

Stressed about Drought Stress: Measuring Plant Physiology in a Rapidly Changing Climate

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ABSTRACT

Climate change is causing widespread forest mortality due to intensified drought conditions. In light of a dynamically changing planet, understanding when forest die-off will occur is vital in predicting forest response to future climate trends. The Environmental Ecology Lab studies plant physiological response to drought stress to determine the lethal level of drought for pinyon pine. This drought research inspired this high school biology lesson, which addresses the NGSS Performance Expectation HS-LS4-6. Students engage in a climate change discussion regarding the devastation of California wildfires. Ongoing research in the lab is then introduced, leading students to design their own drought experiment using radish plants. Students determine an effective drought detector as a solution to mitigate human-induced climate change. Experimental data are statistically tested using R, to determine the effectiveness of drought detectors. To place their observations in a global context, students research the NASA Global Climate Change website to provide evidence to support their claim of human-induced climate change and relate this to a reduction in biodiversity. In a final presentation, groups share their most effective physiological measurement and propose potential applications of drought detection in mitigating adverse impacts of climate change.

Key Words: climate change; tree mortality; drought; wildfire; inquiry; 5E; NGSS.

○ Introduction

Human-influenced carbon emission is the driving force of modern climate change. Carbon dioxide emissions, along with other gases in Earth's atmosphere, create the greenhouse effect (Karl & Trenberth, 2003), which causes global temperature to rise as heat from the sun gets trapped in the atmosphere. Warming leads to changes in climate all over the planet, driving many regions to undergo progressively worsening drought conditions (Allen et al., 2010, 2015). Intensified drought has led to widespread forest mortality in the Northern Hemisphere (Carnicer et al., 2011). When forests die off, there are many adverse consequences – including variance in the carbon, water, and energy exchange (Adams et al., 2009) that affects biodiversity globally (Anderegg et al., 2013).

Investigating drivers and mechanisms behind forest mortality is a pressing issue. This is important for making predictions of how global change affects our ecosystems, which in turn can result in devastating losses for wildlife and society alike (Adams et al., 2010; Breshears et al., 2011; IPCC, 2014). Scientists in the Environmental Ecology Lab at Oklahoma State University, led by Adams, and including Kant and Hammond (authors of this manuscript), work in the field of plant ecophysiology. They study how global change affects ecosystems and are currently interested in this intensified-drought tree-mortality phenomenon. One of the lab's ongoing research projects aims to determine how eastern redcedar (*Juniperus virginiana*) is influenced by drought conditions and particularly how this affects its role in fire risk (Jolly & Johnson, 2018). When leaf moisture levels are high, this grassland-encroaching species has a strong tendency to be fire resilient (Ortmann et al., 1998). However, under dry conditions redcedar readily combusts – leading to potentially catastrophic wildfire risks (Weir & Scasta, 2014). Another species of interest is *Pinus edulis*, or pinyon pine, which has experienced a vast die-off due to intensified drought conditions (Breshears et al., 2005). Author Hammond studies lethal thresholds for pinyon under a drought gradient. He imposes drought on trees at various levels of physiological stress to determine the “point of no return” – defined as a level of drought stress beyond which it is more likely a tree will die than survive – for pinyon pine (Hammond & Adams, 2019; Hammond et al. 2019). Future implications of this research include formulating more accurate tree mortality models to predict climate responses, understanding traits related to tree survival, and figuring out how they can be identified across broad scales (Adams et al., 2013; McDowell et al., 2016; Fisher et al., 2018; Hammond & Adams, 2019).

The drought research conducted by the Environmental Ecology Lab was transitioned into an inquiry-based lesson that takes place intermittently over a seven-week span, allowing the teacher to fill the gaps with other lessons as the plants grow (see Supplemental Material: Resource 1; a numbered key to the Supplemental Material is provided at the end of this article; hereafter they are referred to as



Figure 1. Teacher-led eastern redcedar fire demonstration. Hydrated branch (left) is fire tolerant. Dried branch (right) is highly flammable.

Resource 1, 2, etc.). The project follows a 5E instructional model and was developed for a high school biology classroom. During the lesson, students are introduced to research in the Environmental Ecology Lab and are tasked to collaborate by designing their own experiment to achieve the final objective of determining an effective drought-detecting measurement to present to their peer researchers. Students use “Lab Notes” provided by the Environmental Ecology Lab and are encouraged to share their research findings with Dr. Adams and the team. The purpose of this inquiry-based lesson is for students to measure drought stress of radish plants as a model species adapted from the tree drought study. This lesson addresses the *Next Generation Science Standards* (NGSS), including HS-LS4 (Biological Evolution: Unity and Diversity), and more specifically, the Performance Expectation HS-LS4-6: “Create or revise a simulation to test a solution to mitigate adverse impacts of human activity on biodiversity” (NGSS Lead States, 2013). Learning objectives include for the students to:

- design an experiment to determine an effective drought detector for radish plants;
- statistically analyze experimental data in order to determine the potential significance of results and provide a solution to mitigate impacts on biodiversity; and
- provide scientific evidence to support the claim of human-induced climate change and loss of biodiversity.

○ Engagement Phase

Students observe a teacher-led demonstration using two eastern redcedar branches of equal size and color. One branch is kept in a moisture-sealed bag, remaining hydrated; while the second branch is dried in a microwave, causing the needles to become dehydrated, flattened, and brittle. Students are tasked to provide observations of the two branches and formulate inferences about the hydrated vs. dehydrated branch. The purpose of these observations and inferences is for students to engage in small-group and

whole-class discussion, in order to arrive at the conclusion that the dehydrated branch will catch fire and burn at a much faster rate than the hydrated one. As supporting evidence, the teacher lights the hydrated branch on fire.

Eastern redcedar has a dynamic relationship with fire; when trees are mature and the leaf moisture level is high, the species is fire resistant. However, under low leaf moisture conditions it is highly flammable (Weir & Scasta, 2014). The hydrated branch does not catch fire, but the dried-out branch catches fire quickly, resulting in a tall flame (Figure 1). Detailed instructions on how to prepare the branches and conduct the demonstration can be found in Resource 2. Safety precautions need to be addressed during this demonstration; we suggest conducting the demonstration outside on a concrete surface, away from flammable objects – or in a fume hood in the laboratory.

The second part of the Engagement Phase is a class discussion addressing the devastations of the 2018 California wildfires. To initiate a conversation, students watch a short (<2 minutes) video clip provided by *Time* magazine. Titled, *California’s Wildfires Have Become Bigger, Deadlier, and More Costly. Here’s Why*, the clip provides students with a real-world example of why drought and climate change are relevant to their lives. Teachers can find detailed instructions for this activity in Resource 3. The teacher displays the discussion questions after watching the wildfire video from the slideshow presentation (Resource 4). Students first write their own ideas about the causes and effects of California’s wildfires before engaging in small-group discussions to share their answers. The slideshow contains shocking images from recent California wildfires that helps students learn about the destruction that occurred. These questions are also used to prompt students to connect worsening drought and natural disasters to a changing climate. Topics within this discussion include defining and distinguishing *climate change* and *global warming*, and the discussion ends with suggestions of potential solutions to mitigate worsening wildfires. The purpose of the lesson’s Engagement Phase is to capture students’ interest and call attention to the implications of climate change. This also encourages students to start thinking about solutions to climate change.



Figure 2. Students plant radish seeds (left) into fertilized soil mix filled pots (right) in planting trays.

○ Exploration Phase

Learning Objective 1 (Design an experiment to determine an effective drought detector for radish plants) is addressed in the Exploration Phase. Students are provided an authentic research learning experience by performing lab procedures conducted in college-level research labs. As an introduction, students read about current studies occurring in the Environmental Ecology Lab from the lab's website (Resource 14). In small groups, students discuss the lab's research goals and the fire risk of droughted eastern redcedars, and then the teacher transitions the discussion into context for students' experimental designs.

Students' experimental designs are restricted to using radish plants (*Raphanus sativus*) as the model species – chosen for their low cost, fast growth, and other reliable qualities that provide students with the specific physiological measurements collected in the Environmental Ecology Lab. Students apply what they learn about this model species to a broader application of forest vegetation later on in the lesson. The following transition statement is read: "For the next several weeks, our class will be collaborating with the Environmental Ecology Lab at Oklahoma State University. You will work in lab groups of three to four to design an experiment to determine an effective drought detector for radish plants. You will be given notes describing methods used in the Environmental Ecology Lab. Data will be statistically analyzed using R software. After data are analyzed, your lab group will give a presentation of your research findings" (Resource 5).

To begin the inquiry-based drought experiment, students use the Resource 15 handout to plant and grow 20 radish plants (Figure 2). After planting the seeds, students are presented with available lab materials (Figure 3). Detailed lab notes from the Environmental Ecology Lab describing methods for measuring plant physiology are found in Resource 18. Teachers are provided alternative, easily accessible material options in the teacher handouts. Student groups collaborate to write a one-page research proposal describing the purpose, methods, and details of their planned investigation (guidelines included in Resource 17).

Lab Materials Available

Rulers	Coin Envelopes
Tape Measures	Safranin Dye
Calipers // alt. string and ruler	Scintillation Vials // alt. empty baby food jars
Scale	Microscopes
Scissors	Microscope Slides
Plastic Vials // alt. empty spice jars	Microscope Covers
Refrigerator	Razor Blades
Oven	

Figure 3. Exploration Phase lab material list provided to students during research proposal.

Student groups decide on at least three testable experimental methods to determine an effective drought detector for the radish model plant. One of these dependent variables must be leaf length, a well-established drought detector, as it will later be used as a proxy response variable to compare to other methods to determine their effectiveness. Once team proposals are approved by the facilitating teacher (Table 1), groups begin setting up their selected experimental design and collecting data. Data are recorded in their group's lab binder datasheet (see Resource 16).

Plants are to be watered for the first three weeks during the growing phase. After week 3, drought is initiated and data are collected for two weeks. Students are encouraged to create their own methods for data collection but may also use provided examples of methods, including the use of prescribed dependent variables like stem height, stem diameter, leaf count, and/or leaf width for their "During Drought Phase" methods (see Resource 7). Students are also at liberty to decide when to collect data and how often to water their control group, but three times a week is recommended. At the end of the experiment (Figure 4), during week

Table 1. Research Proposal Rubric used to approve lab group experimental design. To be accepted, all criteria must be “satisfactory.”

Criteria	Unsatisfactory	Satisfactory
Measurements	<3 methods described; none of the methods are designated EOE measurements	≥3 methods described; at least one of the methods is a designated EOE measurement
Materials/equipment	Missing method descriptions specifying what materials/equipment will be used	All methods are described, specifying what materials/equipment will be used
Timing/frequency of measurements	Incomplete timing and frequency of measurement descriptions	Complete timing and frequency of measurement descriptions for all methods
Rationale of measurements	Missing measurement rationale	Complete measurement rationale provided for all methods.
Hypothesis	Hypothesis missing	Hypothesis present
Level of drought	Level of drought not specified	Level of drought specified



Figure 4. Radish plants at end of experiment. Control group (left) and drought group (right).

five, lab groups are required to have one of their three chosen data collection methods be a designated “End of Experiment” measurement. For these measurements, plants are harvested and dissected to collect data on either biomass, percent wilted leaves, relative water content, or functional xylem area.

Biomass is measured by drying and weighing out the shoots and roots (Figure 5). Percent wilted leaves is calculated by dividing the number of wilted leaves over the total number of leaves on an individual plant (Figure 6). Relative water content is a measure of the amount of water in a leaf compared to its maximum water capacity when turgid (Figure 7). Functional xylem area is measured by allowing a dye to pass through the xylem vascular bundles that stains lignin red, exposing how water moves through the stem (Jacobsen et al., 2007; Figure 8). These methods require more detailed instructions and longer time to conduct than the “During Drought Phase” methods (e.g., stem height and stem diameter). Procedures are fully described in Resource 7 and Resource 8.

○ Explanation Phase

Learning Objective 2 (Statistically analyze experimental data in order to determine the potential significance of results and provide a solution to mitigate impacts on biodiversity) is addressed during the Explanation Phase. Now that students have all their data collected, what does it all mean? During this phase, students first conduct an analysis of variance (ANOVA) to determine significance, followed by visualizing their data by creating boxplot graphs using R – a statistical software used in scientific research all around the world, including the Environmental Ecology Lab. Exposure to R provides students with a unique experience with data science, in a frequently used programming language in STEM. While utilizing an open-source statistical analysis program used in professional research is our recommendation, we acknowledge that other software programs with ANOVA capabilities will suffice. If this is students’ first experience with ANOVA, we recommend utilizing



Figure 5. Biomass method sample laying out to weigh mass of shoot and roots. Control sample (left) and drought sample (right).



Figure 6. Percent wilted leaves samples. Control sample (left) with no wilting and drought sample (right) with 66% wilting.

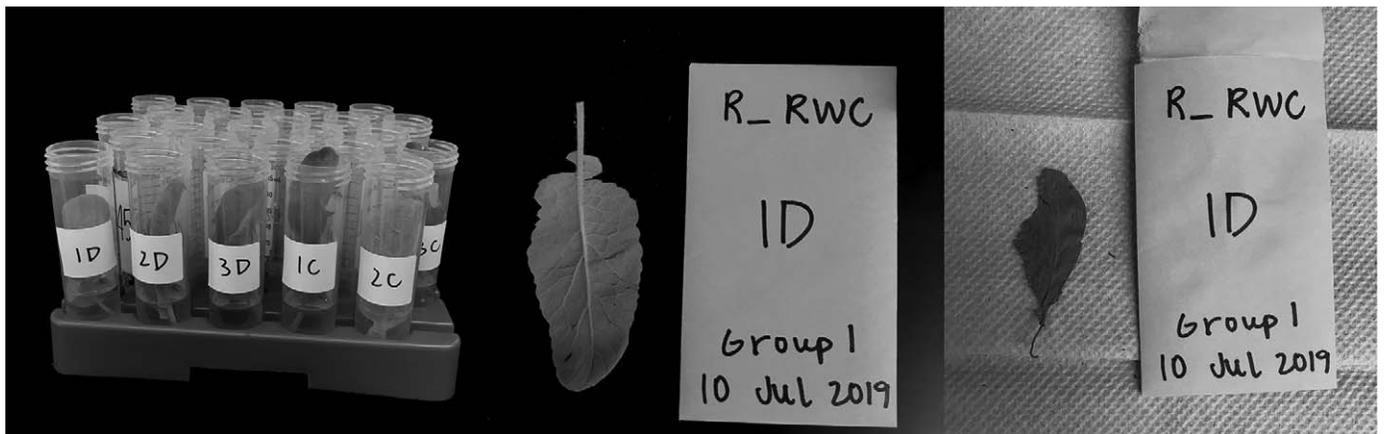


Figure 7. Relative water content method. Fresh leaf samples placed in vials filled with 15 mL of water for turgid mass (left), turgid sample before drying in oven (center), dry sample after oven (right).

resources from Khan Academy (see link under Additional Online Materials below).

For classrooms utilizing R to run ANOVA, students first transfer their lab binder data onto an Excel spreadsheet file. An example Excel spreadsheet file format is included in Resource 19. If R and RStudio have not been installed previously, students can follow the instructions to download the software using Resource 20. Once installed, students upload the prewritten R script file (see Resource 21) to R, and first run the ANOVA test with their collected data. Next, students plot their data using the boxplot function in R. After plotting the boxplots, students visually inspect their data and discuss trends (or absence of trends) between the watered and droughted groups of radish plants, recording these in their lab binder. A review to refresh students on statistical tests and determining sta-

tistical significance is included in Resource 10. Once students run the R script and obtain their statistical results, they are tasked with determining whether the methods used (e.g., plant height, leaf width, water content, biomass) were effective drought detectors.

○ Elaboration Phase

Learning Objective 3 (Provide scientific evidence to support the claim of human-induced climate change and loss of biodiversity) is addressed in the Elaboration Phase. Up to this point in the lesson, students have focused on determining an effective drought detector in response to the intensified drought conditions driven by climate change. Now that students have tested a potential solution to monitor global-change-type drought, the lesson takes a broader approach to investigate climate change. Here, the focus is not on just one effect of climate change – drought – but rather on many of the causes of climate change, and how the results are affecting life on our planet. Students explore the *NASA Global Climate Change, Vital Signs of the Planet* website to answer questions from Resource 22. The first topic students are tasked with is providing potential evidence for human-induced climate change. Are humans

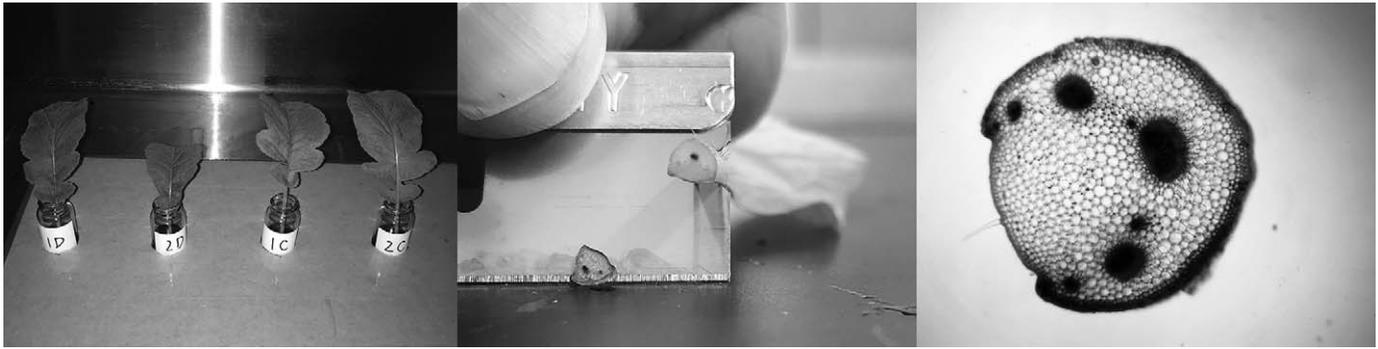


Figure 8. Functional xylem area. Leaf samples sit in safranin stain in vials for ~1 hour (left), stem cross section wet mount is prepared to view under microscope (center), safranin stains the vascular bundles moving the solution up the stem (right).

causing this warming pattern, or is it just another natural warming cycle? These are important skills for high school students to possess (e.g., providing scientific evidence to support a claim and understanding specific content to explain how human activity may be adversely impacting the planet).

The second part of the Elaboration Phase involves students navigating the *NASA Climate Time Machine Interactive* to make observations about various topics: sea ice, sea level, carbon dioxide, and global temperature. Students work independently, selecting two of the four topics to research and recording any dramatic changes and trends they might observe. Next, students research how climate change might affect biodiversity if the identified trends continue.

Finally, the third part of the Elaboration Phase includes a “NASA Images of Change and Solutions” class discussion. Students survey real photographs posted on the NASA website to compare before-and-after shots of the same locations. Students share their observations and proposed implications for biodiversity within their groups and then bring the conversation to the whole class. To wrap up the discussion, students research the differences between “mitigation and adaptation” in regard to climate change and prepare to voice their answers. Guides, questions, and discussion points can all be found in Resource 22 (without answers) and in Resource 12 (with answers).

○ Evaluation Phase

In research, it is vital for scientists to share their findings for the advancement of science. This can be done through journals, presentations, and/or personal collaboration. During the Evaluation Phase, students present their research to the class. Student groups create slideshow presentations to share their experimental design from the Exploration Phase and findings from the Explanation Phase with the class. Experimental methods are explained in detail along with experimental data obtained from their research, including plot interpretations and findings from their statistical analysis. Students are encouraged to take pictures of their plants and materials and use graphs to display their results, just as if they were presenting at a scientific conference. Lab groups share future research directions to further understand and mitigate the adverse impacts of climate change. Presentation guidelines with detailed instructions and a rubric outlining how students will be graded are given in Resource 23. Lab groups are highly encouraged to tweet or email the Environmental Ecology Lab team a picture of their research, but this is not required.

○ Conclusion

Informing students on climate change and the adverse impacts humans can have on our planet is a pressing matter. In this lesson, students explore the effects climate can have on plant stress and survival – of consequence to all life on Earth. Supporting evidence includes global tree mortality being driven by heat and drought (Allen et al., 2010) in recent decades. We hope that this lesson will grant high school students the opportunity to study reliable, peer-reviewed, data-based publications in order to understand how scientists know that our climate is changing. Our aspiration is for students to connect the changing climate to the loss of biodiversity and to comprehend the seriousness of the issue.

This lesson was designed to provide students a research experience to better develop their understanding of the practices and methods of scientific research. Nature of science tenets, such as making observations and inferences, analyzing empirical data, utilizing creativity in scientific work, the influence of (and on) society, and reliance on scientific models are embedded throughout this lesson to instill the epistemology of how scientific knowledge is generated in the biology classroom (McComas, 2015). These two components – science practices and nature of science – in combination with the increased content knowledge addressed in this lesson, provide students three components of science literacy. Our ultimate goal in developing this lesson with an integrated focus on these three components is to formulate a more scientifically literate student populace.

○ Supplemental Material: Resources 1–24

- (1) TeacherPreLessonHandout1-LessonTimeline
- (2) TeacherEngagementHandout1-WateredvsDroughtDemonstration
- (3) TeacherEngagementHandout2-CaliforniaWildfires
- (4) TeacherEngagementHandout3-CaliforniaWildfireSlides
- (5) TeacherExplorationHandout1-ResearchIntroduction
- (6) TeacherExplorationHandout2-PlantingPhase
- (7) TeacherExplorationHandout3-ProposalPhase
- (8) TeacherExplorationHandout4-PlantPhysiologyLabNotes
- (9) TeacherExplanationHandout1-StatisticalAnalysis
- (10) TeacherExplanationHandout2-StatisticalAnalysisSlides
- (11) TeacherExplanationHandout3-InstallingRandRStudio
- (12) TeacherElaborationHandout1-VitalSignsofthePlanet

- (13) TeacherEvaluationHandout1-DroughtResearchPresentationGuidelines
- (14) StudentExplorationHandout1-ResearchIntroduction
- (15) StudentExplorationHandout2-PlantingPhase
- (16) StudentExplorationHandout3-DataSheet
- (17) StudentExplorationHandout4-ProposalPhase
- (18) StudentExplorationHandout5-PlantPhysiologyLabNotes
- (19) StudentExplanationHandout1-ExcelExample
- (20) StudentExplanationHandout2-InstallingRandRStudio
- (21) StudentExplanationHandout3-RScript
- (22) StudentElaborationHandout1-VitalSignsofthePlanet
- (23) StudentEvaluationHandout1-DroughtResearchPresentationGuidelines
- (24) StudentEvaluationHandout2-PresentationRubric

○ Additional Online Materials

- Introduction to the ANOVA Test: <https://www.khanacademy.org/math/statistics-probability/analysis-of-variance-anova-library>
- Environmental Ecology Lab Website: <http://henrydadams.com/research.html>
- NASA Climate Change Time Machine: <https://climate.nasa.gov/interactives/climate-time-machine>
- NASA Vital Signs of the Planet: <https://climate.nasa.gov/evidence>
- R and RStudio Download: <http://cran.us.r-project.org/>
- R and Rstudio Installation Instructions: <https://www.andrewheiss.com/blog/2012/04/17/install-r-rstudio-r-commander-windows-osx/>
- Time magazine video “California’s Wildfires Have Become Bigger, Deadlier, and More Costly. Here’s Why”: <http://time.com/4985252/california-wildfires-fires-climate-change/>

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