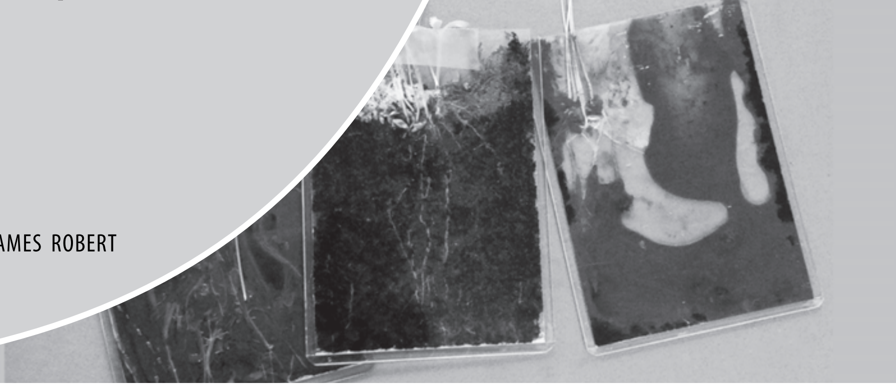
TIPS, TRICKS & TECHNIQUES

Turning Everyday Materials into Living Microscope Slides



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Abstract

Teachers have reported that students enjoy growing plants but that logistical constraints such as limited space and inadequate lighting make it difficult to incorporate living plants into their classrooms. We present a method that takes familiar materials from the students' world – trading-card holders – and uses them to make interactive, cost-effective "plant pouches" that can function as living microscope slides. Students grow plants in card holders and are able to observe both the roots and shoots for several weeks, including making observations with a compound or dissecting microscope. The plant pouches require minimal space or resources, and the system is flexible enough to accommodate different types of plants and is amenable to experimentation.

Key Words: *Botany; plant science; microscopy; observation; plant blindness.*

○ Introduction

Plants provide the foundation for terrestrial life, and their significance cannot be overstated. Yet people of all ages tend to overlook the importance of plants to their everyday lives and to life on this planet, a phenomenon known as "plant blindness" (Wandersee & Schussler, 1999, 2001). Educators can counter plant blindness and promote the development of botanical literacy and appreciation by increasing students' exposure to plants (Lindemann-Matthies, 2005; Schussler & Winslow, 2007; Strgar, 2007; Uno, 2009; Fancovicova & Prokop, 2011; Hemingway et. al., 2015).

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Logistical constraints such as limited space and access to adequate light and water may impede teachers' abilities to grow plants in the classroom (Link-Pérez & Schussler, 2013). The method presented here overcomes logistical and other concerns that could dissuade teachers from incorporating living plants into their curriculum. Students grow plants from seeds in rigid plastic top-loading card holders like those used for trading cards (pack of 25 for under $5.00 on Amazon; see "Materials" for details). Absorbent paper towels provide a soilless (and mess-free) medium to support growth inside the card holders, and a string extending from inside the card holder to an external water source keeps the absorbent paper adequately moistened via capillary action, with little or no need for constant monitoring or additions of water. The space requirement is minimal, as 21–28 "plant pouches" can be accommodated along with a lamp for a light source in as little as one square foot of counter space (Figure 1).

The card holders function as both a growth environment and a microscope slide (Figure 2). The path of roots and shoots can be observed by the unaided eye or with a hand lens or microscope daily for up to three weeks using grass seeds (oats, wheat, or barley) – or longer, depending on the type of plant (Figure 3). These plant pouches are low-cost (less than 30 cents each for materials, excluding seeds), highly visual and tactile, and likely to stimulate students' interest since the card holders – a familiar object for many – are being used for a different purpose than intended by the manufacturer.

A particular benefit of the plant pouches is that the card holders, being clear, expose the typically hidden realm of roots. The ability to examine the plant pouches under a microscope focuses students' attention on previously unobserved details of root growth (Figure 4). The plant pouches are suitable for microscope viewing up to 100× and, to a limited extent due to the thickness of the pouches, 400× total magnification; microscopic examinations need to be conducted only for short periods of time to prevent damage to the roots due to overheating and before condensation on the inside of the card holder prevents a clear view. Students can document their observations by taking photographs using their smartphones or tablets (note: all images in this article were obtained using an iPad or

The American Biology Teacher, Vol. 78, No 4, pages. 328–333, ISSN 0002-7685, electronic ISSN 1938-4211. © 2016 National Association of Biology Teachers. All rights reserved. Please direct all requests for permission to photocopy or reproduce article content through the University of California Press's Reprints and Permissions web page, www.ucpress.edu/journals.php?p=reprints. DOI: 10.1525/abt.2016.78.4.328.

Figure 1. Plants can be grown in card-holder "plant pouches" even when space is severely limited. An ice-cube tray turned upside down provides a suitable support for the plant pouches, including adequate spacing between pouches. A lamp for overhead light and a small glass of water provide the other growth requirements.

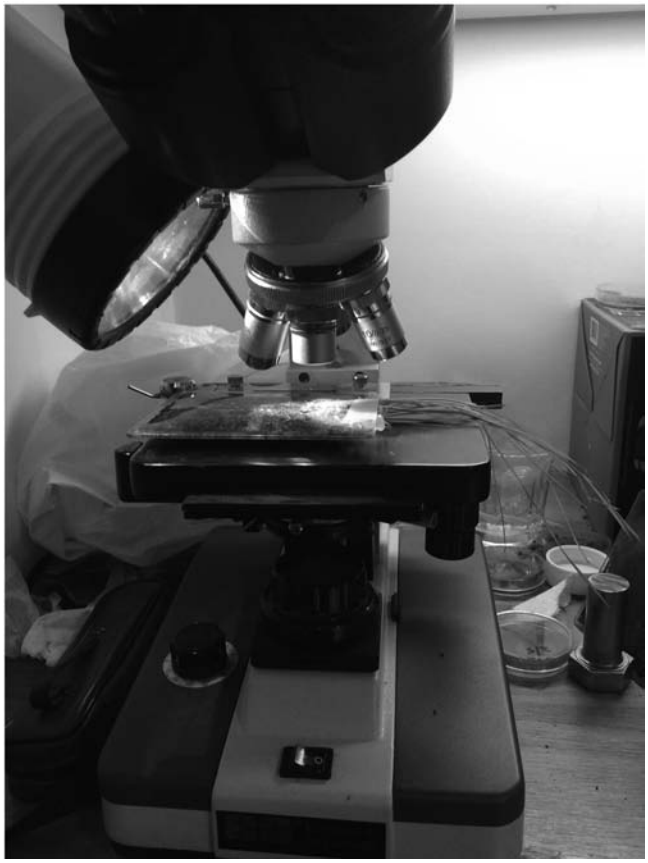

Figure 2. "Plant pouch" being viewed with compound microscope; note the flashlight providing additional incident light to enhance visibility.

smartphone, including photographs taken by holding the smartphone camera to the eyepiece of a microscope). Whether used in conjunction with microscopy and hand lenses or not, the plant pouches can provide an interesting opportunity to help students develop their observational skills and alleviate some symptoms of plant blindness.

○ Materials

- **Rigid top-loading trading-card holders.** Note that soft card holders easily distort and are not amenable to use as microscope slides. We use Ultra Pro 3 × 4 inch Toploader Series, available from http://www.amazon.com for under $5.00 for a package of 25.
- **Strong, absorbent paper towels ("shop towels").** These must be the blue shop towels sold in the automotive section, because they are extremely tough, lint free, and absorbent. The paper towels hold water and function as the growing media within the card holders.
- **Standard 3× 5 inch notecards.** These are used to help insert the paper towel into the card holder.
- **Cotton string or baker's twine (6–16 ply).** This is used as a "wick" to draw water into the card holder via capillary action. Thicker string does a better job of wicking sufficient volumes of water.
- **Dissecting needle or craft popsicle stick.** This is used to position the string and seeds. Craft popsicle sticks (100/package) can be purchased for under $5 online (http://www.amazon.com).
- **Seeds.** The following grass species work well, are easily obtained through online sources, and are important agricultural crops familiar to students as food items: oats (*Avena sativa*), wheat (*Triticum* spp.), and barley (*Hordeum vulgare*). Grass seeds germinate within four days of planting, and the grass blades attain lengths of 16–24 cm within 14 days. A benefit of using grass species is the presence of an intercalary meristem at the junction of the root and the shoot, which enables students to "trim" the grass blades periodically to maintain them at a manageable length (this mimics grazing by animals or mowing of lawns; grass continually grows from the intercalary meristem, meaning that the "newest" part of the shoot is always adjacent to the roots). Other common garden seeds would work, too, and provide many options for observing

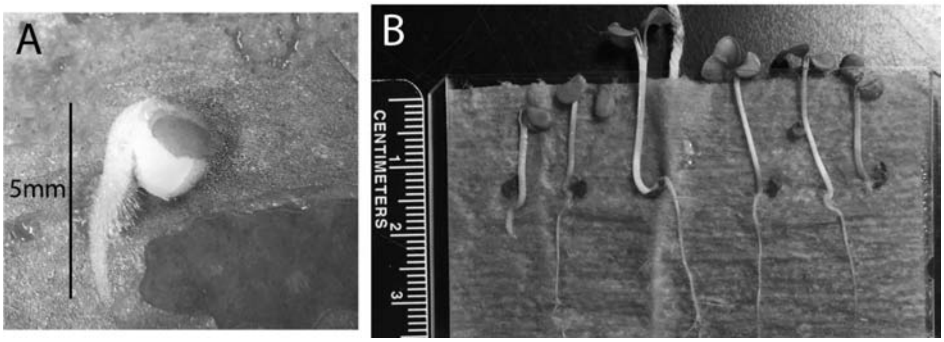

Figure 3. (**A**) Fast Plant shown 22 hours after sowing in a "plant pouch," viewed with a dissecting scope at 7× total magnification. (**B**) Fast Plants eight days after sowing; roots extend up to 5 cm in length (not shown in their entirety).

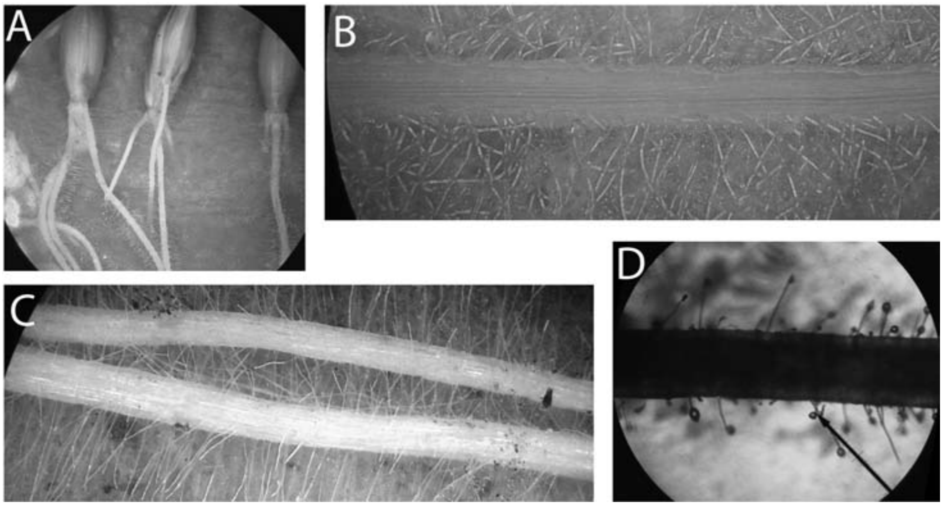

Figure 4. (**A**) Oat seeds soon after germination, with multiple roots emerging from each grain ("caryopsis"); viewed with dissecting microscope at 10× total magnification. (**B**) Translucent and delicate root hairs extending from near the tip of a growing root, providing large surface area for absorption of water and minerals; viewed with compound microscope at 40×. (**C**) Root hairs extending from root growing across the absorbent paper towel of the "plant pouch," viewed with compound microscope at 40×. (**D**) Detail of root hairs on a plant growing in a plant pouch containing potting media instead of paper towel, observed with compound microscope at 400×.

plant diversity. Basil, radish, lettuce, and herbs with small seeds are recommended, though the plants will not attain flowering size in the plant pouch environment. Sunflower seeds require presoaking in tap water for 24 hours prior to sowing; they grow large rapidly, so only dwarf varieties should be used. If the goal is to explore an entire plant life cycle, Wisconsin Fast Plants can be used (available from http://www.carolina.com); flower buds appear within 14 days of sowing, although the plants themselves tend to be dwarfed in the plant pouches with the paper towel media (normal-sized Fast Plants are obtained when peat moss is used as the growth media in the card holder instead; see "Modifications & Extensions" below).

- **Water-soluble plant fertilizer.** It is imperative to provide mineral nutrients to the plant pouches; once plants have consumed the nutrients stored within the seeds, they quickly become mineral deficient. The fertilizer can be added to the external water source or directly to the card holder.
- **Light source.** If a window is available, the plant pouches can be placed near it. Regardless, supplemental light will likely be needed. A desk lamp (or two) with a bright, compact fluorescent bulb generally is sufficient. Incandescent bulbs must be avoided since they emit excessive heat that is damaging to the plants. Lamps should be left on continuously for best results (e.g., Fast Plants require 24-hour light and the bulbs should be positioned approximately 5–10 cm above the plants).

○ Steps for Preparing Card-Holder "Plant Pouches"

1. Cut absorbent paper towel (shop towel) to the size that will fit into the 3 × 4 inch top-loading card holder (standard trading-card size). Generally, this will be 2¾ inches wide and 3¾ inches long.
2. Cut a notecard in half lengthwise and place the piece of paper towel between the two 1½ × 5 inch notecard pieces (Figure 5A) to make a "sandwich"; slide the paper towel sandwiched between the index cards into the card holder, position the paper towel, and then remove the notecards one at a time. The notecards are simply to provide some support to ease the insertion of the paper towel since the paper towel is flimsy and awkward to insert on its own.
3. Cut a 15 inch length of string, thoroughly wet it with water, and insert one end clear to the bottom of the card holder. Gentle pressure applied to the edges of the rigid card holder will cause the sides to bow outward, which allows the string to be dangled in from above. Use a popsicle stick or dissecting needle to position string.
4. Add six seeds to the card holder, positioning them such that the ends of the seeds are approximately 0.5–1.0 cm from the top of the card holder (Figure 5B). This position will ensure that the

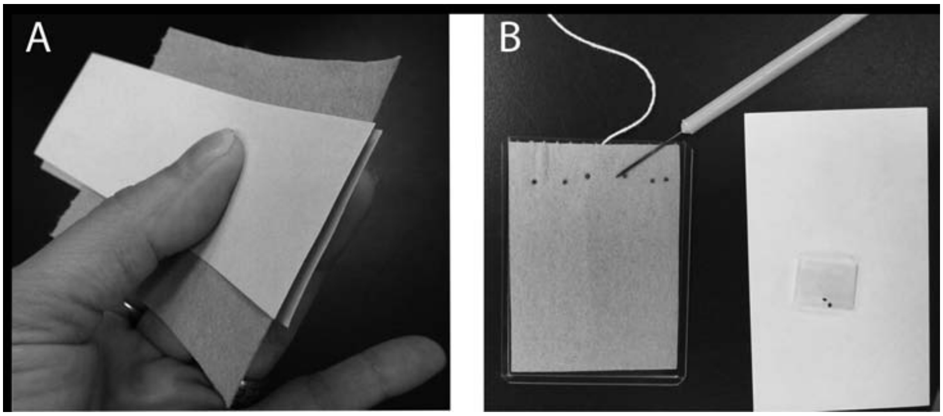

Figure 5. (**A**) Paper towel placed between the two pieces of a notecard that has been cut in half lengthwise. Sandwiched in this manner, the paper towel is relatively easy to insert into the card holder. (**B**) Seeds being positioned with a dissecting needle before water is added to the "plant pouch"; note that the wicking string is on the opposite side of the paper towel from the seeds. To the right is a notecard bearing a loop of transparent tape, with the adhesive side outward, on which seeds have been sprinkled to make them easier to handle (the loop of tape keeps the seeds from scattering and makes it easy to pick up individual seeds).

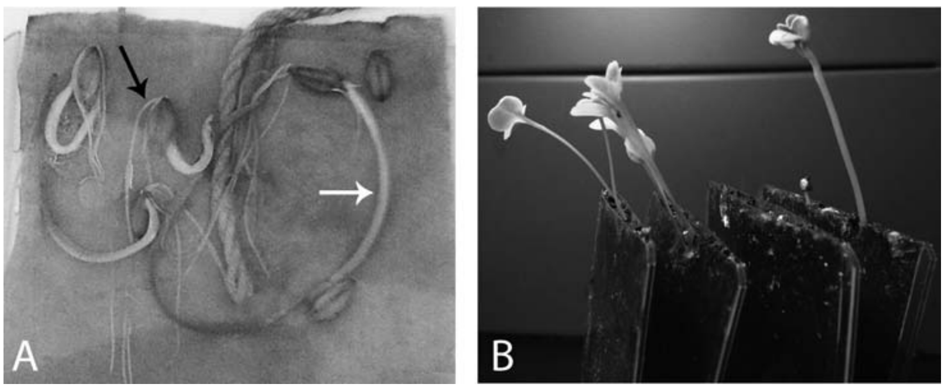

Figure 6. (**A**) Barley seeds shortly after germination, with shoots (white arrow) exhibiting negative gravitropism (growth away from gravity) and roots (black arrow) demonstrating positive gravitropism (growth directed toward gravity). Because the seeds were placed within the "plant pouch" oriented "upside down," the roots and shoots had to curl around the seed in order to resume their normal growth pattern in response to gravity. The shoot indicated by the white arrow has curled around until the tip has reached the top of the card holder (difficult to discern in grayscale photo). (**B**) Seedlings, six days after germination, bending toward the light source. From left to right: Fast Plants, lettuce, basil (not germinated; seeds were three years old and not viable), and radish. These plant pouches were filled with milled peat moss as the growth medium instead of paper towels.

seeds are properly supported once they germinate. Be sure to place the seeds on the opposite side of the paper from where the string is located; otherwise the plant pouch will not be flattened enough and the seeds may slip (Figure 5B).

5. Label the card holder using sticky notes or 2 inch sticky filing tabs attached to the side of the card holder like a flag (Figure 1). The label can include the names of the plant and the student, along with the date the seeds are sown.
6. Add ~5 mL of water to the card holder using a plastic transfer pipette or eyedropper until the paper towel is damp, then place the other end of the string in the cup containing water (external water source).
7. Lean the card holder against the water source or turn an ice-cube tray upside down and place the plant pouches between the molds (Figure 1); all cards can use the same water source unless you are testing different fertilizers.
8. Liquid fertilizer should be added to the water container *after* the seeds have germinated. Once the seedlings have consumed the stored nutrients within the seeds, they will not survive much longer unless provided with mineral nutrients.

○ Observational Opportunities

When using grass seeds (oats, wheat, or barley), germination takes place within two to three days. The roots appear first, followed by the shoots. After seven days, those seeds that are going to germinate will have done so. After two weeks, most plants have reached their maximum height attainable under these conditions. If using multiple species, the rate of growth can be compared between them. Observations made with the unaided eye reveal the germination process and the structure and growth of the roots and shoots. If a seed is oriented "upside down," the shoot can be seen curling around to get above the "ground" and the root can be seen curling downward, both in response to gravity (negative gravitropism in the shoot, positive gravitropism in the roots; Figure 6A). Once shoots have emerged from the top of the plant pouch, the plants can be seen to bend toward light (phototropism) if light is unidirectional or stronger from one side (Figure 6B). With a microscope more details of the roots can be observed, including the protective root cap, delicate root hairs, and microorganisms (if present).

If seeds become contaminated, microorganisms may grow and be observed. Rather than being considered a "failed" experiment, these outcomes should be viewed as welcome opportunities. For example, if a seed does not germinate quickly, students often can observe fungal growth covering the seed coat. A race then ensues between the fungus – extending its threadlike mycelia into the inner part of the seed to consume the stored food within – and the baby plant that is converting the stored food into energy and materials required for its germination and early growth.

Technology & Enrichment

Encouraging students to obtain high-quality photographs using smartphones or tablets can support development of observational skills as students document the changes that occur over several weeks. Digital images can be imported into software, such as PowerPoint, and labels can be added to identify structures; students can write informative captions to accompany these images as further ways to document their observations (see figure captions in this article for exemplars) and share them with others, thus overcoming plant blindness.

○ Modifications & Extensions

The basic plant pouches described above can be used to address many of the *Next Generation Science Standards* (Table 1). Teachers are encouraged to explore some of the modifications and extensions suggested below to fit their learning objectives.

Growing Media

Absorbent shop towels provide the most suitable growth medium, since they retain moisture and do not allow the seeds to slip down into the card holder. However, other media can be used to explore different aspects of plant growth and interaction with the environment. Standard potting mix, milled peat moss (Figure 6B), or soil collected from the school grounds (Figure 7) can be placed as a thin layer within the card holders; the thickness of the potting media obscures some seed and root growth but generally contains soil arthropods and/or nematodes that can be observed interacting with the roots. A plastic transfer pipette can be used to obtain a small sample of liquid from the plant pouches that can be placed on a standard slide with coverslip for viewing with high magnification (400×); we have observed numerous protists, nematodes, and

Table 1. ***Next Generation Science Standards*** **that may be addressed by use of the plant pouches.**

Disciplinary Core Idea (DCI) Code	Performance Expectation
K-LS1-1	Use observations to describe patterns of what plants and animals (including humans) need to survive.
1-LS3-1	Make observations to construct an evidence-based account that young plants and animals are like, but not exactly like, their parents.
2-LS2-1	Plan and conduct an investigation to determine if plants need sunlight and water to grow.
2-LS4-1	Make observations of plants and animals to compare the diversity of life in different habitats.
3-LS4-3	Construct an argument with evidence that in a particular habitat some organisms can survive well, some survive less well, and some cannot survive at all.
3-LS1-1	Develop models to describe that organisms have unique and diverse life cycles but all have in common birth, growth, reproduction, and death.
3-LS3-1	Analyze and interpret data to provide evidence that plants and animals have traits inherited from parents and that variation of these traits exists in a group of similar organisms.
3-LS3-2	Use evidence to support the explanation that traits can be influenced by the environment.
4-LS1-1	Construct an argument that plants and animals have internal and external structures that function to support survival, growth, behavior, and reproduction.
5-LS1-1	Support an argument that plants get the materials they need for growth chiefly from air and water.
MS-LS2-1	Analyze and interpret data to provide evidence for the effects of resource availability on organisms and populations of organisms in an ecosystem.
MS-LS1-5	Construct a scientific explanation based on evidence for how environmental and genetic factors influence the growth of organisms.
HS-LS1-2	Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms.
HS-LS1-3	Plan and conduct an investigation to provide evidence that feedback mechanisms maintain homeostasis.
HS-LS1-4	Use a model to illustrate the role of cellular division (mitosis) and differentiation in producing and maintaining complex organisms.

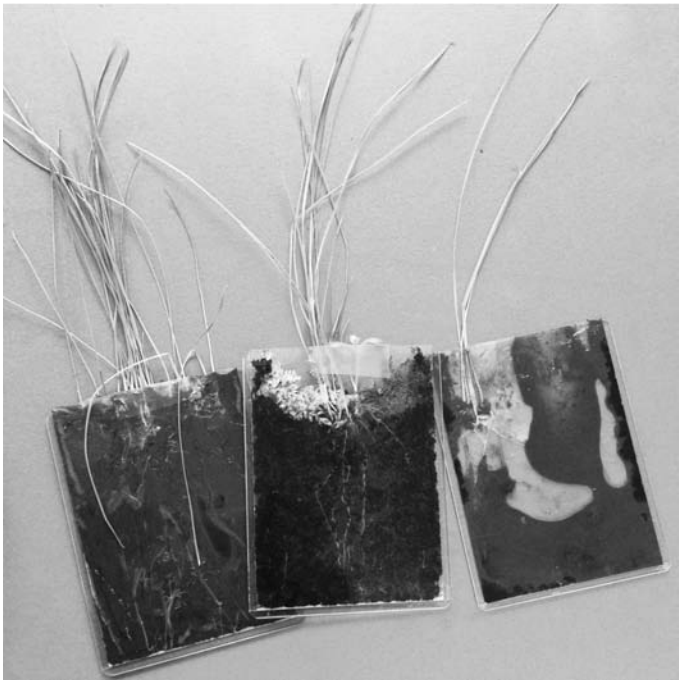

Figure 7. Three rigid card-holder "plant pouches" containing various growth media. From left to right: mud collected from a riverbank, standard potting mix, and watery/thin mud. All pouches contain grasses that were grown from seeds sown directly into the plant pouches; the middle pouch also contains a Mother of Thousands succulent (*Bryophyllum daigremontianum*) that was transplanted there. (Note: several plant pouches containing soil, grass, and *Bryophyllum* have been maintained for close to one year. The soil has broken down considerably and the grass has been trimmed many times, but the pouches have continued to do well simply leaning against a sunny window and watered periodically.)

insects from plant pouches containing various forms of potting media or soil. These plant pouches are easy to water directly by using a transfer pipette or eyedropper.

Fertilizer Effects

Experimentation with different concentrations of mineral nutrients is accomplished easily by establishing different external water sources for each treatment and placing the capillary string in the appropriate treatment.

Plant Selection

A variety of seeds can be used to explore plant diversity. Students also can examine effects of intraspecific competition for resources by growing different quantities of seeds in separate card holders, or they can investigate interspecific competition by growing two or more different types of seeds within one card holder. Alternatively, students can collect small "weedy" plants from lawns next to sidewalks or parking lots and transplant them into a plant pouch after rinsing the roots with tap water.

○ Acknowledgments

We thank Cody Ledford for his help in preparing the figure plates. Comments from several anonymous reviewers greatly improved the quality of the manuscript.

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