Core practical 4: Investigate the effect of sucrose concentration on pollen tube growth

Objectives

- Observe the growth of pollen grains
- Develop the skills of planning investigations and carrying out dilutions
- Carry out research and cite sources of information to support planning and conclusions

Safety

- The solutions used are of low hazard at the dilutions suggested.
- Take care with glass slides and mounted needles to prevent breakages and stick injuries.
- Students allergic to pollen should inform you and take precautions appropriate to the severity of the allergy.
- Wash hands after handling flowers or plant stems.

Specification links

- Practical techniques 1, 3, 4, 5, 8
- CPAC 1a, 2a–2d, 4a, 4b, 5b

Procedure

1. Carry out research to find out what concentrations of sucrose might be a suitable range to test in this experiment. Use reliable sources of information and cite these correctly in your practical write-up.
2. The basic method for germinating and observing the pollen grains is provided in steps 3 to 8 below. Prior to starting this investigation, plan the detailed procedure that you will use. Include the following information.

- How will you change the independent variable of sucrose concentration? What concentrations will you use and how will you make them using the 2 mol dm^{-3} stock solution? You will need to use equal volumes of sucrose and mineral salt solution to germinate the pollen. The mineral salt solution will be provided for you. Only very small volumes are needed.
- How will the dependent variable of pollen tube growth be measured? Bear in mind that tube growth may take up to an hour to occur. How many pollen grains will you measure? How often will you observe the slides?
- What variables must you control and how will you do this?

Basic method

3. Make up the solutions of sucrose as in your plan. Remember that these must contain equal volumes of sucrose solution and mineral salt culture medium when added to the slides.
4. Collect one Petri dish per planned sucrose concentration and label the dishes. Place a filter paper in each dish, moisten the paper with water and replace the lids. The Petri dish will act as a humid chamber in which the pollen slides will be placed to prevent them from drying out. The slides will be used without a coverslip to prevent anoxic conditions developing that might prevent pollen tube growth.
5. Collect one microscope slide per sucrose concentration and label the slides. Be careful not to touch the centre of the slides as they must be absolutely clean. Place a few drops of the sucrose plus mineral salt medium in the central cavity of each slide. Label the slides with the sucrose concentrations you are investigating.
6. Collect a flower that has several mature anthers and is shedding pollen, as riper pollen has a better chance of successful germination. Gently rub the point of a mounted needle over the anthers so that pollen falls on to the medium on each slide. Dislodge any pollen stuck to the needle by tapping the needle against a pencil or forceps. Do not add a coverslip. Repeat for each sucrose concentration, using the same flower if possible.
7. Note the time at which the pollen was added to the medium and place the slides in the Petri dishes. Remove only when observing with the microscope.

8. Use a microscope with ×100 magnification and an eyepiece graticule calibrated using the stage micrometer to observe the slides for pollen germination (see **fig A** in the Student sheet). Measure pollen tube growth as you have planned. To prevent the samples drying out or overheating, make your observations quickly before turning off the microscope lamp and returning the slide to the Petri dish.

Notes on procedure

- Students may need guidance on what makes a reliable source for reference and how to cite references correctly.
- The procedure has been adapted from various publications including the commonly used SAPS protocol (www.saps.org.uk) and there is further useful information on this site (search for 'pollen tube'). While many protocols use the 'hanging drop' method, the approach used here is simpler in terms of equipment and is less messy than some versions.
- This practical provides an opportunity to develop the skills of research and planning. Students' planned dilutions will need to be checked, after which a class protocol could be followed to allow collation of results. The sucrose added to the slide will be diluted by half on mixing with the mineral salt culture medium. This can be done at the sucrose dilution stage, or dropwise when adding to the slide. Various sources suggest success with concentrations from 0.2 to 1.3 mol dm^{-3} (before adding an equal volume of mineral salt growth medium). Percentage germination could be calculated. Measurements of pollen tube growth at regular time intervals will allow students to plot a suitable graph of the mean.

Answers to questions

1. Advantage: the use of pollen from the same flower improves the validity of the results because the maturity of the flower is a controlled variable. This means that any differences in the results between treatments are more likely to be a result of the differences in sucrose concentration.

   Disadvantage: the results may not be reliable. Different results may arise if the experiment is repeated because of differences between flowers. Flowers may differ slightly in maturity or there may be genetic differences in pollen tube growth. Ideally, the investigation would be repeated with a large number of flowers and a mean would be taken.
2. The pollen tube carries the male gametes to the ovule for double fertilisation.
3. Pollen tube growth may be attracted towards the micropyle by chemicals secreted by the embryo sac. The pollen tube may be positively chemotropic.

Sample data

Pollen germination and mean pollen tube length after 50 minutes at a range of sucrose concentrations. Note that students' data may take a variety of forms depending on individual plans.

Sucrose concentration/mol dm^{-1}	Germination (%)	Mean pollen tube length/μm
0.0	6.2	92
0.2	45.7	225
0.4	72.6	275
0.8	23.3	198
1.6	0.0	0

Core practical 4: Investigate the effect of sucrose concentration on pollen tube growth

Objectives

- Observe the growth of pollen grains
- Develop the skills of planning investigations and carrying out dilutions
- Carry out research and cite sources of information to support planning and conclusions

Safety

- The solutions used are of low hazard at the dilutions suggested.
- Take care with glass slides and mounted needles to prevent breakages and stick injuries.
- If you are allergic to pollen, inform your teacher and take precautions appropriate to the severity of your allergy.
- Wash hands after handling flowers or plant stems.

All the maths you need

- Recognise and make use of appropriate units in calculations.
- Recognise and use expressions in decimal and standard form.
- Use ratios, fractions and percentages.
- Use an appropriate number of significant figures.
- Find arithmetic means.
- Make order of magnitude calculations.
- Plot two variables from experimental or other data.

Equipment

- sucrose solution 2 mol dm^{-3}
- mineral salt culture medium
- microscope (×100 and ×400) with eyepiece graticule
- stage micrometer
- plants in flower
- five Petri dishes
- filter paper
- marker pen
- distilled water
- measuring cylinders or syringes
- stop clock
- balance
- scissors
- forceps
- mounted needle
- dropping pipette
- very clean cavity slides

Diagram

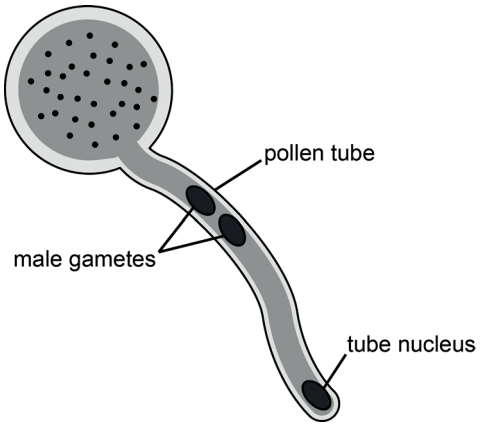

fig A Germinating pollen grain displaying growing pollen tube.

Procedure

1. Carry out research to find out what concentrations of sucrose might be a suitable range to test in this experiment. Use reliable sources of information and cite these correctly in your practical write-up.

2. The basic method for germinating and observing the pollen grains is provided in steps 3 to 8 below. Prior to starting this investigation, plan the detailed procedure that you will use. Include the following information.
   - How will you change the independent variable of sucrose concentration? What concentrations will you use and how will you make them using the 2 mol dm^{-3} stock solution? You will need to use equal volumes of sucrose and mineral salt solution to germinate the pollen. The mineral salt solution will be provided for you. Only very small volumes are needed.
   - How will the dependent variable of pollen tube growth be measured? Bear in mind that tube growth may take up to an hour to occur. How many pollen grains will you measure? How often will you observe the slides?
   - What variables must you control and how will you do this?

Basic method

3. Make up the solutions of sucrose as in your plan. Remember that these must contain equal volumes of sucrose solution and mineral salt culture medium when added to the slides.
4. Collect one Petri dish per planned sucrose concentration and label the dishes. Place a filter paper in each dish, moisten the paper with water and replace the lids. The Petri dish will act as a humid chamber in which the pollen slides will be placed to prevent them from drying out. The slides will be used without a coverslip to prevent anoxic conditions developing that might prevent pollen tube growth.
5. Collect one microscope slide per sucrose concentration and label the slides. Be careful not to touch the centre of the slides; they must be absolutely clean. Place a few drops of the sucrose plus mineral salt medium in the central cavity of each slide. Label the slides with the sucrose concentrations you are investigating.
6. Collect a flower that has several mature anthers and is shedding pollen, as riper pollen has a better chance of successful germination. Gently rub the point of a mounted needle over the anthers so that pollen falls onto the medium on each slide. Dislodge any pollen stuck to the needle by tapping the needle against a pencil or forceps. Do not add a coverslip. Repeat for each sucrose concentration, using the same flower if possible.
7. Note the time at which the pollen was added to the medium and place the slides in the Petri dishes. Remove only when observing with the microscope.
8. Use a microscope with ×100 magnification and an eyepiece graticule calibrated using the stage micrometer to observe the slides for pollen germination (see **fig A**). Measure pollen tube growth as you have planned. To prevent the samples drying out or overheating, make your observations quickly before turning off the microscope lamp and returning the slide to the Petri dish.

Analysis of results

1. Work out the average growth over time for each sucrose concentration. Record your results in a table.
2. Draw a graph to show how mean growth of pollen tubes varies with concentration of sucrose.
3. Write a conclusion that summarises your findings. Compare your findings with those from other sources found in your research. Remember to cite your sources of information correctly.
4. Evaluate your results and conclusion. What were the major sources of error?

Learning tips

- Make sure that you know how to cite references from scientific journals correctly. For example: Butler, K.G., 2000. Pollen germination across the seasons. *School Science Review*, 82 (298), 93–94. This format should be followed, rather than just the web address, even if sources are available online.

Questions

1. You were instructed to use pollen from only one flower for all the concentrations. Comment on any advantages and disadvantages of this approach in terms of the validity and reliability of results.
2. What is the role of the pollen tube?
3. Suggest what might cause pollen tubes to grow in the correct direction in the style.

Core practical 4: Investigate the effect of sucrose concentration on pollen tube growth

Objectives

- Observe the growth of pollen grains
- Develop the skills of planning investigations and carrying out dilutions
- Carry out research and cite sources of information to support planning and conclusions

Safety

For making the mineral solutions:

- Calcium nitrate is oxidising and irritant at concentrations greater than 0.8 mol dm^{-3}. Wear eye protection.
- Potassium nitrate is oxidising, explosive with some metals and dangerous with some combustible materials. Wear eye protection. Dilute to less than 5% (w/v) before pouring the solution down a foul-water drain.
- 1.0 mol dm^{-3} ammonium hydroxide is irritant at this concentration, meaning that eye protection should be worn. It is corrosive at 6 mol dm^{-3} and above and explosive with some halide and metal salts and with oxygen. If making the 1 mol dm^{-3} solution from concentrated stock, use a fume cupboard, avoid inhaling vapour and wear splash-proof goggles or a face shield and chemical-resistant gloves.
- Magnesium sulfate and boric acid are a low hazard.
- Wash hands after handling flowers or plant stems.
- Refer to CLEAPSS Hazcards 6, 19A, 59B and 82 for further guidance.

Equipment per student/group	Notes on equipment
sucrose solution 2 mol dm^{-3}	2 mol dm^{-3} sucrose stock concentration (68.46 g in 100 ml). This solution will keep for some months in a refrigerator.
mineral salt culture medium	Make up immediately before use. To 1 litre of distilled water add: ● 0.417 g calcium nitrate ($Ca(NO_3)_2$), 0.200 g boric acid 0.101 g potassium nitrate (KNO_3) ● 0.217 g magnesium sulfate ($MgSO_4 \cdot 7H_2O$) 3.5 cm^3 of 1.0 mol dm^{-3} ammonium hydroxide (NH_4OH). Equal volumes of the mineral salt and sucrose solutions should have a pH of 8.8. Vary the NH_4OH in the salt solution to adjust the pH if necessary.
microscope (×100 and ×400) with eyepiece graticule	One per group
stage micrometer	These can be shared between groups.
plants in flower (for example, fast-cycling brassica, *Pelargonium* sp., *Impatiens* sp., *Narcissus* sp., Liliaceae)	There is useful information on the plant species suitable for different seasons at www.saps.org.uk (search for 'pollen tube growth'). Older flowers that are fully open work best.
Petri dishes	Five per group

filter papers	
marker pens	These should be suitable for labelling dishes and slides.
distilled water	
measuring cylinders or syringes	These should measure 1 cm^3 and 10 cm^3.
stop clock	One per group
balance	These can be shared between groups.
scissors	These can be shared between groups.
forceps	One per group
mounted needle	One per group
dropping pipette	One per group
very clean cavity slides	If not available, ordinary slides can be used as long as care is taken not to disturb the drop.

Notes