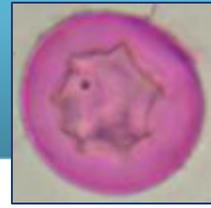


Activity Set B: Exploring Pollen in the Atmosphere



Summary: Students explore wind-pollination through investigating pollen in the atmosphere. They construct simple pollen traps and set them outdoors. Using a staining technique to distinguish pollen from other material on microscope slides, students conduct pollen counts from the collected pollen. They analyze their data and try to answer their research question. Activities can be extended to try to identify or classify the types of pollen collected. Students communicate with scientist mentors online about their investigations.

Learning Activities:

1. Main Activity: Students investigate questions based on collecting atmospheric pollen
2. Extensions: Make or use a pollen reference library to try to identify or classify types of collected pollen

Skills:

1. Develop a research question
2. Design and conduct an investigation based on a research question
3. Use a simple pollen trap to collect pollen
4. Use staining techniques for pollen
5. Use compound microscopes for pollen observation
6. Analyze data and develop conclusions
7. Communicate ideas, questions, observations, conclusions

Concepts:

- Pollen from some plants can be carried in the atmosphere, sometimes for great distances.
- Atmospheric conditions can affect atmospheric pollen counts and pollen types.
- Pollen in the local atmosphere can originate from local plants, but also from distant plants.
- Pollen in the local environment is related to the diversity and distribution of plants covering a wide geographical area.

Evidence of Understanding:

- Students successfully pose an inquiry question related to atmospheric pollen.
- Students collect evidence in relation to their inquiry question.
- Students make sense of their evidence and form logical conclusions based on evidence.
- Students share and communicate their findings.

Juicy Questions for Discussion and Investigation:

- *How much pollen do we find in our air?*
- *What conditions affect the amount of pollen in the air?*
- *What kinds of plants do the pollen grains we find come from?*
- *What types of plants do you have around your pollen traps and how does that affect what you collect?*

Prepare for Field Sampling and Data Collection

(For the Teacher)

You Will Need:

- Access to outdoor sites with flowering grasses, crops, trees, shrubs (review Background information, p. 46 and Examples of Scientific Inquiry Questions, p. 35 for appropriate locations)
- Field / lab notebooks for students
- Pollen Traps – see procedure for preparing them, and relevant materials, on p. 37
- Calberla's Solution for staining



Order!

- **You will need to order Fuchsin Basic Stain crystals in advance – see p. 37 for ordering information**
- Make sure you have other chemicals on hand for preparing Calberla's solution – see p. 37
- Compound microscopes
- Classroom / lab storage space for students' collected pollen traps (trays or labeled cabinet shelves)
- Calculators
- Graph paper and/or computer database (e.g., Excel) for data analysis

Safety Concerns:

- Check with students for allergies to pollen, insect bites or bee stings
- Check for ticks
- Take cover immediately in a storm involving lightning or strong winds

Preparation:

- Investigate field locations yourself, noting what wind-pollinated plants may be flowering -- look for catkins on trees, grasses with anthers and/or feathery stigmas visible, conifer trees with pollen-producing cones, herbaceous plants such as ragweed (*Ambrosia* sp.) or lamb's quarters (*Chenopodium album*) – or many other wind-pollinated plants.
- If students investigate atmospheric pollen at different sites in order to compare them, you might also want to find sites that do not have many wind-pollinated plants nearby.

Important Considerations:

- We recommend investigations that do not require pollen identification, as this is a difficult task and generally requires specialist knowledge. Therefore, the suggestions that follow are written to use general atmospheric pollen counts (from pollen collected on a microscope slide surface) as the dependent variable data. However, if students want to try to classify what kind of pollen they find, they might devise their own key based on pollen shapes they find. For advanced investigations, some resources are suggested for pollen identification in the Extensions section (p. 47).

For the Teacher: Instructional Procedure

1. Introduce the concepts of wind pollination through class discussion or thought provoking questions for homework. (Refer to: Develop a Research Question, p. 34, and Background information, p. 46)
2. Explain the general idea of the investigation and have students communicate with mentors about it.
3. Have students decide on a research question (pp. 34-35) and develop an experimental design (p. 36). Note: we recommend that the data students collect is *pollen counts per field of view*. That is, they will sample pollen on a microscope slide, stain it, and view the slide under a microscope at low to medium magnification, counting the number of pollen grains (regardless of pollen type) in a field of view. Consider having students use multiple samples by sampling multiple fields of view for each slide, as well as using multiple slides (pollen traps). (Refer to Data Collection Procedure, p. 43)
4. Have students communicate with mentors about their investigation and experimental design and incorporate mentor suggestions.
5. Finalize research question, hypothesis and experimental design, and post on the PlantingScience website.
6. Send teams outside to find their sampling sights based on their research questions – have them describe and/or illustrate it in their field notebook.
7. Follow the procedure to create pollen traps (pp. 37-38).
8. Based on student experimental designs, have them place their pollen traps at their sampling sights and leave for 1-24 hours. (Get a practical sense of how many days / where they will be sampling.)
9. Have them collect their slides (p. 39).
10. Using Calberla's Solution, students stain slides then observe them under the microscope and record data (pollen grains per field of view) (pp. 40-43).
11. Have students communicate with mentors online and describe what they are doing.
12. Have students analyze data, graph it and form tentative conclusions (pp. 44-45).
13. Have students share and discuss their conclusions in class and with their online mentors.
14. Have students post final results and conclusions on the website.
15. Be sure to leave time to have students share their data with mentors, and to have discussion with them about the meaning and significance of their findings.

Main Activity: Exploring Pollen in the Atmosphere



Develop a Research Question

Pollen from wind-pollinated plants is transported through the atmosphere. People who are allergic to pollen will tell you that there is more or less pollen in the air on any given day, as they see their symptoms come and go. Sometimes people are allergic to only certain types of pollen, reacting only when particular types of pollen are in the air, but don't react to other types of pollen.

What do you think might affect how much pollen is in the air?

Think about different types of atmospheric conditions? Do you think they affect the amount of pollen in the air?

Do you think time of year might affect how much, or what type, of pollen is in the air?

When do different types of wind-pollinated plants produce pollen? Think about pines, spruces, junipers, cedars, oaks, maples, elms, alders, birches. What about grasses? What about ragweed?

Do you think time of day might affect how much pollen is in the air?

Think about what time of day you might expect plants to shed pollen.

How far do you think pollen travels?

How high in the air is pollen found?

Think about plant heights (including trees), and where the target is for pollen to land.

Background investigation:

Go to the website: www.pollen.com

Type in your zip code at the top of the page and click "enter."

Investigate the forecasted pollen counts in your area.

Look at the section labeled "Today's Prominent Pollen" and see what plants are highlighted. You can click on these plants and learn more about them.

Develop a team question:

Given your investigations, what are you curious about related to atmospheric pollen?

Allow time to share your question with your online mentor and get some feedback.

Examples of Scientific Inquiry Questions for Atmospheric Pollen:

Your online scientist mentor will have additional ideas. Discuss your potential questions with them.

- Impacts of weather on pollen count
 - Rain (rain typically tends to clear air of pollen)
 - Humidity
 - Barometric pressure
 - Wind vs. still
 - Direction of wind (wind can carry pollen from far away)



- Compare pollen counts near different wild or cultivated plants (such as wind vs. insect pollinated)
 - Fruit or nut orchard (insect pollinated) versus corn field (wind pollinated)
 - Goldenrod (insect pollinated) versus ragweed (wind pollinated)



- How far is local pollen traveling?
 - Corn pollen, for example, is relatively heavy. You could set up pollen traps next to the field, a few yards away, a block away, a quarter mile away, etc.

- When do you catch the most pollen (when is pollen released)?
 - Different times of the day
 - Different times of the year



- Are there differences in pollen collected at different heights?
 - For example, setting pollen traps on window sills at the 3rd story level versus ground level
- Advanced Study: Regional versus distance pollen – compare pollen on slides with what's in flower locally (or is pollen traveling from somewhere else?)
 - For example, if you find pine or juniper pollen in January, but these trees are not producing pollen locally, from where might that pollen originated?

Develop an Experimental Design

1. **What is your research question?**
2. **Based on your research question, what is the condition (or variable) you want to vary?** For instance, if you want to investigate pollen counts at different locations, then LOCATION is the condition you want to vary. (Other examples might include, but are not limited to, HEIGHT, DAY, DISTANCE, TYPE OF NEARBY PLANT, TIME, etc.) The condition you want to vary will be your **independent variable**.
3. **What is your dependent variable?** (What data will you collect?)
4. **What is your general procedure for data collection?** For instance, where and when will you set your pollen traps? How many pollen traps will you set (number of samples)? How and when will you count pollen grains? What magnification will you use to count pollen grains (40X or 100X is recommended)? Be sure everybody collecting data uses the same procedure so that you can truly compare the results.
5. **Discuss your research question, independent and dependent variables, and experimental design with your classmates. Post these onto the PlantingScience website and ask your mentor for input on your ideas.**

Make Pollen Traps, Collect Pollen, and Record Data

In order to investigate your research question and collect atmospheric pollen, you will need to make a simple pollen trap.

Materials:

- Microscope slide for each location
- Cover slip for each slide
- Petroleum jelly or double sticky-side tape
- Duct tape
- Scotch tape
- Pasteur pipette (as thin as possible)
- Paper towels
- Scissors
- Compound microscope (best if it can magnify up to 400X or higher)
- Calberla's solution (pollen stain – see instructions below)
 - 5 ml glycerol
 - 10 ml 95% ethanol
 - 15 ml distilled water
 - A few crystals of fuchsin basic stain
 - Distilled water



ORDER IN ADVANCE

Be sure to order Fuchsin Basic Stain (crystals).

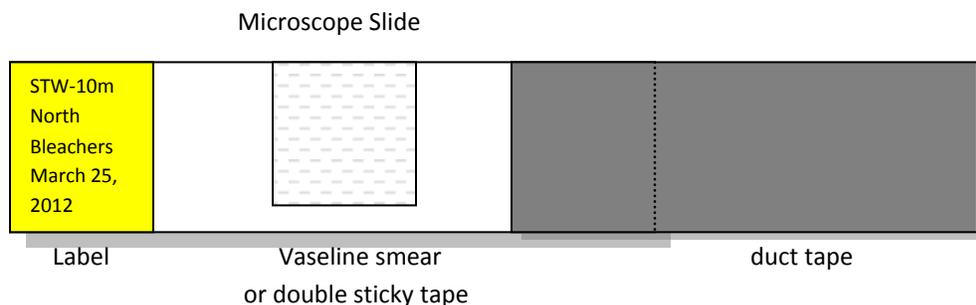
It can be ordered from Science Kit at

<http://sciencekit.com/fuchsin-basic-stain/p/IG0015250/>

Making Simple Pollen Traps

Set Up:

- Label one end of the microscope slide with your group name, date(s) and location for each slide.
- Smear a very thin layer of Vaseline in the middle of your slide, just under where a cover slip will set. (Or cut a piece of double sticky-side tape and place in the middle of the slide.)
- Cut a piece of 3-6" duct tape, and then tear it in half longitudinally.
- Place the duct tape on the other end of the slide which you will use to secure your slide outside (such as to a bleacher seat, window sill, air conditioner, etc.).



Making Simple Pollen Traps (continued)



Start with a microscope slide.



Put just a little petroleum jelly on your finger. (Double sticky-side tape can also work.)



Place a thin layer of petroleum jelly just in the middle of the microscope slide. Immediately use the paper towel to clean your finger so you don't smear the petroleum jelly all over.



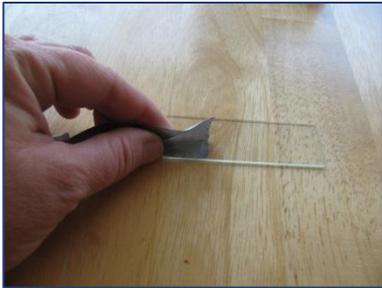
Take a 3-6" strip of duct tape, and cut (or tear) it longitudinally so it's just about the width of a microscope slide. Place the tape on one end of the slide to secure it at your outdoors location.



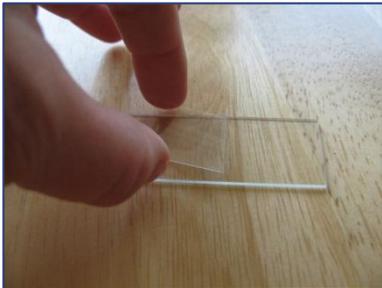
Set your slide at an outdoor location, petroleum jelly side up, and secure it with the duct tape. Leave it for 1-24 hours and collect it.

Setting Traps and Collecting Pollen

- Take your pollen trap to a quiet, dry location which will not be disturbed or get wet if it rains.
- Secure in place with duct tape.
- Retrieve after 1-24 hours.
- Discard the duct tape, but keep the labels for further comparison.
- Stain pollen with Calberla's solution to distinguish the difference between pollen and dirt (see staining directions below).
- Use a cover slip to protect and preserve what you have collected.
- If you wish, you can use a very thin piece of Scotch tape to secure the cover slip.



After collecting the slide, remove the duct tape.



Stain with Calberla's solution (see below), and place a cover slip on the slide, setting one end down first, and gently setting the other end down.



If you wish, you can secure the cover slip by placing a small piece of Scotch tape on the very edge of the cover slip.



Use scissors to trim the tape, so that when folded over, it does not occlude the field of vision.

If you store slides to be viewed later, be sure they have a cover slip on to prevent additional collection of dust/pollen from the air.

Stain Pollen with Calberla's Solution and View Pollen

(Directions for making Calberla's solution on page 41)

1. After collecting atmospheric pollen on your slide, lay the microscope slide on a flat surface.
2. Drop one to three drops of Calberla's solution onto the slide.
3. Cover with a cover slip by laying one edge down first and gently laying the rest down.
4. Use the edge of a paper towel placed at the edge of the cover slip to draw excess stain away.
5. Place slide on microscope stage and observe. Start with lowest power first and focus on pollen grains. Once those are in focus, move to the next highest power and use the fine focus. Repeat at the highest power.
6. Using Calberla's solution, pollen will be stained a fuchsia color (deep purple-pink), and other matter will be stained dark red or brown.

Draw and write your observations below. Use your observations to address your research question(s).

Scan or take a photo of your drawings to share with your online mentors. Be sure to use their expertise to address questions and additional thoughts you may have.

Making Calberla's Solution (Pollen Stain)

Materials:

- 5 ml glycerol
- 10 ml 95% ethanol
- 15 ml distilled water
- 5-15 drops saturated aqueous solution of basic fuchsin
 - A few crystals of fuchsin basic stain
 - Distilled water

ORDER IN ADVANCE

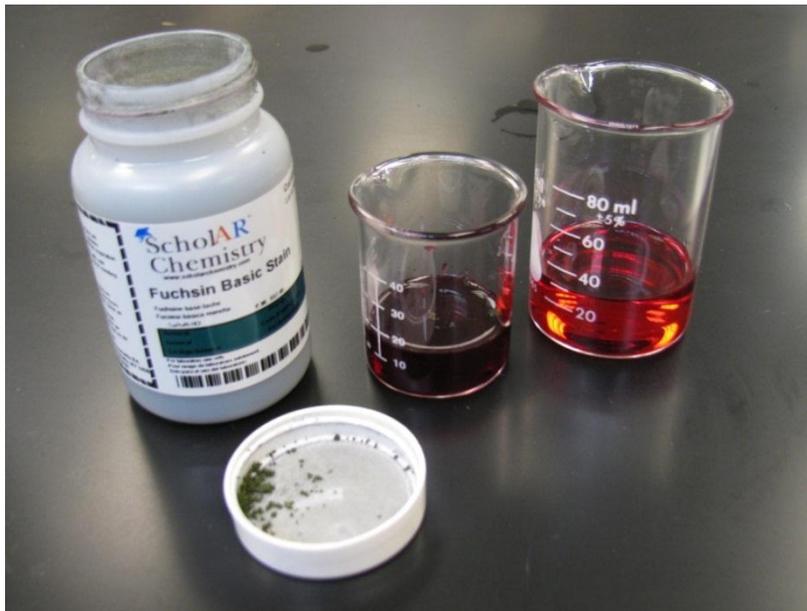
Be sure to order Fuchsin Basic Stain (crystals).

It can be ordered from Science Kit at

<http://sciencekit.com/fuchsin-basic-stain/p/IG0015250/>

Place a few crystals of fuchsin basic stain in a small beaker, and a small amount (~20 ml) of distilled water over it. Stir until crystals will not dissolve any further. If crystals completely dissolve, add a few more crystals. This will be your saturated aqueous solution of basic fuchsin.

Measure out the glycerol, ethanol and distilled water into a medium sized beaker (<100 ml). Add the drops of saturated aqueous solution of basic fuchsin slowly. Adjust the fuchsin concentration so that a microscope slide is stained a light red (some suggest a "claret" color – see photo below) and not a dark red. If the solution is too dark, it is difficult to see the structures, but if it is too light it will hardly stain the pollen. The fuchsin stains components in the outer wall of pollen, but not fungus spores and most other debris. You may occasionally observe some pink-stained debris, but it can usually be distinguished from pollen because it is irregularly shaped and not spherical (or nearly so) like pollen.

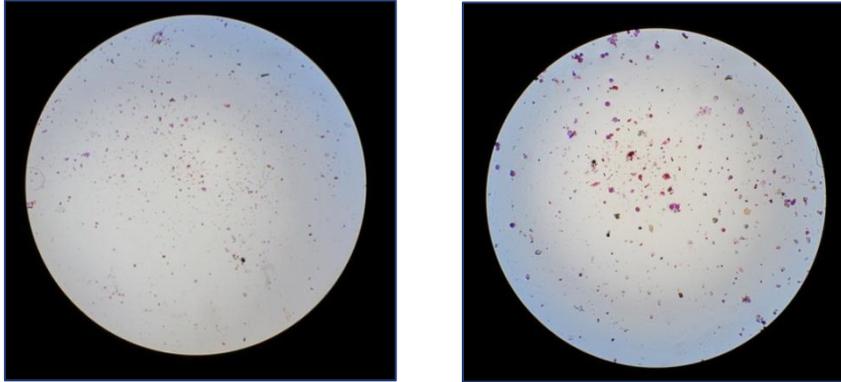


Left: Jar of Fuchsin Basic crystals, and crystals shown in the lid of the jar in front.

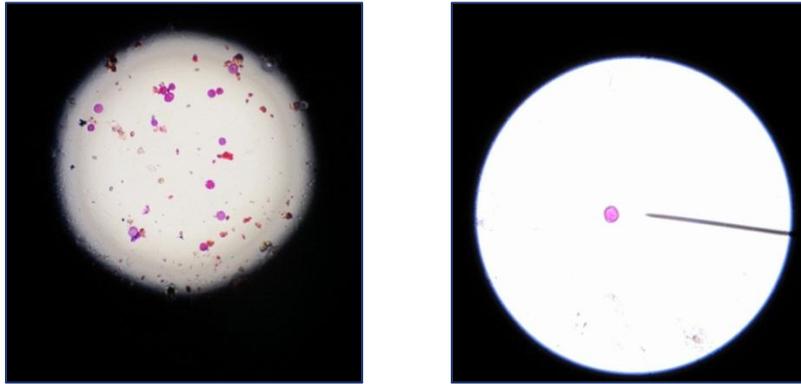
Middle: Saturated aqueous solution of fuchsin basic, made by pouring a small amount of distilled water over fuchsin basic crystals, and mixing until crystals stop dissolving. Notice the dark red color.

Right: Calberla's solution. Note the much lighter red ("claret") color. This is the stain you will use on your microscope slides.

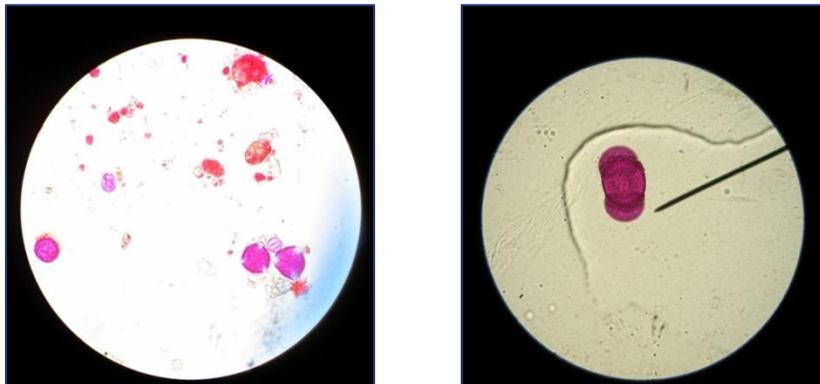
Images of Atmospheric Pollen



Above: Field of view at low magnification (40X).



Above: Field of view at medium magnification (100X).



Above: Field of view at high magnification (400X and 600X).

Note above left the difference between the pollen that is stained fuchsia (pink-purple), and the other material that is stained red. Pollen on the right is pine with the classic air bladders (which reminds some people of Mickey Mouse ears).

Data Collection Procedure

1. Once you feel confident in being able to recognize pollen from other matter on the slide, adjust the magnification back to low or medium power (the level your team agrees on for pollen counting) and count the number of pollen grains per field of view. Record your pollen count.
2. Move the same slide so you have a new field of view, and count and record the number of pollen grains per field of view.
3. Repeat Step 2 until you have the number of samples your team agreed to collect from one slide, then collect data from a new slide. Be sure that the data you record is well labeled – you should record the label of the slide along with each count. The following table is a suggestion of how you might record your data (the data are hypothetical, not real, data). Note that for each condition, two slides are used, and for each slide, four fields of view are observed.

Condition (independent variable)	Slide Label	Pollen Count at 40X	Average per slide	Average per condition
10m from tree	STW-10m, 3/25/2012	15 grains/field of view	14.5 grains/fov	13.25 grains/fov
		10 grains/field of view		
		19 grains/field of view		
		14 grains/field of view		
	LCM-10m, 3/25/2012	12 grains/field of view	12.0 grains/fov	
		8 grains/field of view		
		11 grains/field of view		
		17 grains/field of view		
20m from tree	STW-20m, 3/25/2012	25 grains/field of view	28.5 grains/fov	26.75 grains/fov
		29 grains/field of view		
		36 grains/field of view		
		24 grains/field of view		
	LCM-20m, 3/25/2012	21 grains/field of view	25.0 grains/fov	
		19 grains/field of view		
		28 grains/field of view		
		32 grains/field of view		
30m from tree	STW-30m, 3/25/2012	9 grains/field of view	7.0 grains/fov	6.25 grains/fov
		7 grains/field of view		
		4 grains/field of view		
		8 grains/field of view		
	LCM-30m, 3/25/2012	5 grains/field of view	5.5 grains/fov	
		6 grains/field of view		
		8 grains/field of view		
		3 grains/field of view		

Analysis and Reflection

Data Analysis

- 1. What type of data is your independent variable?** Data can be quantitative (measured), such as number of feet from a tree, or number of meters from the ground, or it can be categorical data (such as the type of nearby plant, or the date, or atmospheric condition).
- 2. What type of data is your dependent variable, and what is its unit of measurement?**
- 3. How is your raw data recorded?** (In a table in your notebook, in a database, etc.?)
- 4. What kind of graph can you create to represent your data?** For instance, consider first what data you will show on each axis (the *x*-axis and *y*-axis). Then consider whether you think a bar graph, a line graph, or some other type of visual representation would be most appropriate. Try plotting your data and discuss it with your team members.
- 5. Try to upload your raw data (from a table or your notebook) and your graph (or other visual representation) to the PlantingScience website to share with your mentors so you can get feedback and thoughts from them.** If your work is in a computer file, you can directly upload your work. If it's handwritten, you can scan or take a digital photo of it, and upload those image files to the website.

Formulating Conclusions and Discussion



Once you collect your observations and data, there are additional things to do as a scientist that are critically important. They include making sense of your results, relating your results back to your original research question, communicating with others and getting feedback. Scientists often formulate new questions to investigate at this point.

In PlantingScience, your online mentors are there to communicate with and get new ideas. Have you been communicating with them? As you try to make sense of your results, continue to get their input.

What was your original research question?

How did you attempt to answer this question?

Did your results provide any answers, partial answers, or surprises? Cite your evidence.

What new ideas or thoughts have you gained from classmates?

What new ideas or thoughts have you gained from your online mentors?

What can you conclude from your investigation? Cite your evidence.

What new research questions could be investigated now, based on your current study?

You can use your answers to these questions to prepare a final report or presentation. It is important to share scientific work, so others can learn from it. Scientists are part of a community, and everyone builds on others' knowledge. Sharing and communicating that knowledge is part of a scientist's responsibility.

Background: Wind Pollination



Have you ever seen television commercials advertising allergy medicines? They often feature large colorful flowers, suggesting that such flowers cause hay fever. This is rather misleading. Why? Large colorful flowers have generally evolved to attract animal pollinators. Plants that are primarily wind pollinated do not need to have large colorful flowers to attract pollinators. In fact, large petals can hinder wind dispersal of pollen. So wind pollinated plants usually possess small inconspicuous flowers, such as in ragweed, or in the catkins of deciduous trees. Many wind pollinated plants do not have flowers at all, such as the conifer trees, which have cones that produce pollen.

Animal pollinated plants can have pollen that is heavy and sticky in order to attach to the pollinator. In contrast, wind pollinated plants have pollen that is light. Wind pollinated plants also produce large amounts of pollen in comparison to animal pollinated plants. If you think about it, you can imagine that chances are better for pollen to be transferred from one flower to another of the same species when an animal pollinator delivers the pollen rather than when the wind blows it. To increase the probability that wind pollinated plants will pollinate successfully, they produce copious amounts of pollen. Sometimes with a gust of wind, you can see a big yellow cloud drift away from a tree releasing pollen. That's a lot of pollen!

Many people in North America think they are allergic to goldenrod flowers (*Solidago* sp.), because they are showy and colorful, and appear in late summer – about the

same time their allergies get worse. Usually, however, people are not allergic to goldenrod, but are rather allergic to ragweed (*Ambrosia* sp.), which flowers at the same time and often grows right next to goldenrod. Ragweed flowers, however, are green and small, and hardly noticeable. Being wind pollinated, ragweed produces lots of pollen that is released into the air. In contrast, goldenrod relies on insect visitors, and its pollen is heavy and sticky.

Another consideration for wind pollinated plants is air movement. Sheltered habitats can restrict air movement, so many wind pollinated plants are found in open habitats. For instance, most plants in the grass family (Poaceae) rely on wind pollination, and they are often found in open meadows, plains and prairies. In fact, the flowers of grasses actually lack petals and sepals, decreasing obstructions to their anthers and stigmas and allowing greater air flow. Many temperate-forest trees are wind pollinated, such as oaks, birches, elms, etc. They flower before the leaves on their trees emerge, allowing for fewer obstructions and increasing air flow among their flowers. These trees produce flowers in early spring, also the windiest time of year. The small air currents around pine cones – formed by the spiraling scales – have been shown to enhance the delivery of pollen inside the cone near the ovule.

Pollen borne on the wind can travel varying distances and to various heights. In general, pollen is released in wind bursts, and most of it falls close to its source. That said, pollen can also travel long distances, sometimes hundreds of miles away!

Extensions: Make or Use a Pollen Reference Library

Make a Pollen Reference Library: In order to try to identify atmospheric pollen you collect, you can make a reference library of your own by collecting pollen of known sources shedding pollen in your area, or you can access online images from some of the sites below.

Prepared slides: To purchase professionally prepared pollen reference microscope slides, see Aerobiology Instruction and Research, LLC (AIR): <http://www.aerobiologylab.com/>

Refer to Online Pollen Microscopy Image Galleries

To make the most of these sites, you may need to know the scientific names, and/or the family names for the relevant plants. Some of these sites provide keys to identify unknown pollen grains based on the characteristics of the pollen.

Also, don't be deterred if some of these sites are for specific geographical regions – there are many common plants in many different regions.

- The USDA website: <http://pollen.usda.gov/Photographs.htm>
- Science and Plants for Schools (UK): <http://www-saps.plantsci.cam.ac.uk/pollen/>
- Center for Pollen Studies, Saint John's University, Minnesota:
http://www1.csbsju.edu/pollen/images/pollensummarysheets/images_new_summaries.htm
- Missouri Pollen Project: <http://davidbogler.com/Pollen/pollen.html>
- Pollen key for selected plants of the San Francisco Estuary Region:
<http://oldweb.geog.berkeley.edu/ProjectsResources/PollenKey/byFamiliesAll-in-1.html>
- Key to the pollen of the Bahamas: <http://www.pollen.mtu.edu/>
- PalDat – Palynological Database – an online publication on fossil and recent pollen and spores:
<http://www.paldat.org/>
- University of Newcastle, Australia Aqua pollen reference
http://www.aqua.org.au/AQUA/Pollen/search_graphics_sketch.htm
- Ask a Biologist (scanning electron microscope – SEM – images):
<http://askbiologist.asu.edu/IMAGES/POLLEN-GALLERY>

Additional Resources and References

Other teaching resources:

Science Kit, source for Fuchsin Basic Stain: <http://sciencekit.com/fuchsin-basic-stain/p/IG0015250/>

SDI Health, Pollen.com for national and local pollen forecasts: <http://pollen.com>

Plant IT resources for pollen investigations, including the case study, *Paul's Puzzle*:

<http://www.myplantit.org/resources.php>

National Allergy Bureau: <http://www.aaaai.org/global/nab-pollen-counts.aspx>

Reiter, C. 1987. Mysterious magic of pollination. *National Wildlife* 25(5): 14.

(Describes how plants manipulate the wind to increase the amount of pollen the plants can capture. Mechanisms by which plants manipulate reproduction; how female plants trap pollen; properties of pollen grains; how pine cones act like turbines causing pollen-containing breezes to spiral around it.)

On methods for official atmospheric pollen counting:

Center for Pollen Studies, St. John University: <http://www1.csbsju.edu/pollen/>

Radboud University Nijmegen: <http://www.vcbio.science.ru.nl/en/virtuallessons/pollencounting/>

St. Louis County, Missouri: http://ww5.stlouisco.com/doh/pollen_site/FAQ.html

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Latorre, F., Romero, E. J., & VirginiaMancini, M. (2008). Comparative study of different methods for capturing airborne pollen, and effects of vegetation and meteorological variables. *Aerobiologia*, 24, 107-120.

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Niklas, K. J. (1988). Equations for the motion of airborne pollen grains near the ovulate organs of wind-pollinated plants. *American Journal of Botany*, 75(3), 433-444.

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