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MILLER, ZHENGQING FU**ABSTRACT**

Plants are a vital component of human life on Earth; they provide us with food and essential nutrients as well as the oxygen we breathe. However, the science education community struggles to find ways to make plant processes less abstract and more understandable for learners. In this article we demonstrate how we make plant processes more understandable for learners by observing the behaviors of a specific plant structure, a stoma, which is a microscopic opening that plays a role in the movement of matter into and out of a plant. Recent research across plant-related science fields centers on plant stomata because they protect plants from various environmental strains, including attacks from pathogens. Translating this research into science classroom instruction has not occurred extensively. A key impediment is that few common methods to make stomata visible or demonstrate their dynamic nature to learners are available. The activities we share here make stomata visible utilizing a specific plant, *Tradescantia zebrina*, and common laboratory equipment. In the first activity, we share how to demonstrate stomata closing and opening by manipulating a combination of these environmental factors. In the second activity, we describe how to create a visual simulation of stomata response to attacks from microorganisms.

Key Words: stomata response visualization; plant pathology; stomata functions; plant processes; guard cells.

○ Introduction

Leading biology education stakeholders identified the lack of a defined set of core concepts in plant biology that undergraduates should learn as an area of major concern (American Society of Plant Biologists & Botanical Society of America, 2011). These same biology education leaders also identified important content that should be emphasized within undergraduate biology curriculum, such as knowing plants are made of cells and other molecules that support plant functions; plants have a diversity of structures for retaining water, exchanging gases, and supporting growth; and plants have specialized structures and systems for defense against disease and

predation. The emphasis on understanding plant structures and functions, and the roles they play in matter cycling and plant health, is consistent with major K–12 science education framework themes (NGSS Lead States, 2013). These foci also highlight the importance of an ongoing issue in the science education community, namely researchers have found few instructional approaches that positively impact learners' plant process conceptions (Brown & Schwartz, 2009; Carlsson, 2002). In response, some stakeholders have argued that more attention should be given to teaching about the interrelationships between specific plant processes (Carlsson, 2002) and that the goal for biology teachers should be to help students develop strong frameworks of interrelated concepts, like plant processes (Mintzes et al., 2001).

In this article we focus on how to make plant processes more understandable for learners by observing the behaviors of a specific plant structure, a stoma, which is a microscopic opening that plays a role in the movement of matter into and out of a plant. Stomata are found on the underside of plant leaves and on stems, and they open and close to support plant processes. For example, stomata open early in the day to allow photosynthesis to occur and then close later in the day. Stomata also work to maintain plant health by responding to environmental conditions. For instance, plant stomata close to reduce water loss from plants when environmental conditions are hot and dry. The responsive nature of plant stomata make them an ideal instructional aid for introducing learners to processes that impact matter movement and transformation in plants.

Unfortunately, methods for demonstrating the dynamic structure and function of stomata are not common, and this is a barrier to translating stomata response behavior into science classroom instruction. For example, a recent internet search revealed that the most common classroom activities that make stomata visible to learners involves making impressions of stomata using clear (see-through) nail polish and tape, resulting in a translucent impression of stomata (for example, see California Academy of Sciences, 2018). The activities shared here respond to these shortcomings by making stomata visible utilizing a specific plant, *Tradescantia zebrina*, and common laboratory equipment. The activities also allow learners to observe how stomata respond to environmental conditions and attacks from microorganisms.

○ Plant Stomata

Plant stomata open and close in response to a variety of environmental factors including light, water content, temperature, and carbon dioxide levels inside the plant. Light is one of the most important factors that promote stomata opening. Higher quantities and intensity of light promote opening. An increase in temperature, usually associated with increased light intensity, also cause stomata to open as plants need to release water to initiate plant cooling. Water content inside a plant affects stomata opening and closing as well. For example, low water levels promote stomata closure. Stomata also take in atmospheric carbon dioxide to initiate photosynthesis. As photosynthesis occurs, stomata open for the plant to obtain more carbon dioxide. While it is not possible to examine the impact of any one of these factors in isolation, we share how to demonstrate stomata closing and opening by manipulating a combination of these environmental factors.

○ Laboratory Exercises on Stomata Responses to Environmental Conditions

Due to the natural circadian rhythm of plants, plant stomata are generally open in the morning when the plant is experiencing ideal conditions and then begin to close automatically as the day progresses, so it is easier to view open stomata in the morning. However, we also present other methods (see our “afternoon viewing option” section) that allow learners to complete the initial viewing of the open stomata later in the day. We provide the material list, procedures, and some background information needed to complete these activities.

Material

Each station with two students needs these items:

- Two leaves from an “inch plant,” also called *Tradescantia zebrina* (see Figure 1)
- Two glass microscope slides and cover slips
- A standard light microscope with a 10× objective



Figure 1. *Tradescantia zebrina*, also known known as the inch plant.

Advanced Preparation

1. **Moist plant preparation.** Water one *Tradescantia zebrina* plant the day before and the day of the activity to ensure that the soil is moist. This will increase the amount of moisture in the plant and subsequently the diameter of the stomata. Placing the plant outside in direct sunlight during warm weather prior to the activities also promotes the opening of stomata.
2. **Dry plant preparation.** Do not water one *Tradescantia zebrina* plant for a week prior to the day of the activity to ensure that the soil is dry. Also, keep it stored in a dark room for 24 hours before the activity.
3. **Microscope preparation.** Set up your microscope in a well-lit area and rotate to its lowest magnification lens, generally the 4× objective. Provide one setup for each lab station.
4. **Afternoon viewing option.** About three hours before the lab activity, place an entire inch plant in a large sealable plastic bag and close it to create an airtight seal (see Figure 2). Ensure that the bag does not physically constrict or damage the foliage of the plant, as this would adversely affect the results. Do not keep the plant in this state for longer than four hours as it may cause cell death and subsequent stomata closure. The overall health of the plant is not impacted if proper precautions are followed. Stomata will open to a maximum state in response to dwindling carbon dioxide (CO₂) (Morison, 1987).

Instructional Procedures

5. We generally use this activity to introduce plant stomata to students. We typically engage middle school students by also asking them to consider and share their ideas about how cells contribute to plant functions. Middle school student responses typically focus on how cells help plants obtain energy and what roles cell parts play in overall plant



Figure 2. Inch plant in a sealed plastic bag.

health. Similarly, we ask high school and college students to consider how they think cells help plants maintain homeostasis. These student responses often focus on cells playing a role in the processing of gases, nutrients, and waste, but without clear understandings of the methods or structures involved.

6. It is useful to solicit and make student ideas public without passing judgement. We use probing questions such as, “How do you know that?” and “What makes you think of that idea?” to clarify students’ thinking.
7. We also discuss lab safety and remind students to wear goggles at all times during the lab.
8. Prior to exploring plant stomata, tell students they will be observing specialized plant cells in order to learn more about how cells contribute to plant functions.
9. Next, harvest one leaf from the moist plant for every station. Pick a flat leaf if possible. Torsion of the leaf body can indicate stress, which will adversely affect the results of the activity. Larger leaves generally provide better results compared to smaller leaves.
10. Distribute leaves to lab stations.
11. Instruct students to place each leaf abaxial (purple) side up on a slide. Then, have students place the leaf and slide on the microscope and focus on it with the 4× objective. Once the leaf is in focus, rotate the 10× objective into place and observe each leaf to verify that the stomata are fully open (see Figure 3). This viewpoint will work best for subsequent observations.
12. After exploration of open stomata, we explain how guard cells, another kind of specialized plant cells, open and close the stomata to allow water to move out of the plant (and plant and atmospheric gas exchange to occur). We also explain that these open stomata are from a plant with moist soil that has been in sunlight for some time (thus engaging in photosynthesis).
13. Next, harvest one leaf from the dry plant for every station and follow the moist plant procedure to view it. You should notice that the stomata are more closed than the moist plant stomata (see Figure 4).

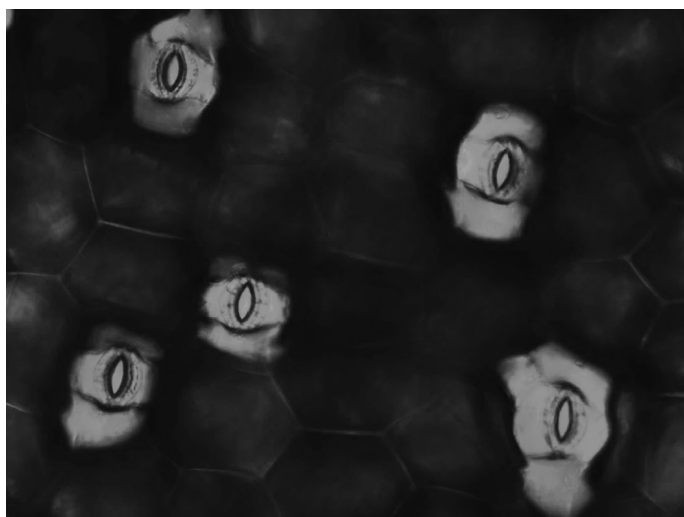


Figure 3. Open stomata on an inch plant.

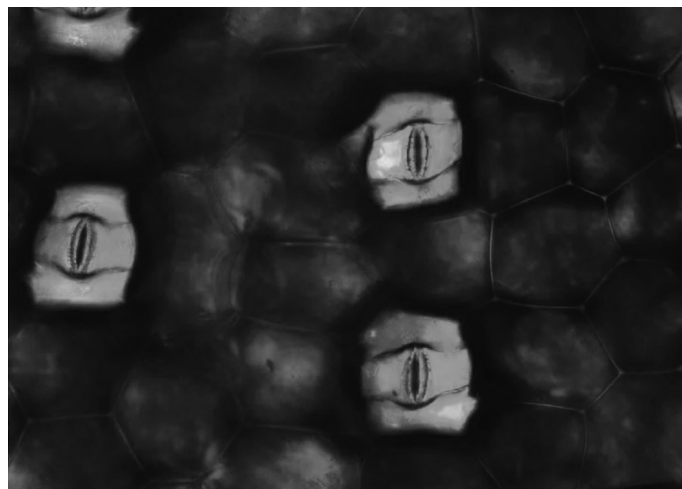


Figure 4. Closed stomata on an inch plant.

14. After exploring the more closed stomata, we explain that these closed stomata were harvested from a plant with dry soil that has been in darkness for a day.
15. Finally, we tell students how stomata respond to a variety of environmental factors and discuss how the open stomata reflect the plant’s response to moist and sunny conditions while the closed stomata demonstrate the plant’s response to dry and dark conditions. We also discuss how these responses to environmental factors might support plant health during environmental stress.

After completing the lab activities, we require middle school students to independently create stomata models (drawings with written explanations that focus on how stomata close and open in response to environmental factors) and share their ideas in writing about how cells contribute to overall plant health. Similarly, we require high school and college students to independently create stomata models and record their ideas about how stomata help plants maintain homeostasis when confronted with environmental stress.

○ Stomata Responses & Plant Pathology

Plant pathologists, scientists who study how plants respond to disease and unfriendly atmospheric conditions, discovered that stomata are also a gateway for plant pathogens to enter and attack plants and that stomata close to defend the plant when threatening pathogens attempt to invade (Melotto et al., 2006). If the pathogen is denied access to internal tissues of the plant through the stomata, infection can often be diminished. This knowledge helps plant pathologists respond to some of the most pressing science issues of our time, such as a plant disease called citrus greening, which has caused around \$1 billion in orange crop losses every year since 2005 in Florida alone and has been identified as one of the most prominent agricultural problems in the United States (Allen, 2015). Our second lab activity allows students to replicate and observe the stomata-closing defense mechanism without introducing actual pathogens into a plant. Instead, methods for observing plant stomata in the fully open state at the onset of the laboratory activities are described. Then a solution is administered that mimics the biochemical signaling mechanisms plants use to cause plant stomata to close, thus defending the plant from infectious microorganisms.

○ Laboratory Exercises on Stomata Responses to Pathogens

In the previous activity we shared how plant stomata are generally open in the morning and then begin to close as the day progresses, so it is easier to view open stomata in the morning. We also presented an option for viewing fully open stomata later in the day (see our “afternoon viewing option” section). Please follow those same procedures to view open stomata if you are completing the following activity in the afternoon or evening. Here we share material, procedures, and some background information needed to complete the activities on stomata response to pathogens.

Material

Each station with two students needs these items:

- Two leaves from an “inch plant,” also called *Tradescantia zebrina* (see Figure 1)
- Two clear sealable plastic petri dishes
- Approximately 2 ounces of H_2O_2 solution (tap water and 3% hydrogen peroxide at a 3:1 ratio)
- Two glass slides and cover slips
- One standard light microscope with a 10 $\times$ objective
- One large plastic bag (clear or opaque) with a zip closure (only for our “afternoon viewing option”).

Material Preparation

1. Water *Tradescantia zebrina* the day before and the day of the activity to ensure that the soil is moist.
2. On the day of the lab, fill one petri dish container with enough H_2O_2 solution to cover a leaf. As a control, fill a second container with only tap water. Be sure to label each container because the solutions appear identical. Each lab station requires one set of petri dishes. To prepare the H_2O_2 solution, mix normal tap water and 3% hydrogen peroxide (from a drugstore) at a 3:1 ratio. Diluting the H_2O_2 solution prevents oxidative damage to plant tissue.
3. Set up your microscope in a well-lit area and rotate its lowest magnification lens into place, generally the 4 $\times$ objective, which will be used to initially focus the leaf. Provide one setup for each lab station.

Instructional Procedures

1. We generally complete this activity after the Laboratory Exercises on Stomata Responses to Environmental Conditions. We typically engage students by asking them to share what they know about plant diseases and how they think plants defend themselves from infectious diseases. Student responses typically focus on prior experiences with diseased plants, their noticing of fuzz and/or mold on some diseased plants, and wonderings about how plants “get sick.”
2. Before starting the lab, we tell students that the two solutions we are using appear to be the same and are labeled so we can tell them apart. We also discuss these lab safety statements:
 - Wear goggles at all times during the lab.
 - The H_2O_2 is harmful when ingested or splashed in eyes.

- Prolonged exposure of skin to H_2O_2 may result in temporary whitish discoloration of the fingers. This effect can be avoided by using a pair of forceps to remove leaf samples from solution.
3. Prior to exploring plant stomata, tell students they will be observing specialized plant cells to learn more about how plants defend themselves from diseases.
 4. Next, harvest two leaves from the *Tradescantia zebrina* plant for every station. Pick flat leaves if possible. Larger leaves will generally provide better results than smaller leaves.
 5. Distribute leaves to lab stations. When transporting the leaves, keep them sealed in the plastic container. This will prevent leaves from experiencing drastic changes in atmospheric conditions (humidity and/or CO_2 concentration) that may cause premature closure of the stomata.
 6. Instruct students to place each leaf abaxial (purple) side up on a slide. Then, have students place the leaf and slide on the microscope and focus on it with the 4 $\times$ objective. Once the leaf is in focus, rotate the 10 $\times$ objective into place and observe each leaf to verify that the stomata are fully open (see Figure 3). This viewpoint will work best for subsequent observations.
 7. After exploration of open stomata, we reemphasize how specialized plant cells called guard cells open and close the stomata. Next we explain that, depending on the viewing method used, the wide open stomata demonstrate the plant’s response to normal morning conditions (as in our “normal viewing option” section) or to environmental stress (as in our “afternoon viewing option” section).
 8. Next, we have students place one leaf in each solution (H_2O_2 and control) abaxial side down, seal the container, and let the leaves soak for 15 minutes. While waiting, we tell students that plant pathologists are scientists who study how plants respond to disease and unfriendly atmospheric conditions. We also explain how plant pathologists discovered that just like stomata respond to environmental stress, they also act as a gateway for infectious microorganisms to invade and attack plants (Melotto et al., 2006). Next, we explain how plants respond to attacks from infectious microorganisms by releasing a signaling hormone and that the H_2O_2 solution is similar to the signaling hormone, so by placing a leaf in the H_2O_2 solution we can observe how plants respond to such attacks.
 9. Then we remove both leaves and prepare them abaxial side up on a glass slide to be observed under the microscope. Observe the leaf from the water control. At this point, the stomata should be open (see Figure 3).
 10. Next observe the leaf treated with H_2O_2 . Use forceps when removing it to avoid temporary discoloration of fingers. Most, if not all, stomata now should be fully closed (see Figure 4).
 11. Here we explain how stomata closure is an essential component of plant responses to infectious microorganisms. When the plant perceives attacks from such pathogens, a signaling hormone is released that triggers the stomata to close, which helps the plant defend itself. We also discuss how plant pathologists use this knowledge to study ways to better support plant health.
 12. After completing the lab activities, we require middle school students to revise their stomata models (drawings

with written explanations that focus on how stomata close and open in response to environmental factors) and share their new ideas in writing about how cells contribute to overall plant health. Similarly, we require high school and college students to revise their stomata models and record ideas about how stomata help plants maintain homeostasis when confronted with attacks from pathogens.

○ Discussion

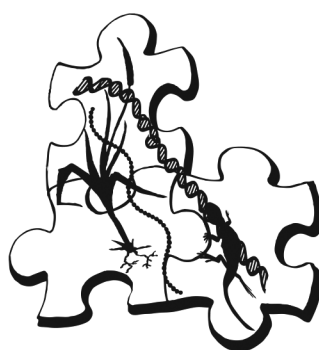
Our experiences leading these laboratory activities reveal that learners are fascinated by the beautiful appearance of these stomata and captivated by their responsiveness to environmental factors. Being able to reveal the anatomical function of the guard cell, and the ability of the leaf to perceive and respond to threats, also provides opportunities to connect classroom investigations to cutting-edge research of pressing societal issues like the citrus greening disease. Further, these activities respond to the science education community's struggle to find instructional approaches that positively impact learners' conceptions of plant processes (Anderson et al., 1990; Brown & Schwartz, 2009; Carlsson, 2002) by making the inter-related nature of plant processes less abstract and more concrete. Creating such understanding ensures that students, and the larger general population, are better positioned to take part in public discourse and decision-making about related societal issues.

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