The Case for Forensic Botany

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Published By: National Association of Biology Teachers
URL: http://www.bioone.org/doi/full/10.1525/abt.2011.73.7.8

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ABSTRACT
Would you rather solve a forensics puzzle or take a lab practical? An alternative to the traditional lab practical can be used to assess students' skills and knowledge in plant cell biology and anatomy. This forensics project challenges students to analyze evidence from crime scenarios. The instructor supplies the scenarios, evidence collected at the crime scene, and type specimens. The students prepare the evidence and type specimens for analysis by light and polarized light microscopy and then document and report their findings. Students enjoy this project and are able to demonstrate their skills as well as their knowledge.

Key Words: Forensic botany; microscopy; plant cell; stomach contents.

A series of labs that engage students in solving forensic botany puzzles can serve as a new kind of practical exam. Boyd (2006) describes a field- and lab-based forensic botany exercise and says “it is clear that students... find forensic investigation a good way to pull together the course content...”. The activity presented here is used following a unit on plant anatomy and cellular ultrastructure. Traditional practical exams assess timed recognition and interpretation of prepared specimens, whereas forensics projects include other skills. This project encourages independent thought, requires proficiency in sample preparation and microscopy, and tests knowledge of plant structure.

The major part of the project is analysis of “stomach contents.” A crime scenario is presented and students are given tubes of stomach contents (manufactured by the instructor) that they must analyze to help solve the crime. Students use knowledge of the beautiful crystals, plastids, fibers, pigments, tissues, and arrangements of cells that comprise and differentiate food plants from one another to answer the question – What was the victim’s last meal? In Forensic Botany: Principles and Applications to Criminal Casework (Coyle, 2005: p. 140) it is reported that the average human diet includes roughly 70 to 100 different plant species! Forensic analysis of stomach contents from the victim of a crime includes light microscopy of the evidence in comparison to slides prepared from reference samples. Pre-lab readings to demonstrate use of botany in real forensics cases can include Silent Witness (Entwistle, 2003), Crime Solving Plants (Graham, 2006), The Secret Life of Plant Crystals (Amato, 2006), Of Murder and Microscopes (Nijhuis, 2007), and Forensic Botany: Case Studies in the Use of Plant Anatomy (Dilcher, 2001).

**Methods & Materials**
Depending on student preparation, the instructor may vary the scenarios and prompts. A directed prompt might be to determine whether the stomach contents contain a particular food. For example, given that the suspect was seen at a fast food restaurant with an unidentified companion at the time of the murder, does the evidence support the idea that the victim may have had a last meal of French fries? At the other end of the spectrum would be the open-ended: Identify at least six plant components of the stomach contents and suggest what the victim’s last meal was. One scenario enjoyed by both high school students in a one-day program and college students in a capstone lab was the mystery of the death of beloved pet ferret Bobo. Bobo passed away while in the care of a sitter. Did Bobo nibble on the leaves of houseplants that contain calcium oxalate crystals (druses or raphides) and secondary metabolites that may have done him in? Did the sitter feed Bobo forbidden foods? What was his last meal? Information such as what the sitter ate for lunch, what Bobo typically ate, and what plants were in the home was provided to guide investigation.

Basic microscopy and histological techniques are used. In previous lab sessions, the students have learned how to prepare thin sections of plant material for light microscopy. See Silvius and Eckart (1997) for discussion of plant specimens and techniques that involve no stains, and Yeung (1998) for a detailed guide to preparation of stains and hand sectioning of plant material. The two most useful stains can be purchased ready-to-use from Carolina Biological: toluidine blue O (available as a 1% aqueous solution) and Lugols solution (I₂KI). If polarized light microscopy is included, it can be used as a
tool to identify plant material on the basis of the Maltese cross evident in amyloplasts when viewed with crossed polars and the birefringence exhibited by cellulosic fibers (for complete instructions for a lab on polarized light microscopy, see McMahon, 2004). Inexpensive Polaroid filters are available from Edmund Scientific.

After the scenario is set, the students are provided with a microfuge tube of stomach contents, small spatulas and transfer pipets, staining dishes, forceps and razor blades, compound microscopes, distilled water, slides and coverslips, Lugol’s solution, toluidine blue, and an array of fresh type specimens. The students make mounts of the stomach contents, both stained and unstained, for viewing under the microscope. For comparison they then section and prepare fresh mounts of the type specimens. Data sheets are filled in as students analyze the stomach contents and are turned in to the instructor at the end of the project (see Figure 1 and Table 1).

Stomach contents are easily manufactured and modified to include specimens with which students are familiar. A leafy green and an apple or banana (mostly non-descript parenchyma) that will oxidize and turn brown make the base for the mixture and give it an unappealing color. Prepare the contents a day ahead of time and refrigerate to allow the brownish color to develop and fresh smell to dissipate. A suggested recipe is equal parts diced fresh potato, pear, apple, banana, leafy green, red bell pepper, pineapple, choice of flours, and sufficient water to make the mixture a thick slurry. Place these materials in a blender and blend on high speed until smooth. Using a transfer pipet with the tip cut high to enlarge the opening, aliquot the mixture into microfuge tubes. The mixture can be refrigerated for over a week.

○ Additional Exercises

(a) Is this real honey, or did the manufacturer adulterate it with other materials? Mix a large drop of honey with a drop of water on a microscope slide and apply a coverslip. Or mix a large drop of honey with

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On this sheet, use the left column (Evidence) to draw and comment on (indicate color, stain used, behavior under polarized light, etc.) single cells or clumps of cells seen in the stomach contents. Use the right column (type specimen) to draw and comment on the type specimen that you believe matches material found in the stomach contents. By comparing characteristics of cells from the type specimens with those found in the stomach contents, you can suggest what foods were eaten. In the comments section, explain your reasoning and indicate whether your evidence rules out certain possibilities or suggests a particular scenario.

Evidence: ___________________  Type Specimen: ___________________

Comments: ____________________________

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Figure 1. Data-sheet template.

Table 1. Expected results.

<table>
<thead>
<tr>
<th>Name of Method</th>
<th>Identifying Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato</td>
<td>Large egg-shaped amyloplasts. Visible unstained; black/purple with Lugol’s solution; large, off-center Maltese cross with polarized light.</td>
</tr>
<tr>
<td>Pineapple</td>
<td>Raphides visible unstained.</td>
</tr>
<tr>
<td>Pear</td>
<td>Clumps of sclereids visible unstained, blue/green with toluidine blue.</td>
</tr>
<tr>
<td>Banana</td>
<td>Small, irregular amyloplasts, clearly differentiated from potato plastids. Irregular parenchyma.</td>
</tr>
<tr>
<td>Apple</td>
<td>Loose parenchyma cells, tiny amyloplasts. Anthocyanin-filled vacuoles visible in red peel.</td>
</tr>
<tr>
<td>Leafy green</td>
<td>Chloroplasts visible unstained. Vascular tissue (especially spiral xylem) visible unstained.</td>
</tr>
<tr>
<td>Red bell pepper</td>
<td>Loose parenchyma cells with small red/orange chromoplasts.</td>
</tr>
<tr>
<td>Begonia house plant</td>
<td>Mace/star shaped druses visible unstained.</td>
</tr>
</tbody>
</table>
a drop of Lugol’s solution and apply a coverslip. Real honey contains pollen grains but no amyloplasts. Pollen grains are beautiful and easily visible unstained under 100× magnification. Amyloplasts are evident after staining with Lugol’s.

(b) What is the composition of this paper sample? If wood, is it hardwood or softwood? Place 1-cm squares of paper in microfuge tubes of hot water and allow to soak for a few minutes. With dissecting needles tease a tiny square of paper apart in a drop of water on a microscope slide – the more dispersed the fibers are, the better. Apply a coverslip. Prior to mounting, samples may be stained with toluidine blue and destained in water for better contrast. Paper made from softwoods show fibers and tracheids (long, thin cells with pits and tapered ends); paper made from hardwoods will show tracheids, fibers of various lengths and thicknesses, and vessel members (large-diameter cells with pits and open ends) (Petracco & Kubic, 2004). Cotton will show long, thin, twisted fibers (no pits) that are birefringent in rainbow colors under crossed Polaroïds. Type samples of each kind of paper should be available for comparison. A good instructor’s resource is Integrative Plant Anatomy (Dickison, 2000), which contains images and descriptions of useful fibers in the section on economic and applied plant anatomy.

(c) Is this coffee creamer bulked with starch? Add a small amount of potato starch to a commercial, powdered coffee creamer such as Coffee Mate (make sure there is no starch in the ingredients). Leave a batch of creamer unaltered. Dispense small scoops of the materials into microfuge tubes. Amyloplasts in the starch-treated samples will be evident with Lugol’s solution or with crossed Polaroïds.

(d) Is this fiber sample plant, animal, or synthetic? Does it match the clothing of the victim or suspect? If plant in origin – what plant? Carolina Biological provides a fiber identification kit (NP-69-98775) with instructions for microscopy and a burn test that students enjoy – the burn rate, odor, and ash of samples held to a flame distinguish fibers. After students work through the kit in a previous lab they are prepared to be given an unknown fiber sample. For the unknowns, tan/white yarns made of 100% wool, cotton, nylon, flax, and bamboo can be purchased at a craft store.

Considerations for the High School Lab

For the high school curriculum, a forensic botany lab can be used to work toward two of the national standards for life science education. The National Science Education Standards (National Research Council, 1996) for grades 9–12 include the cell. This lab demonstrates plant cell structure and can link with discussion of function in the classroom. Science as inquiry is a key standard emphasized for all grade levels under Teaching Standard B and Content Standard A of the National Science Education Standards. This lab asks students to trust their eyes and skills and to make conclusions on the basis of data they gather. If lab time is limited and safety considerations prevent students from using razors or scalpels, the following abbreviated version of the labs can be used.

Session 1: With 30 minutes of prep work, the instructor can prepare 10 slides each of thin sections of pear stained with toluidine blue, apple stained with toluidine blue, potato stained with Lugol’s solution, and unstained samples of all three specimens. The specimens are mounted in 30% glycerol, if available, to prevent them from drying out. Slides can be kept on trays in a refrigerator until class time or a coat of clear nail polish can be applied around the edge of the coverslip to preserve the slide. In the lab, students view the stained and unstained type specimens and draw what they see (they will keep their drawings to use as reference for the following lab). Students can prepare their own slides of crushed pineapple by taking a small amount of pineapple from the can, placing it in a drop of water, teasing it apart with dissecting needles, and applying a coverslip.

Session 2: Students are given a scenario and individual samples of “stomach contents.” Their job is to determine the composition of the victim’s last meal. No sectioning of type specimens is needed if students bring their drawings from the previous session or if the type specimen slides from session 1 are available. Students prepare fresh slides of small scoops of material onto the slides) of stomach contents (both stained and unstained), view them under the microscope, and report their findings.

Assessment

In a sophomore-level, semester-long General Botany course for biology majors, a forensics project similar to the one outlined above was done after a unit on plant anatomy. The instructor maintained a spreadsheet with a number for each student and the identities of samples provided for each category. For example, student 1 got cotton resume paper, creamer without starch, stomach contents batch A, etc. With several variants for each category, students had different samples from their lab neighbors and had to rely on their own skills and judgment. Students were free to discuss the projects and use outside resources as they would in a real-world situation. Grades were based on correct identifications with appropriate supporting evidence.

A traditional lab practical (24 timed stations each with specimens under the microscope) was also given at the semester’s end. Out of 31 students, 85% scored higher on the forensics project than on the practical. In particular, some students who performed at a C or lower level on written exams and traditional practicals showed excellent work on the project. Without a time constraint and with the ability to use the text and lab notes, the forensics project allowed students to show their capabilities in a different way.

In an anonymous survey administered after the semester, 80% of respondents indicated via answers to objective questions that they enjoyed the lab, that it reinforced microscopy skills, that it tested knowledge of plant structure, and that it should be used again. All written comments received were positive and included the following: “Applying our knowledge to an actual task was a nice and fun way to learn and review,” the lab series was “memorable,” and the “open-ended structure of the lab allowed for experience in the true research aspect of biology.”

References


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