POLLEN (Dead or Alive)?

by Oscie B. Whatley, Missouri

We would never be so foolish as to pollinate a dead flower, because we can easily see that it is dead. Yet, unbeknownst, we will use dead pollen, store pollen improperly, and transport it with no idea as to what it may be subjected. All because we *see* pollen the same whether it be DEAD or ALIVE.

Viable pollen is as essential to seed production as is the fresh live pistil.

Heretofore, most of us could test pollen only by hybridizing, and the cause of failures still remained a mystery. Isolating each of the causes of sterility is far too ambitious for the average breeder to consider at this time. However, one common and very essential part of the fertilizing phenomenon is the germination of the pollen tube from the pollen grain. This activity can be observed under a microscope (100X power) when properly prepared on a slide.

It is generally believed that pollen which will germinate and grow healthy pollen tubes will also carry the necessary nuclei for fertilization of the egg.

With the means to observe pollen-tube growth, we can isolate one common cause of pollen viability or the lack of it. Converted tetraploid pollen in particular needs to be scrutinized for viability because sterility is very common among new conversions. I have observed several first and second year conversions with mixed diploid and tetraploid pollen where the diploid was fertile and the tetraploid was not. Many conversions will not become tetraploid pollen fertile until after three years from treating.

Viability of any pollen is subject to water and temperature exposure. Wet pollen appears to be thoroughly dead from only a short exposure while temperature affects the viability by duration and intensity.

This article has two purposes—first to share my observations of pollen germination and tube growth, and second, to describe the method so that interested hybridizers can have the same access to expand their knowledge.

Observations & Some Conclusions

1. STORAGE

Pollen from the garden and stored pollen (50° F in a dehydrated container) were tracked simultaneously at one

hour intervals, starting when the pollen sacs broke open (dehised) and fluffy pollen could be collected. One diploid, 'Stella de Oro,' and two tetraploids, 'Isosceles' and 'Solar Music,' were used on six different days. All varieties of fresh pollen germinated in approximately 30 to 40 minutes, and the number of grains that germinated were between 30 and 60%.

Stored pollen remained consistent for several weeks, but garden pollen reduced its number of germinating grains by half in two or three hours when exposed to between 80° F and 85° F. After exposure to between 85° F and 90° F for one (1) hour, most tetraploid pollen was less than one-fourth the original count (or about 10% of total grains). Diploid was a little better. After six hours from pollen sac opening, all garden tetraploid pollen was dead on a moderately hot day. Later experiments revealed pollen taken from storage would accumulate time exposure and have similar deterioration of germination.

Conclusion:

Garden pollen must be used while fresh for best results. Pollen can be stored (at 50° F) for two to three weeks and works well provided it is protected against warm temperatures while being used. Frozen pollen (10° F) from the same varieties showed fairly good germination after four months. More experiments will be conducted on this pollen next spring. Similar to 50° F storage, one would expect rapid deterioration in a few hours if the container was removed from the freezer.

2. VIABILITY PEAK

Pollen was checked from six (6) varieties that differed in time when pollen sacs broke open. Collection started one hour before opening, at the time of opening, and one hour after opening. All pollen acted about the same one hour before opening (no germination). At opening, the greatest percentage germinated; one hour after opening, some drop in germination was noted at 75° F.

Conclusion:

Pollen should be collected when pollen sacs open, which may vary from two days before flower opens to one to two hours after flower opens. Pollen from the anthers collected before normally opening and then forced under lights showed similar peak values.

3. POLLEN TUBE GROWTH RATE

Fresh pollen from tetraploids had an average growth rate of *1 micron per second.*

Fresh pollen from diploids had an average growth rate of *1 micron per 4 seconds.*

Older pollen and variation in sucrose percentage will cause different speeds, but usually the comparison, tetraploid to diploid, is one-fourth to one-third slower for the diploid.

Conclusion:

This is a method of determining ploidy that is more positive than measuring pollen grains and stomata guard cells since the speeds never overlap. **This is a new concept of MID (Microscopic Identification)** that I did not cover in

the article on identification (tetraploids and diploids). I discovered it while looking for variations in pollen-tube speeds that might correlate with viability.

NOTE: If these speeds hold true to the speeds in the styles, then even the tetraploid would require 28 hours for pollen tubes to grow to the ovary.

General Notes:

• Diploid pollen germinates best at 10% sucrose. Tetraploid pollen germinates best at 14% sucrose.

• All pollen germinated in-vitro was at 75°F (microscope light may have added a little heat).

• Percentage readings were taken at one (1) hour after starting, however, germination continued up to four (4) hours.

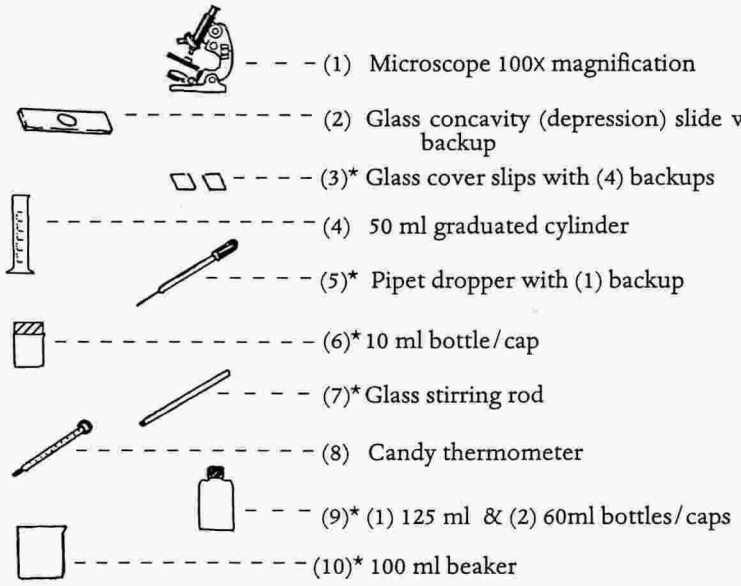
• The cell sap from tetraploid broken styles was substituted for the in-vitro solution, and very little germination was noted. (Could the styles on tetraploids be less than the ideal sucrose percentage?)

• The Ready-to-Use solution 14% was applied to stigmas on about fifty (50) crosses before pollination. Results were not conclusive. However, some sets on difficult pod parents were noted.

OPEN QUESTION: Is the pistil's viability affected by temperature and time similar to the pollen's viability?

Method

Equipment required:



Material:

(1) Daylily pollen tube study kit (see AUTHOR'S NOTE, page 27) *OR*
(A)* Sucrose (sugar); (B) Distilled water (deionized preferred)

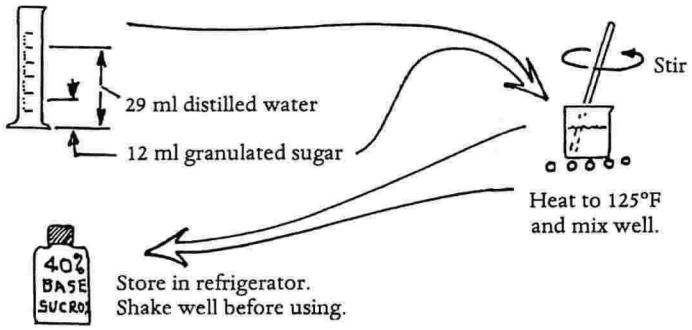
* Items included in kit, note page 27.

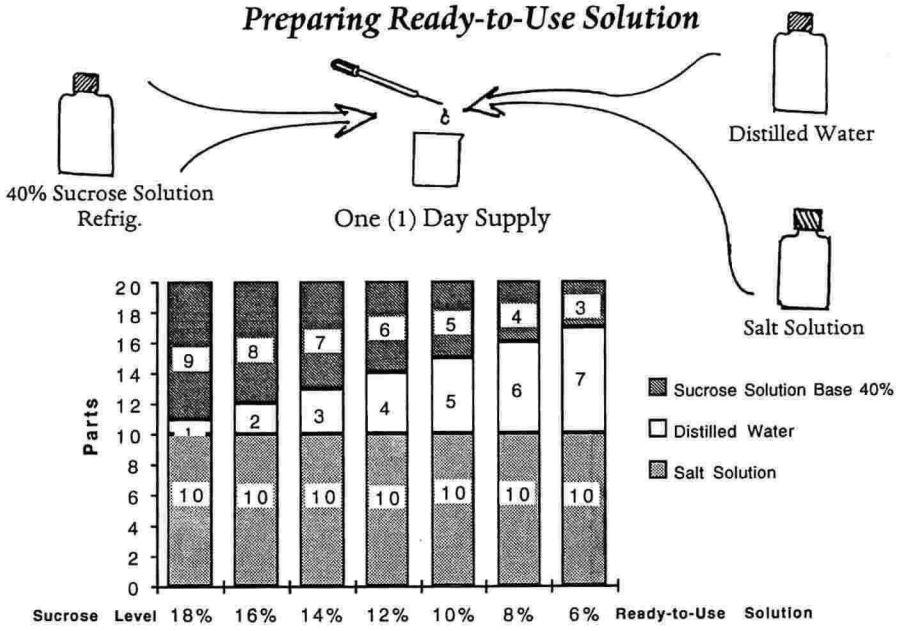
(C)* Prepare a mineral salt solution that is 2.54 mM $Ca(NO_3)_2$ (calcium nitrate), 1.62 mM H_3BO_3 (boric acid), 1.00 mM KNO_3 (potassium nitrate), 0.88 mM $MgSO_4 \cdot 7H_2O$ (magnesium sulfate), 3.5 mM NH_3 (ammonia). Add the following amounts of substances to distilled water and bring the final volume to 1.00 liter:

0.417 g $Ca(NO_3)_2$
0.100 g H_3BO_3
0.101 g KNO_3
0.217 g $MgSO_4 \cdot 7H_2O$
3.5 ml of 1.0 M NH_4OH

*Items included in kit.

Prepare 40% 1.2 M Sucrose Solution





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Preparing Ready-to-Use Solution (cont.)

Example: To make twenty (20) drops of 14% Ready-to-Use solution, place seven (7) drops of sucrose base in small beaker, add three (3) drops of water, and add ten (10) drops of salt solution.

NOTE: Both R-to-U and base sucrose solutions are subject to contamination from air spores at room temperature. I suggest keeping base solution out of the refrigerator at a minimum and keeping it covered as much as possible. R-to-U solution should be made fresh each day that it is to be used.

Preparing the Slide Specimen

Lay out necessary equipment and material on a stable, hard surface where access is easily available from a sitting position.

Start with clean, dry slides and cover slips so surfaces will form neat cohesive drops on the glass covers. Be sure slide is with concavity down while locating it near the slide cover.

Be sure pipet is clean and free of fluids before starting each operation by shaking like a fever thermometer and squeezing nipple. Pull up R-to-U solution about ¼ length of pipet and expel a drop or two (to waste) before applying to cover as shown in illustration. Apply pollen to the specimen drop by touching the fluffy pollen to the center of the drop. Pollen from a capsule should be raked out (with a toothpick) in a minute quantity and dropped on the solution. It may be necessary to stir the drop with a clean toothpick to spread excess to the edges.

With the slide concavity down, center the slide concavity over the specimen drop and gently lower to contact the cover. If the seal drops spread around the edges of the cover and the specimen drop does not contact the slide, all is well. Now, turn this assembly over with the concavity up.

See Pollen Tubes Grow

The specimen drop should hang from the cover and not touch the slide. Under the microscope, focus on the bottom of the drop where grains should be separated enough to allow germination and tube growth.

In approximately twenty to forty minutes, the grains should start to germinate when a bright translucent bump begins to form on a grain. This bump will grow into a root-like tube at a rate of between one micron per second to one-quarter micron per second.

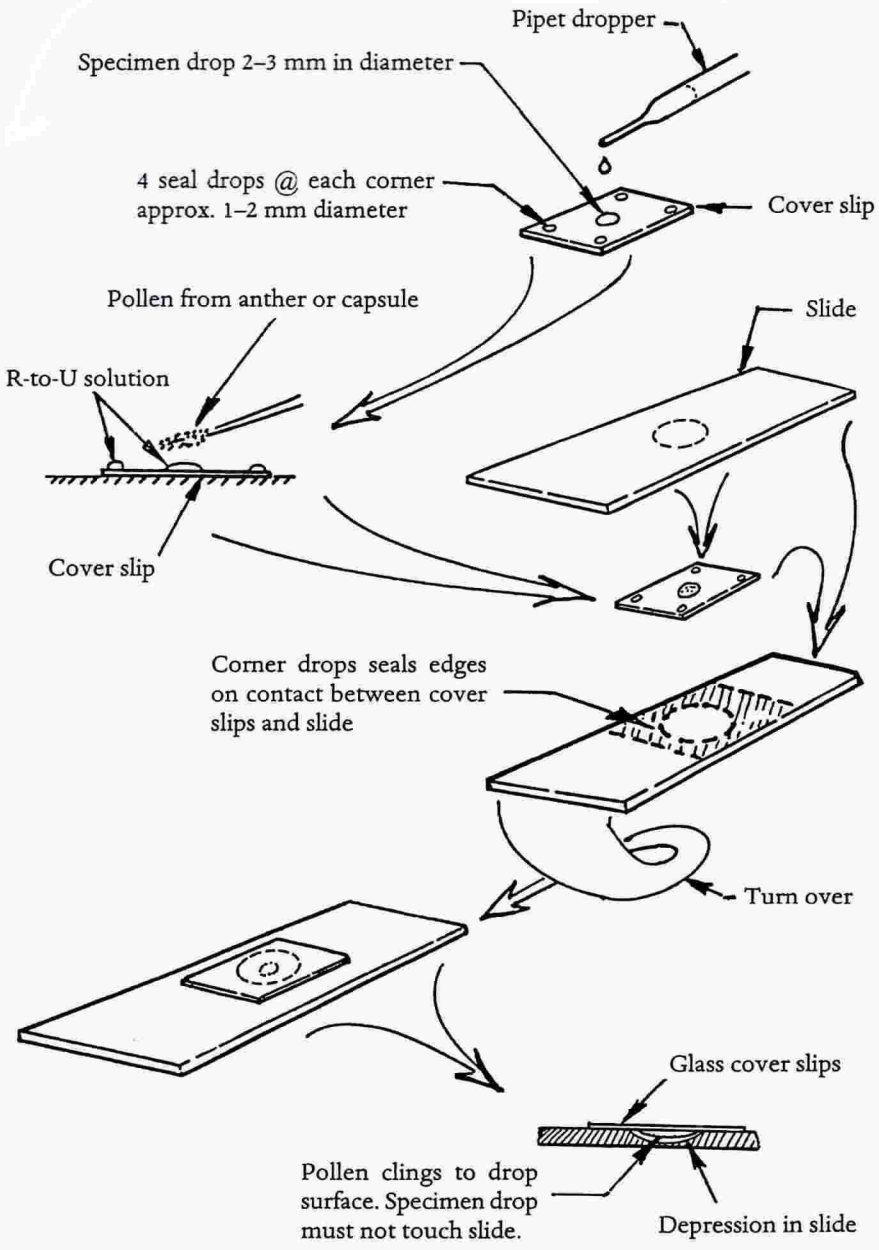
To evaluate the percentage of germination, use the field of view and count all the grains in this view. Then count the germinated grains and divide the total into the germinated grains (example: 20/80 = .25 = 25%). I have chosen one hour from the preparation point as a time to judge germination percentage.

*AUTHOR'S NOTE ABOUT "THE KIT"

To accomodate hybridizers who may not wish to go to the trouble of assembling all the materials listed in this article, I am making available in kit form the starred items at near cost. [See small ad, pg. 30.] This is offered only as a convenience. I am not generally in the kit business, but I would not want anyone discouraged at the outset by the prospect of a shopping trip. Anyone who has sources and the means to control the formula has all the information necessary to circumvent the kit.—*O.B.W.*

POLLEN (DEAD OR ALIVE)

Method



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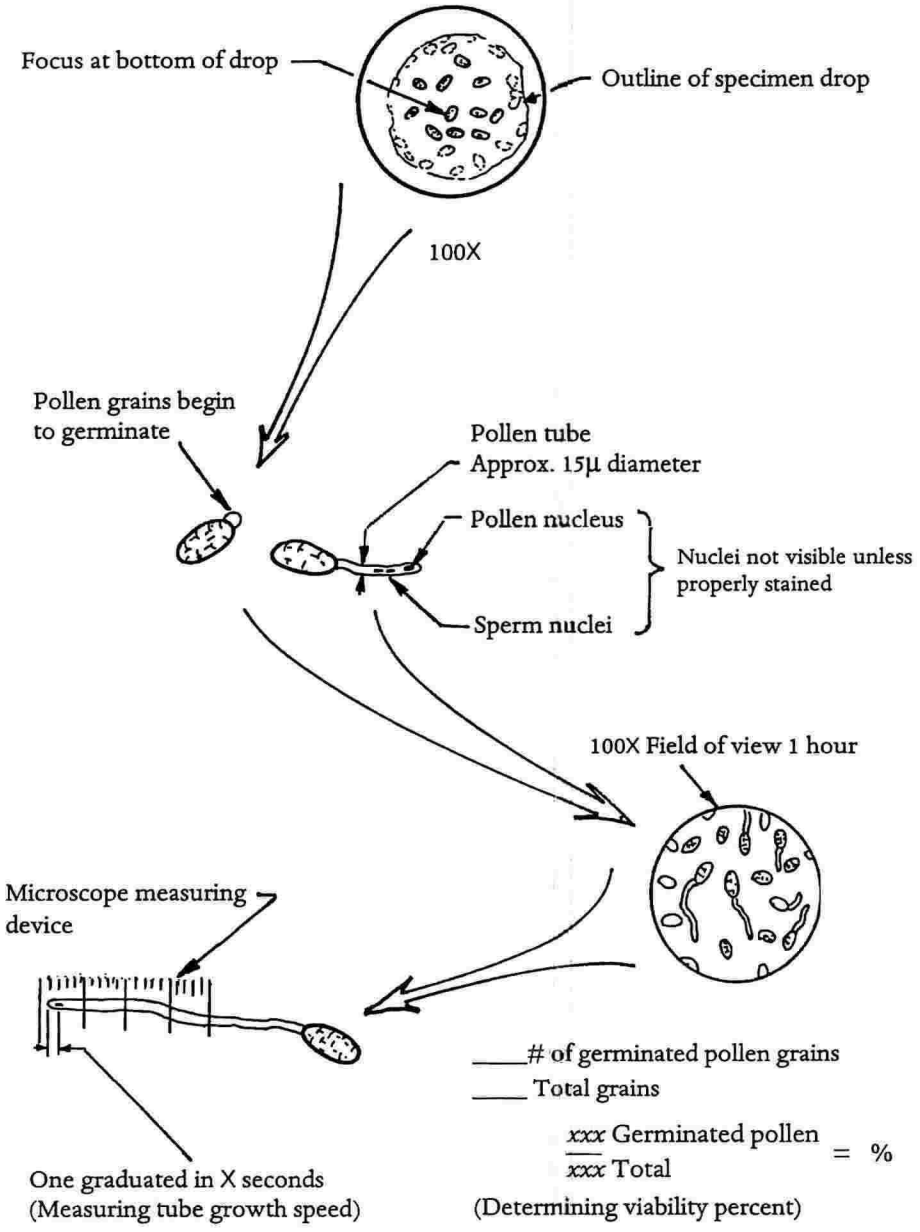
POLLEN VIABILITY (POLLEN TUBE STUDY)

Method



The author wishes to thank educators Dr. Robert A. Greisbach. and Richard Henderson for their invaluable advice and encouragement on this project.—*O.B.W.*