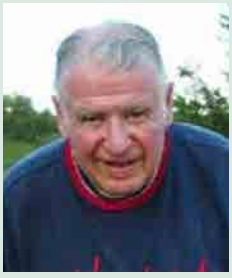
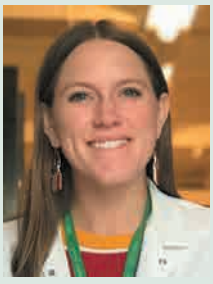
Discovering the Microscopic World of Live Tree Bark

A Model Instructional Experience for Students and Teachers Using a Virtual iAdventure, Teacher Preparation Guide, Student Worksheets, and Moist Chamber Cultures





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RATIONALE FOR INSTRUCTIONAL EXPERIENCE

Patricia (Trish) A. Smith, now retired, was a seventh-grade Life Science teacher at Warrensburg RVI Middle School (WMS), at Warrensburg, MO during 2004–2007. Her expertise in finding grant funds supported her laboratory classroom activities with extramural funding. These grant funds came from the Missouri Department of Elementary and Secondary Education and local private organizations. She shared her classroom and laboratory experiences on how live animals and trees were integrated into her laboratory activities in presentations given nationally and in Missouri (Figure 1). Her connections to the University of Central Missouri (UCM) led her to explore a possible National Science Foundation-Research Experience for Teachers (NSF-RET) grant

Figure 1. *Trish Smith in her classroom laboratory wetting moist chamber tree bark cultures. Note labeled plastic Petri dishes with moist chamber tree bark ready for observation. (Photo by H.W. Keller.)*

as a supplement to the iAdventures that was already part of her synergistic laboratory experiences. The intent of the NSF-RET was to provide funds for professional development targeted for teachers K–12 on the cutting edge of science, to strengthen partnerships between institutions of higher learning and local school districts. She consulted in 2004 with then NSF grant-holder, and Principal Investigator, Harold W. Keller at UCM, who had an NSF grant titled "Biodiversity and Ecology of Tree Canopy Biota in the Great Smoky Mountains National Park." The objectives of this tree canopy biodiversity research project were chronicled in previous publications and will not be repeated here (Keller, 2004, 2005, 2019; Smith and Keller, 2004; Kilgore et al., 2008). This partnership appeared to be a good fit for an NSF grant proposal to the Division of Environmental Biology, Biodiversity Surveys and Inventories Program.

Prospective applicants for RET grants must prepare a cooperative grant proposal after first consulting with the appropriate NSF Program Officer. This grant proposal application included a three-page descriptive narrative, a two-page teacher curriculum vitae, a prepared budget, and justification for up to a limit of $10,000. This RET supplemental funding application was submitted electronically through the grant-holder's university by NSF Fastlane.

Current application instructions are included in opportunity announcement NSF18-089. Some of these details have changed (for example, teacher budget costs are now up to $15,000). The following quotation represents in part current NSF priorities: "Another goal of the RET supplement activity is to build collaborative relationships between K-12 science educators and the NSF research community. BIO is particularly interested in encouraging its researchers to build mutually rewarding partnerships with teachers at urban or rural schools and those in school districts with limited resources."

EXPERIENCING TREE CANOPY BIODIVERSITY IN GREAT SMOKY MOUNTAINS NATIONAL PARK: CONNECTING SEVENTH GRADE STUDENTS THROUGH AN iADVENTURE

This activity was part of the virtual field tree canopy experience in the Great Smoky Mountains National Park (GSMNP - iAdventure) during 2004. The field collection of live tree trunk bark samples of Eastern Red Cedar (*Juniperus virginiana*) trees took place at Pertle Springs, the land laboratory for UCM (see Teacher Preparation Guide and Student Worksheets below). Seventh-grade life science students from WMS were bused to Pertle Springs where they collected live tree bark samples and prepared moist chamber bark cultures in the classroom laboratory. Many life forms, including myxomycetes, were observed during laboratory class sessions.

The iAdventure live link with all its web content was removed from the Warrensburg Middle School site when Trish and Stan Smith retired. However, the papers, images and documents and original Uniform Resource Locator were preserved by the authors. In September 2021, Jason Best,

the Director of Biodiversity Informatics at the Botanical Research Institute of Texas (BRIT), was able to revive the original website through GitHub (https://britorg.github.io/GSMNP_iAdventure). Interested persons can now access and experience the full content of the iAdventure in GSMNP, as well as the Teacher Information Page that has Student Worksheets related to the collection of field samples. The intent is to extend the benefits of these field experiences for students and teachers worldwide through this linked inquiry-based iAdventure available as an interactive web-based activity. Additionally, historical snapshots of the iAdventure site can be accessed through Archive.org at: https://web.archive.org/web/2016*/http://warrensburg.k12.mo.us/iadventure/gsmnpiadventure/.

SYNOPSIS OF iADVENTURE, TEACHER PREPARATION GUIDE, AND STUDENT WORKSHEETS CONTENT

In the summer of 2004, Trish and Stan Smith arrived at GSMNP, pitched a tent, and recorded daily activities of the tree canopy research team as part of the iAdventure. *Exploring Life in the Forest Canopy* highlights and tracks the activities of five undergraduate UCM students (Amber, Ashley, Cheryl, Erin, and Tommy) as they participate in the tree-climbing school at Pertle Springs and the field experience climbing giant trees in the GSMNP collecting tree trunk bark samples. On the iAdventure website, visitors can explore topical headings such as *Research Objectives; GSMNP All Taxa Biodiversity Inventory,* with geographical area description; *Field Trip Organization Pre-trip Planning; Knott Clinic; Tree Climbing School; Meet Charly Pottorff,* professional arborist;

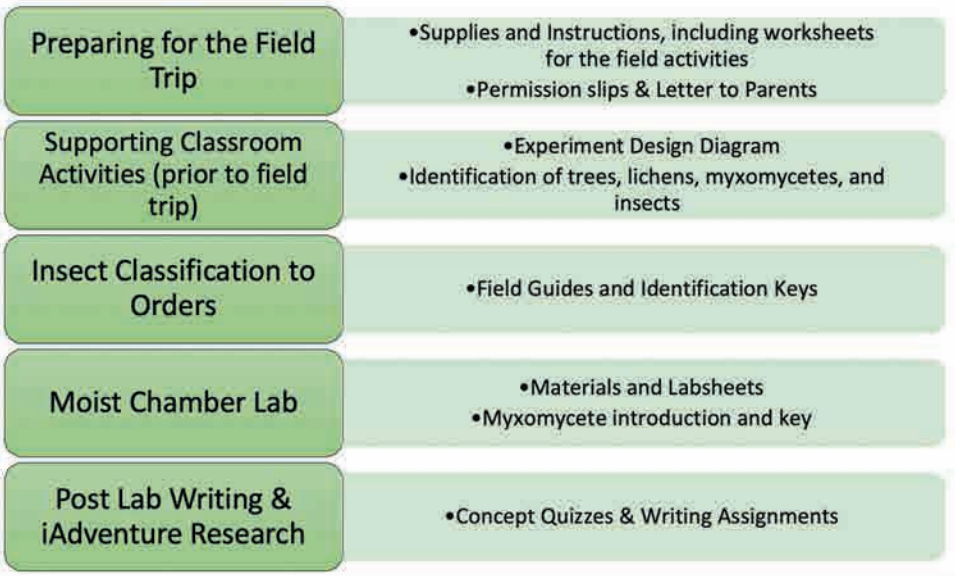

Figure 2. *Summary of resources provided for teachers on the Teacher Information Page, iAdventure website.*

meet Dr. Steve Wilson, entomologist world authority on plant hoppers; *meet Dr. Joe Ely,* biometrician and plant ecologist; *Field Work*, climbing trees and collecting bark samples, as well as preparing and raising insect flight intercept canopy traps; *Life at the Research Station; about Trish and Stan Smith*; and *Stories from the Field*, about the discovery of a new tree canopy myxomycete species, *Diachea arboricola*, by Melissa Skrabal, among others.

The Teacher Information Page has a list of resources needed, as well as a list of questions for students about their observations of the iAdventure. It also includes links to worksheets that provide more detailed information about how to prepare for the field trip, as well as information about lichens, myxomycetes, and insects (Figure 2). Some examples included a *List of Field Tasks; Supply List; Tree Tags; Field Task Instruction Sheets: Meadow Sweepers; Canopy Catchers; Barking up the Right Tree; Myxo-O-Masters; Red Cedar Database Sheet; Lichen Log; Tree Sleuths; Entomology Worksheet; Insect Identification Key; Moist Chamber Laboratory Supplies* (used for preparation of moist chamber cultures); *Bark pH Procedure Document; Examination of Moist Chamber Cultures Labsheet;* and *Key to the Myxomycete* Orders, among others. Lectures describing the illustrated myxomycete life cycle, color images of myxomycete fruiting bodies using Smart Board presentations, and question-and-answer sessions enabled students to interact with the presenter. References were available for student reading and picture keying of myxomycete fruiting bodies observed in moist chamber cultures (Keller and Braun, 1999).

The two main student activities are nested under the Title Page (Tier One) and iAdventure (Tier Two) (Figure 3). The Tier 1

Figure 3. *Snapshot of the 'Site Map' of the iAdventure website*

iAdventure website allows worldwide access to the tree canopy field experiences in GSMNP and the parallel field research at Pertle Springs. This was a problem-solving activity that helped students determine the direction and outcome of a content-rich storyline using resources available on the internet, particularly resources providing real-world data and primary documents. Participating students should experience the three phases of research emphasized in the original GSMNP NSF grant: the Adventure Phase (tree climbing using ropes and collecting bark samples for moist chamber cultures (Kilgore et al., 2008); the Laboratory Phase (sample sorting and preparation of moist chamber cultures); and the Publication Phase (poster and oral platform presentations for local, regional, and national scientific meetings).

The Tier 2 site emphasizes the collection of live tree trunk bark of Eastern Red Cedar, American Elm (*Ulmus americana*), and White Oak (*Quercus alba*) at Pertle Springs. Students were divided into four groups and then subdivided into task groups of one or two students (Figures 4 and 5). UCM faculty

Figure 4. *Two seventh-grade WMS students collecting trunk bark samples from a live Eastern Red Cedar tree at Pertle Springs. Note tree tag number, collecting gear, and students enjoying this field experience. (Photo by H.W. Keller.)*

Figure 5. *Author and student measuring tree trunk diameter of Eastern Red Cedar tree at Pertle Springs. Note student on ground recording tree data. (Photo by T. Smith.)*

and students from the Biology Department, along with student parents, assisted with field collections. Six groups of 20 WMS students were transported to Pertle Springs for one-hour field trips on September 28 and 29, 2004, for a total of 240 students. Safety of the seventh-grade WMS students was a priority. Therefore, they did not climb trees, use knives, or shoot slick lines with the Big Shot… much to their dismay! Tree bark samples were used to prepare moist chamber cultures so students could observe a miniature ecosystem composed of myxomycetes, fungi, lichens, mosses, liverworts, green algae, cyanobacterial algae, myxobacteria, tardigrades, insects, and nematodes, among others. This is only an overview of the two tiers; more information is available on the website.

INSECT FLIGHT INTERCEPT TRAPS

Steve Wilson at UCM was in charge of the aerial installation of the Sante insect flight intercept tree canopy traps at GSMNP, Big Oak Tree State Park, and Pertle Springs (Figure 6). Students assisted in raising the fine-meshed canopy traps with two open pyramid structures (9 feet high by 4 feet wide) with a top and bottom 500-mL collector bottle (killing jar) filled with 70% isopropyl alcohol. This canopy trap was tethered to a horizontal branch at 50 to 60 feet for five days. Top canopy collection bottles tended to trap insects that hit the trap then climbed upward such as leafhoppers, tree hoppers and planthoppers, and moths; flies and beetles tended to hit the trap and drop downward into the bottom bottle (Wilson et al., 2003). Students also collected from ground sites using sweep nets. The collected insect specimens were used to perfect the taxonomic keys and create a basis for understanding diversity and adaptation.

Figure 6. *Flight intercept tree canopy insect trap installed in a tree at Pertle Springs. (Photo by H.W. Keller.)*

PERTLE SPRINGS FIELD COLLECTIONS AND LABORATORY PREPARATION OF MOIST CHAMBER CULTURES USING LIVE TREE TRUNK BARK

General credit for the use of moist chamber bark cultures from living trees goes back to the early 1930s, when tiny species of myxomycetes new to science were discovered by graduate student Henry C. Gilbert working under the supervision of Professor Dr. George W. Martin at the University of Iowa Mycological Laboratory (Gilbert and Martin, 1933; Gilbert, 1934). Since then, many papers and books have described preparations of moist chamber cultures that may differ in methodology but involve wetting field collections of bark from living trees (Keller, 2004; Keller et al., 2004; Everhart et al., 2009; Scarborough et al., 2009; Snell and Keller, 2003), herbaceous plants (Kilgore et al., 2009), and decaying wood or leaves from ground sites, usually at times when myxomycete fruiting bodies are not present (Keller et al., 2008). This technique gives the observer the opportunity to create a self-contained moist environment where the myxomycete plasmodium and developing fruiting body stages are present, although they are not always seen in the field.

Seventh-grade WMS life science students field-collected live tree trunk bark from Eastern Red Cedar trees at Pertle Springs (Figures 4 and 5). This is a short 10-minute bus trip from WMS to a series of trees that line the paved roadway at the entrance of the area (Scarborough et al., 2009). This tree species was targeted because it

has the highest species diversity of life forms, which provided students with the best chance of success observing moist chamber bark cultures (Keller and Braun 1999; Keller and Marshall, 2019; Scarborough et al., 2009; Perry et al., 2020). Two-student team members were briefed on the safe collection of tree trunk bark following instructions on Data Worksheets that recorded species of tree, overall estimated size of the tree (height and diameter; Figure 5), characteristics of bark surface, presence of other life forms (for example, lichens), and height on tree where the bark sample was collected.

Bark samples collected in paper bags were transported to the WMS class laboratory, where students prepared moist chamber cultures in oversized sterile plastic Petri dishes (150 × 25 mm) that were lined with sterile filter paper. About six pieces of bark sample covering the bottom of the dish were arranged without overlapping. Thirty mL of sterile deionized water was added around the bark, avoiding directly wetting the bark surface areas. These moist chamber bark cultures were allowed to soak for 24 hours, and any excess water was decanted during the next laboratory period. Observations were made during normal laboratory class sessions twice a week for approximately four weeks. Students recorded pH values using litmus color-coded papers and observed life forms over this period using the naked eye and 20 to 50× power dissecting microscopes (Figures 7–9).

Angela Scarborough (senior undergraduate student, Figure 8) and Courtney Kilgore (master's degree graduate student, Figure 9) from UCM served as mentors for the seventh-grade students, helping them locate and identify life forms in the moist chamber bark cultures. They were also available to answer questions about their tree canopy–climbing experiences in the GSMNP.

Figure 7. *Two WMS students scan moist chamber bark cultures with dissecting microscope. (Photo by H.W. Keller.)*

Figure 8. *Undergraduate UCM student Angela Scarborough assisting WMS students search for life forms in moist chamber bark culture. (Photo by H.W. Keller.)*

Figure 9. *UCM graduate student Courtney Kilgore scanning moist chamber cultures using a dissecting microscope. Two WMS students on the left, and Harold Keller on the right. Note the red shirts worn by the UCM tree canopy research team highlighting the iridescent myxomycete sporangium Diachea arboricola, a tree canopy species new to science. (Photo by T. Smith.)*

MOIST CHAMBER BARK CULTURE HOW-TO VIDEO

In 2021, the first moist chamber culture instructional video, *How to Create a Moist Chamber Culture to View the Biodiversity Growing on Live Tree Bark*, was made available by Ashley Bordelon, Herbarium Digitization Coordinator, of BRIT's Urban Ecology Program. A PDF with accompanying written instructions is also available and can be found at https://brit.org/research/research-projects/urban-ecology-program/ with the title: *Preparation of Moist Chamber Tree Bark Cultures: A Beginner's Primer for Use at Home* by Ashley Bordelon and Harold W. Keller (Fort Worth Botanic Garden Botanical Research Institute of Texas).

This video emphasizes the moist chamber culture technique using live tree trunk bark samples and store-bought low-cost supplies readily available at local stores for teachers, students, and hobbyists that may want to use this technique. Many teachers cannot afford the more expensive supplies used by the seventh-grade students supplied by an NSF grant–funded activity and the more reproducible protocols required by some publication formats. Nevertheless, this video was created for teachers and community enthusiasts based on live trees in their own backyards or nearby forested areas. Examples of myxomycete fruiting body development are highlighted in this video. This moist chamber technique sometimes results in the discovery of species new to science, as well as rare species seldom or never collected in the field (Keller, 2004; Keller and Marshall, 2019; Perry et al., 2020). This can be an added incentive for beginners to share their discoveries with other myxomycetologists, mycologists, and botanists.

RESULTS AND OUTREACH ACTIVITIES

Each fall for a four-year period (2004–2007), Keller met with six different seventh-grade life science classes (approximately 120 students) for a total of 18 hours. More than 500 WMS students were involved in this teaching activity over the course of 90 hours. On September 28 and 29, 2004, six groups of 20 WMS students were transported to Pertle Springs for one-hour field trips to collect trunk bark samples from living trees. During much of this time, Angela Scarborough and Courtney Kilgore also assisted students (Figures 8 and 9).

These activities were presented in popular media such as newspapers, television, websites, and exhibits. For example, UCM highlighted our research with a color image and short storyline on the front page of the university website. *The Daily Star Journal* ran two color images under the banner headline featuring Local Nature Lesson, that described the seventh-grade life science students collecting activities at Pertle Springs, and another front-page article titled *Junior Scientists at Work*, showing a color photograph of students and Dr. Keller observing moist chamber cultures with a description of the RET-NSF Program.

Local interest in this RET-NSF funded project was noted in *UCM News* under the title *Grant Provides Experience in Scientific Research.* Campus Today featured Trish Smith collecting bark samples, and another article *More Than a Bug's Life Fascinates*, showed students collecting insects using flight intercept canopy traps at Pertle Springs. Television station KMOS, housed at UCM, sent film crews to shoot footage of WMS students at Pertle Springs that aired as a five-minute segment on *University Magazine*. The RET-NSF

poster (Keller et al., 2005) presented at the Fifth International Congress on Systematics and Ecology of Myxomycetes (ICSEM5) in Tlaxcala, Mexico, was displayed at WMS and also at the UCM Morris Science Building.

One striking example of student observations was the surprise discovery of nematodes. These attention-getting nematodes, with their S-shaped wiggling and writhing movements in thin films of water, were frequently observed by students in moist chamber cultures from bark of living trees. Nematodes in some bark cultures were attached by their posterior ends, standing and waving in a behavioral pattern known as nictation. Nematode movements were also observed in the video of moist chamber bark cultures highlighted here by Ashley Bordelon.

Our efforts to involve seventh-grade students in the research objectives of this project was to transfer knowledge about how biodiversity is documented to the next generation of students. Websites and posters also disseminated field biology to a broader audience of students and teachers alike (Figure 10).

CONCLUSIONS

"Teaching has been an extremely rewarding job in many ways, but bringing the GSMNP research project into my seventh-grade life science classroom through the RET Program is one of my proudest moments," Trish Smith said. The highest tribute or reward Keller could ever receive is the twinkle in the eyes and glow and smile on the faces of the seventh-grade life science students at WMS when they said that "it's awesome" or "it's cool" after observing a myxomycete sporangium or plasmodium. These students learned to picture key and recognize different myxomycete species, insect taxa, and general life forms of lichens, mostly crustose and foliose types. The aim of this activity was to assist and encourage students and teachers to experience field trips and laboratory exercises that will create excitement and interest in exploring, collecting, and discovering life forms often overlooked in nature.

Figure 10. *Tree canopy research team in GSMNP. Far left, Stan and Trish Smith; UCM undergraduate students Amber, Tommy, Ashley back row, bottom row Erin and Cheryl; far right, Steve Wilson. (Photo by H.W. Keller.)*

ACKNOWLEDGMENTS

Special thanks go to Trish Smith, who was responsible for preparing the iAdventure, Teacher Preparation Guide, Student Worksheets, and logistics for the student field experience at Pertle Springs and laboratory observations. Without her and Stan's help, this activity would not have been possible. Ashley Bordelon used her creative talents to brainstorm the video content, organize the visual images, and narrate the moist chamber instructions. Dr. Joe Ely, ecologist and biometrician, and Dr. Steve Wilson, entomologist from UCM, were essential co-investigators on this research project. Charly Pottorff, a professional arborist, taught UCM students the double rope climbing method and how to shoot the Big Shot at a climbing school at Pertle Springs. Many people contributed their volunteer expertise and we thank them all, including parents, faculty from the UCM Biology and Education Departments, the UCM student mentors and tree climbers, and investigators from other institutions. This student activity would not have been possible without the help of many people not named but who contributed time, effort, cooperation, and authorship on publications. Multiple grants provided financial assistance in part from the National Science Foundation, Discover Life in America, National Geographic Society, Sigma Xi, The Scientific Research Honor Society, Missouri Department of Natural Resources, the U.S. Department of Education McNair Scholars Program, and the UCM Summer Undergraduate Research and Creative Projects Program. Photographic credits are given after each photograph, and image release forms were obtained from all WMS students. We trust that this spirit of discovery described here will lead others to explore, enjoy, learn, and share their results with others.

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