may be tested for viability.
- From a graph of the average rate of increase in pod length over time, is there a period in post-fertilization development in which the increase in pod length is fastest?
- Is there a relationship between the number of developing seed and pod length?
- How do graphs of pod length increase over time compare between the pollinated and unpollinated sets of plants?

Extension

For an activity investigating the role of plant hormones in the regulation of pod enlargement, see *WFPID Hormone-Induced Parthenocarpy in Rapid-Cycling Brassica rapa*.

