TIEE

Teaching Issues and Experiments in Ecology - Volume 11, March 2016

EXPERIMENTS

Effects of plant quality on caterpillar performance

Caralyn B. Zehnder

Springfield Technical Community College, Office of Learning Engagement & Advancement, Springfield, MA 01105; czehnder@stcc.edu

ABSTRACT
Fertilizers are commonly applied to plants in both agricultural and ornamental settings and these fertilizers can affect insect herbivores. Beet armyworms, *Spodoptera exigua*, are phytophagous insects that are easy to rear in the lab. In this exercise, students design an experiment that manipulates plant quality via fertilizer application and then measure the effect of fertilization on caterpillar performance. In the first class, students design the experiment and apply the treatments. In subsequent weeks, students conduct a feeding trial where they collect and analyze data on caterpillar performance. Each student writes a lab report based on class data.

KEYWORD DESCRIPTORS

- **Ecological Topic Keywords:** Community ecology, herbivores, plant quality, population ecology, species interactions, herbivory, plant-animal interactions

- **Science Methodological Skills Keywords:** collecting and presenting data, data analysis, experimental design, graphing data, hypothesis generation and testing, quantitative data analysis, scientific writing, statistics

- **Pedagogical Methods Keywords:** assessment, cooperative learning, guided inquiry, peer editing

CLASS TIME

One 2-3 hour class period for design and implementation of experiment (week 1). 15-30 minutes in class for caterpillar weighing during weeks 4 and 5 (or you can end the experiment after week 4). One 2-3 hour class period to review t-tests and graphing (week 5). One hour for peer review (this could be accomplished outside of class).

OUTSIDE OF CLASS TIME

Students will spend approximately 1 hour per week during weeks 1 – 4 designing their experiment and taking care of plants and caterpillars. They will spend several hours analyzing class data, finding and reading journal articles, commenting on a peer review and writing papers based on their results.

STUDENT PRODUCTS

Each student submits a short description of a proposed experimental design. Each student writes a lab report in the style of *Ecology* based on the class data.

SETTING

The experiment is carried out entirely in the lab. You will need a small amount of space to grow plants and rear caterpillars. For a lab section of 24 students, you will need approximately 1m^2 for growing plants and another 1m^2 for rearing caterpillars.

COURSE CONTEXT

The experiment as described is used in a sophomore-level ecology course with a maximum of 24 students per lab section.

INSTITUTION

This experiment has been conducted at a small public liberal arts university.

TRANSFERABILITY

This experiment is a guided-inquiry exercise, so it can be transferred to other levels by providing more or less guidance to the students in terms of experimental design and data analysis. This experiment could be conducted with wide variety host plant species (cotton, soybeans, collards, lettuce). Other phytophagous insects that are easily reared in the laboratory such as the tobacco hornworms (*Manduca sexta*) and the cabbage white butterfly (*Pieris rapae*), could be used. Besides fertilizer, other treatments could be used such as comparing different plant species, the presence or absence of a mutualism if legumes and rhizobia are used, or soil moisture levels.

TIEE

Teaching Issues and Experiments in Ecology - Volume 11, March 2016

ACKNOWLEDGEMENTS
This submission was greatly improved by comments and suggestions from Christopher Beck and the TIEE anonymous reviewers. I thank A. VandeVoort and K. Manoylov for helpful course discussions. Finally, I thank the many students who have enrolled in my Ecology course at Georgia College and participated in this laboratory exercise.

SYNOPSIS OF THE EXPERIMENT

Principal Ecological Question Addressed

How does variation in plant quality affect caterpillar performance? Specifically, how does fertilization alter caterpillar larval mass, development time, or pupal mass?

What Happens

The length of this experiment can vary depending on how much prep work the instructor does beforehand and how long the caterpillars are allowed to develop. In the first class, students collaboratively design an experiment and apply the first fertilizer treatment. Students treat the plants over the next 3 weeks. In week 4, students receive their caterpillars. Each student is assigned a treatment (fertilizer or control), feeds their caterpillar leaves from the appropriate treatment, and records the dependent variables from their caterpillar. Class data are combined. Then students analyze data and write lab reports.

Experiment Objectives

By completing this laboratory experiment, students will be able to:

1. Design an ecological laboratory experiment.
2. Explain how to randomly assign experimental treatments to subjects and explain why this is important.
3. Collect data on insect herbivore performance.
4. Statistically analyze data using two-tailed t-tests.
5. Correctly present and interpret statistical results.
6. Create bar charts and box plots in EXCEL.
7. Write a lab report in the format of the journal *Ecology*.
8. Find appropriate journal articles and correctly reference these articles.

Equipment/ Logistics Required

Plants can be grown from seeds or purchased directly from a garden supply store. Plants should be at least 1 month old at the start of the experiment.

Spodoptera exigua are generalists and will consume many easy to grow plant species including: soybeans, cotton, collards, and lettuce. For a class of 24 students, you will need ~50 plants if you are rearing the caterpillars from egg to pupation. The fertilizer treatment can be a general fertilizer such as Miracle-Gro ®, or a specific nutrient, such as nitrogen in the form of ammonium nitrate. *Spodoptera exigua* can be ordered from http://www.benzonresearch.com/. The instructor, or the institution's laboratory coordinator, will need a USDA 526 permit for shipping of live insects. Permit applications can be made through the USDA website: http://www.aphis.usda.gov/plant_health/permits/organism/index.shtml.

Instructors can also order *Pieris rapae* eggs from Carolina Biological Supply Company (no permit required, http://www.carolina.com/butterfly-cultures/brassica-butterfly-pieris-rapae-eggs-1-strip-living/144100.pr?question=). *P. rapae* are specialists on plants in the brassica family, so the instructor will need to grow an appropriate plant such as collards, Brussels sprouts, or broccoli. One order of eggs from Carolina only includes 5-6 eggs.

Caterpillars can be reared in plastic sandwich containers. Each container will need a few holes poked in it, for air ventilation. To help prevent the clipped leaves from desiccating, include a moist paper towel in each container. Caterpillar development is faster in warmer temperatures. If an incubator is available, then set the temperature to 25°C and the day-night light cycle to 16:8 hours. Space on a lab bench or some space in a greenhouse can also work. The containers can be stacked. Ideally students should be able to access their caterpillars outside of class so that they can add leaves, moisten the paper towel and clean out the containers as necessary.

Below is a list of supplies for a class of 24 students:

- 50 1-month old plants.
    - 50 small plastic pots (10 x 10cm pots have worked for us).
    - Trays for carrying plants (not necessary, just makes it easier to move them around).
    - Potting soil.
- 24 *Spodoptera exigua* larvae ordered from http://www.benzonresearch.com/. *S. exigua* are shipped in a batch of 1000 eggs. Eggs will hatch in 3-4 days at room temperature (25°C). Allow the larvae to develop for 2-3 days before beginning the experiment because younger larvae are more difficult to move. *S. exigua* can be easily reared on artificial diet if you want to establish a colony.
- Fertilizer (Miracle-Gro ® or another brand). Follow package instructions for mixing. Total amount depends on the total number of plants in the experiment. A class of 24 only uses a few teaspoons of fertilizer.

- 24 plastic sandwich containers with small pin holes poked in the top.
- 3-4 small paintbrushes for transferring caterpillars.
- Sharpies.
- Labelling tape.
- Small scissors.
- Paper towels.
- 0.1mg analytical balance for weighing caterpillars. 10 day old caterpillars weigh 0.027-0.087 grams. It is helpful to have more than one balance for lab days when everyone needs to weigh their caterpillars.
- Weigh boats or weigh paper.
- Graduated cylinders (for applying the fertilizer and control treatments). I use 10 in a class of 24, so that multiple students can apply the treatments at the same time.
- Glassware for mixing and storing the fertilizer.

Summary of What is Due

Students submit an experiment description before the first day of class. After collecting and analyzing data, each student writes a lab report based on the class data. Students submit rough drafts for peer and instructor review.

DETAILED DESCRIPTION OF THE EXPERIMENT

Introduction

Plant quality matters to insect herbivores. Plant quality is a broad term that encompasses any physical, chemical, or biological plant trait (e.g., size and structure, nutritional value, secondary compounds, and phenology) that influences herbivore preference or performance. Variation in host-plant quality can influence insect herbivore survival, development time, and fecundity (Rossiter 1988, Haggstrom and Larsson 1995, Lill and Marquis 2001, Tsai and Wang 2001, Ladner and Altizer 2005).

Nitrogen is considered the most limiting macronutrient for insect herbivores (Mattson 1980). Nitrogen fertilization has been linked with increased insect density, shorter development time, higher survival rates, increased insect mass, and higher fecundity (Mattson 1980, Cisneros and Godfrey 2001, Nevo and Coll 2001, Tsai and Wang 2001, Chen et al. 2004, Stiling and Moon 2005). However, other studies have found negative or no effects of nitrogen fertilization on insect abundance or performance (Bethke et al. 1998, Casey and Raupp 1999, Muller et al. 2005).

Most insects in the order Lepidoptera (butterflies and moths) are herbivores during their larval (caterpillar) stage, and some of them are major agricultural pests. The beet armyworm, *Spodoptera exigua*, is native to Asia and it was introduced into the US around 1875. *S. exigua* is now common throughout the southern and western U.S and it is a pest insect of asparagus, cotton, corn, collards, soybean, tobacco, alfalfa, sugar beets, pepper, tomato, potato, onion, pea, sunflower, and citrus (Smits et al. 1987). It is known as a generalist or polyphagous (poly=many, phagous = eat) insect because it successfully develops on many different host plants. The life cycle of the beet armyworm lasts 30-40 days. It is a holometabolous insects meaning that it undergoes complete metamorphosis. The adult female lays eggs on a host plant, within a couple of days the eggs hatch and the larvae (caterpillars) begin doing what they do best: eating and growing. The greenish-brown larvae regularly shed their exoskeleton and go through 5 instars over ~20 days. Once a caterpillar reaches the final instar it pupates. The pupal stage lasts for approximately 1 week before the adult emerges.

Materials and Methods

Week 1:

Part 1: Fifty 1-month old soybean, *Glycine max*, seedlings will be available for you in lab today. Additionally, you will have access to Miracle-Gro ® plant fertilizer.

After reading the introduction answer the following questions (2-3 paragraphs): *You are a scientist working for the USDA studying soybeans, an economically important crop. Soybeans are regularly fertilized. Soybeans are attacked by multiple insect herbivores. You are interested in studying how soybean fertilization affects S. exigua, a caterpillar species that attacks soybeans.*

1. *What is your hypothesis?*

2. *Design a lab experiment to test this hypothesis. State the independent and dependent variables and briefly describe your methods.*

Come to class prepared to present your experimental design and you should also submit a copy of your answers online. You will then work with your group members to develop a group experiment that you will present to the class. We will then develop an experimental design that the entire class will follow. Over the next 3 weeks, you will apply the appropriate treatment to each plant. Then during week 4, each student will receive one *S. exigua* caterpillar to rear. You will regularly care for your caterpillar and collect data on larval mass, development time and pupal mass.

Part 2: After our class decides on an experimental design, it is time to **randomly assign** each plant to either the control or fertilizer treatment. We want to do this in an unbiased manner, meaning that for each plant there is an equal chance of it being assigned to the control or fertilizer treatment. This is one way that researchers eliminate **confounding variables** (or lurking variables) in an experiment. For example, it would be extremely biased if we ranked the plants by size and then put all of the large plants in the fertilizer treatment and all of the small plants in the control treatment. Then plant size would be confounded with the independent variable and we wouldn't be able to determine if plant size or fertilizer treatment was affecting caterpillar performance.

We will use a random number table to assign each plant to either the control or fertilizer treatment in an unbiased manner. Half of you will assign plants to treatment and label the plants, while the other half of the class mixes up the fertilizer solution and applies the fertilizer or control solution to each plant. Using the random number function in EXCEL (=RANDBETWEEN(0,1)), I created a random table of 1s and 0s. For our purposes, 0=control and 1=fertilizer. Start anywhere on this table and then work either down a column or across a row and assign each plant to the appropriate treatment. It is OK if there are a slightly uneven number of control and fertilizer plants. Clearly label each plant.

Meanwhile, anyone who is not labelling plants needs to follow the instructions on the Miracle-Gro ® box and prepare a fertilizer solution. We will be applying 40mL of the Miracle Gro ® solution to each fertilizer plant and 40mL of water to each control plant. Each plant will receive the fertilizer application once a week for 3 weeks, so we need approximately 300mL of fertilizer solution (25 plants x 3 weekly applications x 40mL per week = 300mL). Using a graduated cylinder apply 40 mL of fertilizer solution to each fertilizer plant and 40mL of water to each control plant.

After all of the plants are labelled and have received their appropriate treatment, place the pots back on the trays. Be sure to mix up the plants. Do not put all of the fertilizer plants in one tray and all of the control plants in another. Again, we want to minimize confounding variables as much as possible. These plants will be growing in the greenhouse and we don't want environmental variation within the greenhouse to affect plant growth. For example, plants at one end of the table receive more direct sunlight then plants at the other end. If the plants are not mixed up in the tray, then our experimental design would be biased and sunlight would be a confounding variable.

End of class discussion:
What details from today will you need to include in your lab report?

Week 2:
Volunteers will water the plants with the appropriate treatment on Wednesday. On Monday and Friday, other students will water all of the plants.

Week 3:
Volunteers will water the plants with the appropriate treatment on Wednesday. On Monday and Friday, other students will water all of the plants.

Week 4:
Today the feeding trial begins! Each of you will receive one 4-day old *S. exigua* caterpillar. Everyone will be responsible for 1 replicate. For our experiment, a replicate is a container with a beet armyworm fed control leaves or fertilizer leaves. For our class, we will have 12 caterpillars fed control leaves and 12 fed fertilizer leaves. Each student will be randomly assigned to the control or fertilizer treatment.

To assemble a caterpillar rearing container, begin by labelling a sandwich container with tape and write your name and treatment on it. Then place a moist, but not dripping wet, paper towel in container. The purpose of the paper towel is to slow down how fast the leaves dry out. Caterpillars can't swim, so if you have lots of water sloshing around in your container then your caterpillar will drown. Then clip one leaf from the appropriate treatment plant. Discard dried or 'unhealthy' looking leaves. Once we have started sampling from a plant, use all of its leaves. This avoids induction, or changes in plant quality because the plant is responding to its leaves being cut off. Use whole, fully expanded leaves (clip leaf at petiole). Once you have this setup, then come over to me to receive a caterpillar. Then close your container and place it on the counter.

It is your responsibility to regularly check on your caterpillar. This means that you should check your caterpillar every day or every other day. Add new leaves as necessary, remove old dried leaves, replace the paper towel as needed, but disturb the caterpillar as little as possible. Before a weekend, provide 2 leaves for your caterpillar on Friday afternoon and add more leaves again on Monday. Record when you feed and how your caterpillar looks on the observation sheet. Initially, 1 leaf will last 1-2 days, but as it grows larger it will consume more.

End of class discussion:

What details from today will you need to include in your lab report?

Week 5:
Today our caterpillars are 11 days old and they will have their first weigh in. Put a piece of weigh paper on the balance and tare the balance. Fold the paper in half so it is easier to handle. Using a paintbrush, carefully place your caterpillar on the paper. Weigh your caterpillar and record this on the class datasheet.

This is a good time to give your caterpillar more leaves and clean out the container. Continue to regularly check in on your caterpillar. Add new leaves as necessary, remove old dried leaves, replace the paper towel as needed but disturb the caterpillar as little as possible. Record when you feed and how your caterpillar looks on the observation sheet in the lab. Don't panic if your caterpillar starts to change color, is not moving as much and looks scrunched up like an accordion. This means that it is getting ready to pupate. Pupation is an energy intensive endeavor and some caterpillars might not survive the process. Leave your caterpillar alone and record the date that you see a small, brown cocoon. You should also record pupal mass at this time. Then you are done!

Give your caterpillar to your instructor. *S. exigua* is a pest species and should not be released in the wild.

End of class discussion:
What details from today will you need to include in your lab report?

Week 6
Part 1: Today our caterpillars are 18 days old and they will have their second weigh in. Put a piece of weigh paper on the balance and tare the balance. Fold the paper in half so it is easier to handle. Using a paintbrush, carefully place your caterpillar on the paper. Weigh your caterpillar and record this on the class datasheet. If your caterpillar has pupated already, then make sure that you recorded the date of pupation and pupal mass. However, don't record a larval mass for day18.

This is a good time to give your caterpillar more leaves and clean out the container. Continue to regularly check in on your caterpillar. Add new leaves as necessary, remove old dried leaves, replace the paper towel as needed but disturb the caterpillar as little as possible. Record when you feed and how your caterpillar looks on the observation sheet in the lab. Don't panic if your caterpillar starts to change color, is not moving as much and looks scrunched up like an accordion. This means that it is getting ready to pupate. Pupation is an energy intensive endeavor and some caterpillars might not survive the process. Leave your caterpillar alone and record the date that you see a small, brown cocoon. You should also record pupal mass at this time. Then you are done!

Give your caterpillar to your instructor. *S. exigua* is a pest species and should not be released in the wild.

Part 2: Bar charts, box plots and t-tests. Read the box plots and t-tests handout before class. I strongly recommend that you complete the practice problems and then check your answers. I will answer questions about t-tests and box plots for 10 minutes at the beginning of class and then you will take a closed-book quiz on this material. After you are done, then you should weigh your caterpillar if you haven't already.

After you have completed the quiz, you should open the caterpillar data EXCEL file. Save this file to your computer and work through the bar graphs, box plots and t-tests handout. Before you leave class, you need to show me:

1. A bar graph with standard error bars of the day11 larval mass data.
2. A box plot of the day11 larval mass data.
3. A t-test table analyzing these data in EXCEL and a results statement in the correct format.

Week 7: Lab report rough draft due.

Week 8: Last week, you received two lab reports to peer review. I need to see (so you can earn a grade) the peer review comments that you have made on the reports. If you did your peer review electronically, then email me a copy of your comments**.** If you made your comments on paper, then please highlight the comments or do something to show me which comments you made before coming to class.

Instruction for peer review: Get together with your partner and discuss your two reports. Using the rubric, grade each report (one rubric per report). Write down additional comments to explain the scores. Be critical but polite. You can give ½ grades. Be sure to write down the number of the report on the rubric. Do not write your name on the rubric. These comments will be returned to the original author.

Week 9: Lab report final drafts due.

Questions for Further Thought and Discussion:

1. When we were assigning the plants to each treatment, we used a random number table to **randomly** assign plants to each treatment. What does it mean to randomly assign treatments? Why is this important?
2. Why is it important to reduce or eliminate confounding variables in an experiment?
3. Imagine you wanted to conduct this experiment under field conditions (outside in a soybean field). Describe how you would modify this experiment. State any new dependent variables you would measure and explain why you included these in your experiment. Describe two potential confounding variables that you would try to reduce and explain how you would do this.
4. Did fertilizer application affect caterpillar performance? What might this mean for agriculture applications of fertilizer?
5. Have other researchers found similar results concerning the effect of fertilizer on herbivore characteristics?
6. Did fertilizer application affect all of the dependent variables in the same way? If there were differences, explain these differences.

References

References

Bethke, J. A., R. A. Redak, and U. K. Schuch. 1998. Melon aphid performance on chrysanthemum as mediated by cultivar, and differential levels of fertilization and irrigation. Entomologia Experimentalis Et Applicata **88**:41-47.

Casey, C. A., and M. J. Raupp. 1999. Supplemental nitrogen fertilization of containerized azalea does not affect performance of azalea lace bug (Heteroptera : Tingidae). Environmental Entomology **28**:998-1003.

Chen, Y.-Z., L. Lin, C.-W. Wang, C.-C. Yeh, and S.-Y. Hwang. 2004. Response of two *Pieris* (Lepidoptera: Pieridae) species to fertilization of a host plant. Zoological Studies. **43**:778-786.

Cisneros, J. J., and L. D. Godfrey. 2001. Midseason pest status of the cotton aphid (Homoptera : Aphididae) in California cotton: Is nitrogen a key factor? Environmental Entomology **30**:501-510.

Haggstrom, H., and S. Larsson. 1995. Slow larval growth on a suboptimal willow results in high predation mortality in the leaf beetle *Galerucella lineola*. Oecologia **104**:308-315.

Ladner, D. T., and S. Altizer. 2005. Oviposition preference and larval performance of North American monarch butterflies on four *Asclepias* species. Entomologia Experimentalis Et Applicata **116**:9-20.

Lill, J. T., and R. J. Marquis. 2001. The effects of leaf quality on herbivore performance and attack from natural enemies. Oecologia **126**:418-428.

Mattson, W. J. 1980. Herbivory in relation to plant nitrogen-content. Annual Review Of Ecology And Systematics **11**:119-161.

Muller, C. B., M. D. E. Fellowes, and H. C. J. Godfray. 2005. Relative importance of fertiliser addition to plants and exclusion of predators for aphid growth in the field. Oecologia **143**:419-427.

Nevo, E., and M. Coll. 2001. Effect of nitrogen fertilization on *Aphis gossypii* (Homoptera : Aphididae): Variation in size, color and reproduction. Journal of Economic Entomology **94**:27-32.

Rossiter, M. 1988. Relationships among defoliation, red oak phenolics, and gypsy moth growth and reproduction. Ecology **69**:267-277.

Smits, P., M. Velden, M. Vrie, and J. Vlak. 1987. Feeding and dispersion of *Spodoptera exigua* larvae and its relevance for control with a nuclear polyhedrosis virus. Entomologia Experimentalis Et Applicata **43**:67-72.

Stiling, P., and D. C. Moon. 2005. Quality or quantity: the direct and indirect effects of host plants on herbivores and their natural enemies. Oecologia **142**:413-420.

Tsai, J. H., and J. J. Wang. 2001. Effects of host plants on biology and life table parameters of *Aphis spiraecola* (Homoptera : Aphididae). Environmental Entomology **30**:44-50.

Links:
University of Florida Featured Creature by John L. Capinera
http://entnemdept.ufl.edu/creatures/veg/leaf/beet_armyworm.htm

Tools for Assessment of Student Learning Outcomes:

There are a variety of ways that student performance can be assessed for this experiment. In the past, students have written individual lab reports based on class data.

- Lab report description
- Lab report rubric

Students can practice evaluating sections of a lab report using this handout, typically followed by a class discussion.

Students can learn about plagiarism and practice ways to avoid it using this handout.

Additionally, students are assessed on their peer review comments using the following grading scale:

10/10: The peer review provides thoughtful, polite comments and suggestions throughout the entire lab report. Comments are explained when necessary. Comments are about content, not only grammar and spelling.

7/10: The peer review provides polite comments and suggestions throughout the entire lab report. Comments are explained when necessary. Most of the comments are focused on grammar and spelling.

5/10: Comments are rude or condescending. Or multiple sections of the lab report does not have comments provided.

NOTES TO FACULTY

Challenges to Anticipate and Solve

Challenge #1: *Determining the appropriate dependent variables*. Students often struggle to identify appropriate dependent variables, because they have very little experience in this plant-herbivore system. Many students will indicate survival rate as a dependent variable, which is appropriate; however, they will not be able to statistically analyze survival using a t-test. Students also may indicate more subjective variables, such as caterpillar color or health. If you are using *S. exigua*, then you can expect a wide range of larval coloration ranging from light green to dark brown (regardless of food quality). And larval coloration changes throughout development. Larval mass, development time, and pupal mass are common variables in this type of experiment and they are easy to measure. You can either gently guide students towards these variables or assign them a paper to read and use as an example, such as the Chen et al. (2004) article.

Challenge #2: *Plants or caterpillars die.* This challenge occurs whenever you work with living organisms. One year we used pansies as the host plant and all the caterpillars died by the second week of the feeding trial. Students only had one dependent variable to analyze in their lab reports. As a result, I required them to hypothesize potential reasons for the caterpillar die-off and describe how they would test this hypothesis. Also, setting up the feeding trial can be a stressful experience for the caterpillars, so I usually give each student 2 or 3 caterpillars to increase the chances that one survives until the first weigh-in. Then, I randomly remove all but one of the caterpillars.

Challenge #3: *Students struggle with interpreting their statistical analyses.* Having students complete their first t-test during lab gives the instructor the opportunity to provide immediate feedback and help struggling students. I recommend that each student writes down the sentence that will go into their results section, so that the instructor can review this work. Additionally, you may want to give a brief lecture introducing these topics, especially the t-tests. I ask students to read the information ahead of time and then take a quiz at the start of class. Then we move on to the computer activity. For the activity, I find it helpful to have students work through the EXCEL handouts at their own pace in a computer lab, I circle around the room and help as needed.

Challenge #4: *Students neglect their caterpillars and it dies*. Grade students on how well they take care of their caterpillar (I usually assign 10 pts worth of caterpillar care credit). If you have a TA or student assistant helping with the lab, then they can help by checking on each caterpillar regularly and noting if a caterpillar is without food. Encourage students to work together and feed their friends' caterpillars if necessary. In my experience, most students want to be good caterpillar parents.

Challenge #5: *Small sample size*. If class size is small, then it may be necessary to assign each student multiple replicates (caterpillars) in order to increase sample size and statistical power.

Comments on Introducing the Experiment to Your Students:

When students are preparing their proposals, you may want to reduce the amount of information that you give them about the experimental setup, since this may influence their proposals.

Because I allow this experiment and the subsequent lab report to proceed for the first half of the semester, I usually introduce this experiment during the first week of lab. Therefore, students have very little prior knowledge about the topic beyond the introductory material.

I expect students to come to the first lab class prepared to discuss their experimental design. Because this is a guided-inquiry activity, students need time to discuss their ideas and design their own experiment. The instructor should facilitate these discussions. Students frequently want to put the caterpillars directly on a plant, rather than feeding cut leaves to the caterpillars. The instructor should help students consider the difficulties associated with that experimental design and guide them towards resources that describe the feeding trial method. If caterpillars are put directly onto plants, then you will need to cage each plant-caterpillar pair and also cage each control plant to account for cage-effects. Additionally, it is much easier to find a caterpillar on one leaf in a small container than it is to find a caterpillar on a whole plant.

Comments on Experiment Description

A variety of host plants can be used for this experiment since *S. exigua* are generalists and will consume many easy-to-grow plant species including: soybeans, cotton, collards, and lettuce. Plants can be grown from seeds or purchased directly from a garden supply store. Plants should be at least 1 month old at the start of the experiment. For a class of 24 students, you will need ~50 plants if you are rearing the caterpillars from egg to pupation. The fertilizer treatment can be a general fertilizer such as Miracle-Gro ®, or a specific nutrient, such nitrogen in the form of ammonium nitrate. Additionally, if you are focusing on nitrogen and using ammonium nitrate, then you can add a nice quantitative challenge by having students calculate the amount of ammonium nitrate needed per pot based on recommended agricultural applications If students are applying the treatment for 3 weeks, then they will need access to the plants and the plants will need to be regularly watered.

Spodoptera exigua can be ordered from http://www.benzonresearch.com/. The instructor, or the institution's laboratory coordinator, will need a USDA 526 permit for shipping of live insects. Permit applications can be made through the USDA website: http://www.aphis.usda.gov/plant_health/permits/organism/index.shtml.

Instructors can also order *Pieris rapae* eggs from Carolina Biological Supply Company (no permit required, http://www.carolina.com/butterfly-cultures/brassica-butterfly-pieris-rapae-eggs-1-strip-living/144100.pr?question=). *P. rapae* are specialists on plants in the brassica family, so the instructor will need to grow an appropriate plant such as collards, Brussels sprouts, or broccoli. One order of eggs from Carolina only includes 5-6 eggs.

Caterpillars can be reared in plastic sandwich containers. Each container will need a few holes poked in it, for air ventilation. To help prevent the clipped leaves from desiccating, include a moist paper towel in each container. Caterpillar development is faster in warmer temperatures. If an incubator is available, then set the temperature to 25°C and the day-night light cycle to 16:8

hours. Space on a lab bench or some space in a greenhouse can also work. The containers can be stacked.

On the first day of the feeding trial, when you are distributing the caterpillars to the students use a small paintbrush to move one or two caterpillars to the leaf, if the caterpillars are small then you may want to add two to each container and then randomly remove one of them if they are both alive after a few days.

Ideally students should be able to access their caterpillars outside of class so that they can add leaves, moisten the paper towel, and clean out the containers as necessary. Some students will want to keep their “pet” caterpillar at the end of class. Don’t let them! *S. exigua* is a pest species and should not be released in the wild. At the end of the experiment, you can either add the surviving *S. exigua* back into the colony, if you are keeping one, or put them in the freezer and then the trash.

Unless all students check on their caterpillars daily, which is unrealistic in most situations, there will be error associated with the development time dependent variable. If students are assigned randomly to the treatment groups, then it is unlikely that one treatment group will have students who are better caterpillar “parents” then others.

Comments on the Data Collection and Analysis Methods Used in the Experiment:
Students enjoy comparing caterpillars and have fun weighing them. Instruct students not to let the caterpillar crawl off the weigh paper and underneath the weigh pan. Sometimes students have trouble calculating development time. Students record the date that their caterpillars pupate, and some students try to analyze this date with a t-test and that doesn’t work. They have to use this date to calculate development time in days, so they need to know what day the eggs hatched. Depending on how quickly the caterpillars develop, some of caterpillars may pupate before the second larval mass weigh-in. As long as there is an adequate sample size, a few missing values won’t influence the results. If you notice that a large proportion of the caterpillars are beginning to pupate, then either drop the second larval mass measurement or quickly weigh all the caterpillars.

Assuming that these data are normally distributed, and they usually are, the correct way to analyze these data is using a two-tailed, two sample t-test. This assumes that the students did not have a directional hypothesis; in which case a one-tailed, two sample t-test would be most appropriate. If students are experienced with hypothesis testing, then you may want to include this distinction. However, in an introductory class you will probably want to coach students to state non-directional hypotheses, in order to validate the use of two-tailed t-tests.

If fertilization improves plant nutritional quality (more nitrogen), then we would expect that the caterpillars fed leaves from the fertilizer treatment would have larger larval mass and short development times than caterpillars fed leaves from the control treatment. However, if you choose a host plant that has a nitrogen-based defense, then the additional fertilizer could potentially increase constitutive defense levels in the plant and cause the fertilizer plants to be better defended. Then the results would be reversed. Many plants in the nightshade family, Solanacea, produce nitrogen-based alkaloids, such as nicotine in tobacco or capsaicin in chili peppers. Typically, there is no difference in pupal mass between the two treatments or only a small difference. Pupal mass seems to be more constrained than larval mass or development time and differences in pupal mass are more likely when there are large differences in plant quality between the two treatments.

In a true research setting, it would be appropriate to determine the sex of each caterpillar by looking at the patterns on the chrysalis. Then, the appropriate statistical analysis would be a 2-way ANOVA with treatment and sex as the two factors. However, then you need a larger sample size. Because I want to use this experiment to teach students about using statistics, and for most of my students this is their first experience doing any type of statistical analysis, I use the simpler experimental design described above. If you are interested in determining caterpillar sex, then this link provides good pictures for sexing pupa: http://www.ukleps.org/sexingpupae.html. Students will need microscopes.

Comments on Questions for Further Thought:

1. **When we were assigning the plants to each treatment, we used a random number table to randomly assign plants to each treatment. What does it mean to randomly assign treatments? Why is this important?**

   The point of this question is to have students think about proper experimental design and the importance of assigning treatments to subjects in an unbiased manner.

2. **Why is it important to reduce or eliminate confounding variables in an experiment?**

The point of this question is to have students think about proper experimental design and the importance of reducing the influence of confounding variables. Eliminating confounding variables provides stronger support for the hypothesis that changes in the independent variables are causing changes in the dependent variable. While many students can repeat the definition for the term confounding variable, many of them have trouble applying this concept and they struggle when asked to identifying confounding variables in experiments.

3. **Imagine you wanted to conduct this experiment under field conditions (outside in a soybean field). Describe how you would modify this experiment. State any new dependent variables you would measure and explain why you included these in your experiment. Describe two potential confounding variables that you would try to reduce and explain how you would do this.**

   This question asks students to think about posing a similar hypothesis in a “messier” setting. There are many possible answers to the question. Some students may describe the exact same type of feeding trial but with field grown plants. Other students may describe an experiment with field cages. Others may describe any experiment where plants are fertilized and naturally occurring herbivores are counted, or herbivore damage is measured. Ideally, this experiment would include plant biomass or yield as a dependent variable. It is much easier to control environmental conditions in the lab rather than in the field, so conditions such as soil moisture and timing (how old are the plants in the experiment) become more important.

4. **Did fertilizer application affect caterpillar performance? What does this mean for agriculture applications of fertilizer?**

   If fertilization improves plant nutritional quality (more nitrogen), then we would expect that the caterpillars fed leaves from the fertilizer treatment would have larger larval mass and shorter development times than caterpillars fed leaves from the control treatment. This means that fertilizing plants can improve plant yield while also increasing the performance of herbivores that attack plants.

   Below are results from Spring 2014 class data. Surprisingly, development time was shorter for the control treatment than for the fertilizer treatment for this class.

   Larval mass at day 10 was significantly higher for larva fed leaves from the fertilized treatments than control treatments (Figure 1: t_{39}= 3.365; p=0.002).

TIEE

Teaching Issues and Experiments in Ecology - Volume 11, March 2016

There was no differences in larval mass between the two treatments on day 17 (Figure 2: t_{25}= 0.668; p=0.510). There was no differences in pupal mass between the fertilizer and control treatments (Figure 3: t_{26}= -1.036; p=0.3096). Development time was significantly shorter for the control group than the fertilizer group- (Figure 4: t_{26}=2.313; p=.0289).

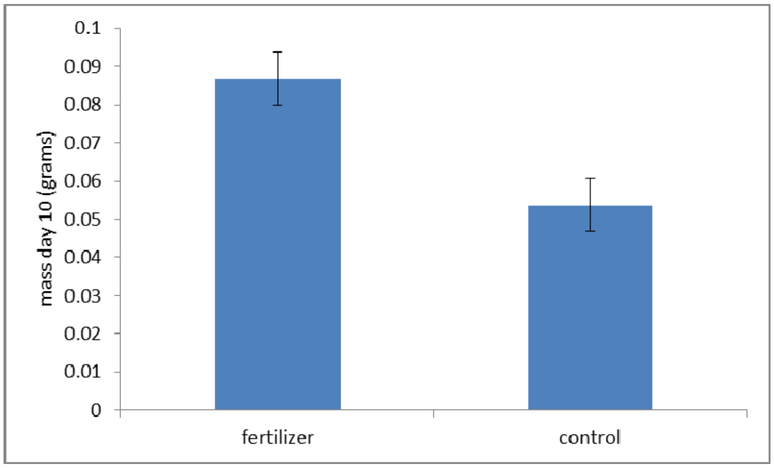

Figure 1: Larval mass (day 10) of *Spodoptera exigua* larva fed leaves from either fertilizer treated (n=20) or control (n=21) soybean, *Glycine max*, seedlings.

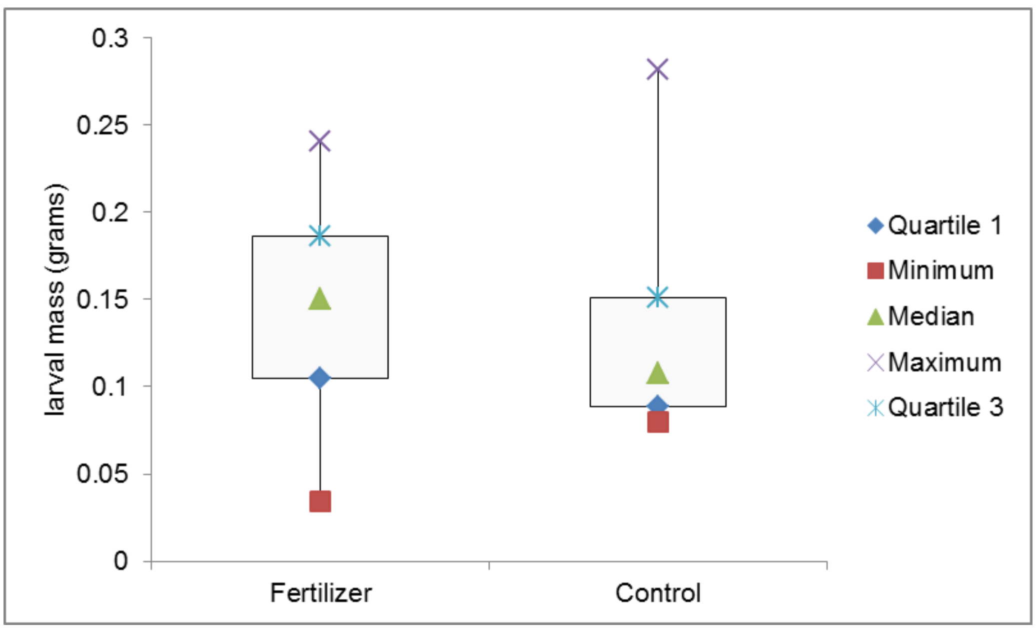

Figure 2: Larval mass (day 17) of *Spodoptera exigua* larva fed leaves from either fertilizer treated (n=15) or control (n=17) soybean, *Glycine max*, seedlings.

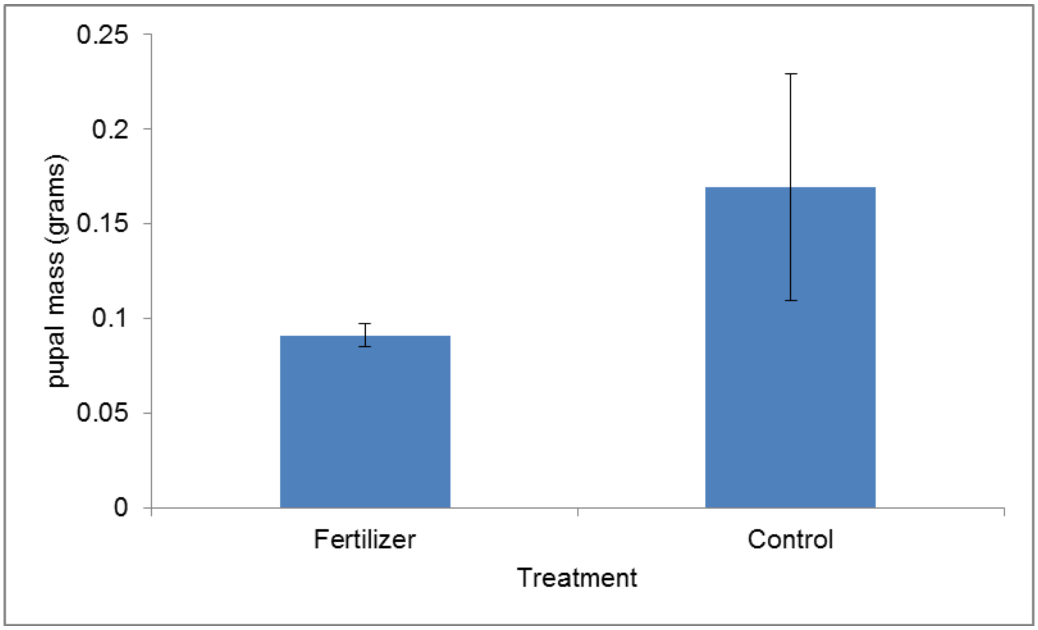

Figure 3: Pupal mass of *Spodoptera exigua* reared on soybeans treated with fertilizer (n=11) or water (n=17).

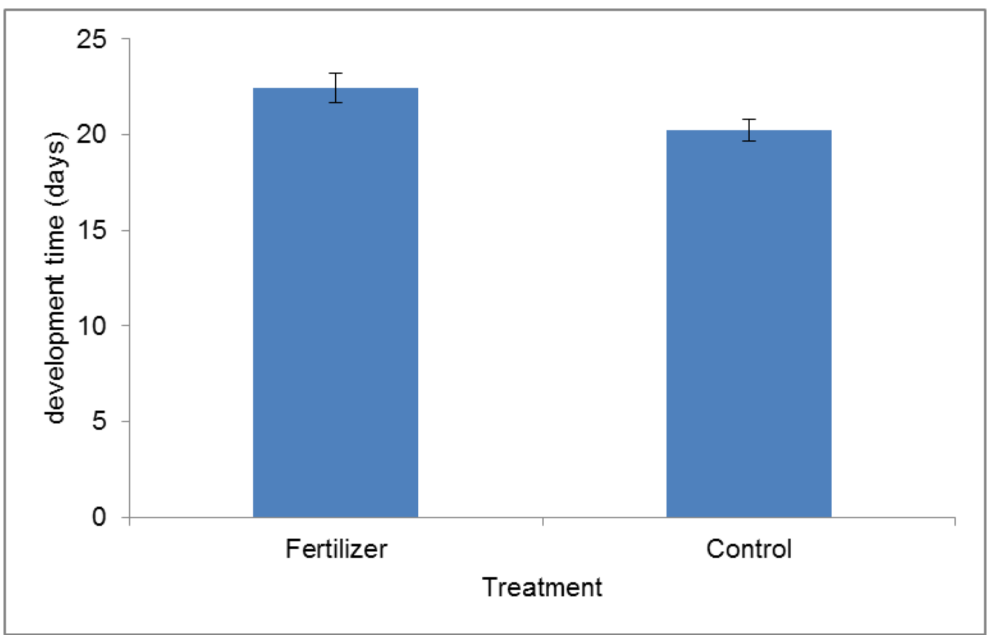

Figure 4: Development time of *Spodoptera exigua* reared on soybeans treated with fertilizer (n=11) or water (n=17).

5. **Have other researchers found similar results concerning the effect of fertilizer on herbivore characteristics?**

   Yes. There are many studies that have examined how fertilization changes plant-herbivore interactions. Mattson (1980) provides a classic review of this topic.

   Other examples include:
   Baylis, M., and N. E. Pierce. 1991. The effect of host-plant quality on the survival of larvae and oviposition by adults of an ant-tended lycaenid butterfly, *Jalmenus evagoras*." Ecological Entomology 16.1: 1-9.

   Chen, Y.-Z., L. Lin, C.-W. Wang, C.-C. Yeh, and S.-Y. Hwang. 2004. Response of two Pieris (Lepidoptera: Pieridae) species to fertilization of a host plant. Zoological Studies. 43:778-786.

   Floater, Graham. 1997. Rainfall, nitrogen and host plant condition: consequences for the processionary caterpillar, *Ochrogaster lunifer*. Ecological Entomology 22.3 (1997): 247-255.

   Mattson, W. J. 1980. Herbivory in relation to plant nitrogen-content. Annual Review of Ecology And Systematics 11:119-161.

   Wilcox, A., and M. J. Crawley. 1988. The effects of host plant defoliation and fertilizer application on larval growth and oviposition behavior in cinnabar moth. Oecologia 76: 283-287.

6. **Did fertilizer application affect all of the dependent variables in the same way? If there were differences, explain these differences.**

   This will vary. Typically pupal mass is more constrained than larval mass and development time. You can see a set of results in from one class (spring 2014) in the answer to question #4 above. Fertilizer application increased larval mass at day10. However, the fertilizer treatment had no effect on larval mass at day17 or on pupal mass. Surprisingly, development time was slightly shorter for the caterpillars fed leaves from the control treatment plants. A large number of caterpillars fed leaves from the fertilizer plants died. If the fertilizer-treated plants were able to increase their production of defensive chemicals, then this could explain the longer development time and lower survival in the fertilizer treatment. Don’t be surprised if your results vary (even from year to year).

TIEE

Teaching Issues and Experiments in Ecology - Volume 11, March 2016

Comments on the Assessment of Student Learning Outcomes:

It is common for students to write lab reports in their science classes, and the lab report format used for the summative assessment of this activity is a typical lab report format. The rubric included here is a very detailed rubric, and some instructors may prefer a more holistic rubric that does not drill down to as many specific points. There are many examples of these online.

- From Cornell College: http://www.cornellcollege.edu/library/faculty/focusing-on-assignments/tools-for-assessment/evaluation-of-lab-reports.shtml
- From North Carolina State University: https://www.ncsu.edu/labwrite/instructors/gradinglwr.htm
- From MIT: http://tll.mit.edu/help/grading-rubrics
- From the University of North Carolina Wilmingtom: http://uncw.edu/cas/samplerubrics.html

If this is the first time your students have individually written lab reports, then having them submit rough drafts and having the instructor comment on each rough draft is extremely important.

I have taught this lab annually for the past 6 years in two sections of Ecology each year, and I have learned that students learn a great deal from the peer review process. To facilitate discussion during the peer review process, I assign each student two reports to review. Another student in the class reviews the same two reports, so in class I pair up these students and they grade each report using the rubric. For many of my students, this is the first time that they have carefully read the rubric. It is a bit of a logistical hassle to arrange this, but I think the benefits to student learning outweigh the costs.

I also assess their knowledge of t-tests and box plots using the in-class quiz as well as using similar questions on exams.

For the initial experiment proposal, due on day 1, I usually evaluate this work based on completeness. Because this is due on the first day of class, I don't expect students to have a good understanding of experimental design and I think it would be unfair to "grade" their proposals for experimental rigor. However, at the end of class I include an experimental design question on the final that asks students to design an experiment to test a given hypothesis. I usually change the system slightly so that students cannot exactly copy what we did in lab. For example, in 2014 I asked students the following question on the final exam: You are a scientist working for the USDA studying cotton, an economically important crop in the southeast. You are comparing caterpillar performance on two cotton varieties (*Deltapine* and *Suregrow*) and an important cotton-herbivore, the cotton bollworm (the bollworm is a caterpillar that specializes on cotton). You hypothesize that caterpillar performance is going to be better on *Deltapine* than on *Suregrow*. Design a **lab** experiment to test this hypothesis. State the independent and dependent variables and describe your methods.

Comments on Formative Evaluation of this Experiment:

I regularly use the SALG (Student Assessment of their Learning Gains: http://www.salgsite.org/) to evaluate how much students report that they are learning from this lab activity. The SALG ask students to rate how well different aspects of the class affect their learning gains and also provides areas for written comments. I administer the SALG during the last week of class. I set up the SALG so that I can see which students have completed the survey, but I cannot link student names with their comments so their comments remain anonymous. I usually give students a few points for SALG completion.

Below are some of the relevant SALG results from my Spring 2013 Ecology course. A majority of the students reported that they made good or great gains in using statistics, using EXCEL, and scientific writing. These are all skills developed by this lab activity. Additionally, 56% of the students reported that the Herbivory Lab Report (my name for this activity in class) provided “much” or “great” help for their learning.

As a result of your work in this class, what GAINS DID YOU MAKE in the following SKILLS?	1:no gains	2:a little gain	3:moderate gain	4:good gain	5:great gain	9:not applicable
Using and interpreting statistics	2%	0%	15%	37%	26%	0%
Using EXCEL	0%	0%	13%	30%	35%	2%
Scientific writing	0%	4%	15%	35%	26%	0%

HOW MUCH did each of the following aspects of the class HELP YOUR LEARNING?	1:no help	2:a little help	3:moderate help	4:much help	5:great help	9:not applicable
Herbivory lab report	2%	7%	26%	24%	22%	0%

Additionally, midway through the semester I ask students to provide anonymous feedback. I distribute questionnaires with the following questions:

Please answer the following questions about our class. ***Do not write your name on this survey.*** *I will have the opportunity to see this feedback and make positive changes now rather than waiting until the end of the semester when you will complete another more in-depth survey. Thank you.*

1. What do you like about this class and the instruction that you are receiving?
2. What don't you like? What changes would you like to see made?
3. Are you learning the information that you were hoping you would learn? If not, what can we do differently?
4. Please write any additional comments on the back.

Comments on Translating the Activity to Other Institutional Scales or Locations:

1. translating this experiment to larger scales if you teach at a smaller school and vice versa.

   S. exigua is easy to rear and you can order batches of 1000 eggs, so it would not be a problem to have enough caterpillars for multiple course sections. The main difficulty would be training graduate teaching assistants in how to facilitate student learning in an guided-inquiry lab setting. However, more resources are becoming available as many large, research universities switch away from cookbook-style labs. The Association for Biology Laboratory Education (ABLE) is a good resource: http://www.ableweb.org/. Additionally, research has shown the teaching guided-inquiry labs also benefits the graduate teaching assistants (Hughes & Ellefson. 2013. Inquiry-based training improves teaching effectiveness of biology teaching assistants. PLOS One: 8 (10). e78540. doi:10.1371/journal.pone.0078540).

   At a larger school it would be important to have a laboratory coordinator or lead TA to help organize the experiment. Additionally, TAs would need to be trained and "calibrated" to fairly grade lab reports. I have found it helpful to lead group grading sessions where all the TAs grade 4-6 lab reports together and discuss how to grade them. This helps TA develop their grading and feedback skills, and prevents a situation where there is large grade variation among lab sections.

2. using this lab in different regions of the country or world, in different seasons, or using different study species or systems,

   Since this is a lab experiment, it can easily be translated to different regions or countries and it works during any season. I designed this indoor experiment because an outdoor lab during the first ½ of the spring semester was not a reliable option.

A variety of host plants can be used for this experiment since *S. exigua* are generalists and will consume many easy-to-grow plant species including: soybeans, cotton, collards, and lettuce. Plants can be grown from seeds or purchased directly from a garden supply store. Plants should be at least 1 month old at the start of the experiment.

Other phytophagous insects that are easily reared in the laboratory such as the tobacco hornworms (*Manduca sexta*) and the cabbage white butterfly (*Pieris rapae*), could be used. Besides fertilizer, other treatments could be used such as comparing different plant species, the presence or absence of a mutualism if legumes and rhizobia are used, or soil moisture levels.

3. using this activity to teach ecology to students with physical or other disabilities

   Assuming that any students with physical disabilities are able to access the laboratory, then they should be able to complete all of the lab activities. Some students may have difficulty weighing their caterpillar, and if this is the case then the instructor could do this for them. If students need to access their caterpillars outside of the classroom, then the instructor needs to make sure that the caterpillars are accessible to everyone or assign students partners to help with this work.

   Students with learning disabilities may need more time on the quiz or with the computer lab activity, as according to their accommodations.

4. using this activity to teach ecology in pre-college settings (K-12).

   As far as I know, this experiment has not been tried in pre-college settings. However, with the right teacher training and support, there is no reason why high school students could not complete this activity and it would be a interesting way to achieve both science and math learning standards in an interdisciplinary manner.

STUDENT COLLECTED DATA FROM THIS EXPERIMENT

I have included an EXCEL spreadsheet with student collected data from this experiment. This spreadsheet includes combined class data from two Ecology sections (n=41 students).

COPYRIGHT STATEMENT

Lastly, we request that you return your students' and your comments on this activity to the TIEE Managing Editor (tieesubmissions@esa.org) for posting at this site.

GENERIC DISCLAIMER

Adult supervision is recommended when performing this lab activity. We also recommend that common sense and proper safety precautions be followed by all participants. No responsibility is implied or taken by the contributing author, the editors of this Volume, nor anyone associated with maintaining the TIEE web site, nor by their academic employers, nor by the Ecological Society of America for anyone who sustains injuries as a result of using the materials or ideas, or performing the procedures put forth at the TIEE web site, or in any printed materials that derive therefrom.