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EXPERIMENTS

How do pawpaws affect tree regeneration?

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ABSTRACT
In this study, students investigate whether and how much the native understory shrub pawpaw (Asimina triloba) may affect tree regeneration in a forested habitat. During one lab period in the field, they collect data on the densities of tree seedlings and saplings under varying densities of pawpaws. During a second lab period in a classroom, they test whether tree densities are lower under pawpaws, and whether plots with higher densities of pawpaws have lower densities of trees. Students then report their findings in a brief lab report. This project introduces students to key components of ecological research including transect and plot sampling, paired designs, statistical analysis and scientific writing.

KEYWORD DESCRIPTORS
- **Ecological Topic Keywords:** Community ecology, competition, conservation biology, deer impacts, forest ecology, invasive species, multispecies interactions, plant ecology, seedling growth, species interactions

- **Science Methodological Skills Keywords:** collecting and presenting data, correlation versus causation, data analysis, experimental design, field work, quantitative sampling, scientific writing, statistics

- **Pedagogical Methods Keywords:** guided inquiry, project-based teaching, student-active teaching

CLASS TIME
Two 3–4-hour lab periods.

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OUTSIDE OF CLASS TIME

Students enter data and write a lab report.

STUDENT PRODUCTS

A brief (2-3 page) lab report—i.e., a miniature scientific paper without references.

SETTING

In the first lab period, students collect data in the field at a forested site where a focal species (e.g. pawpaw) has the potential to affect tree regeneration. This works best during the growing season. In the second lab period, in a classroom where students can bring or use computers, they analyze data and prepare to write a lab report.

COURSE CONTEXT

This activity is designed for use near the beginning of a general ecology course, in an introductory course covering ecology, or in a field ecology or natural history course that may be open to nonmajors. It is intended for students with little to no background in ecology, statistics or scientific writing. Class size should be conducive to field work (10–25).

INSTITUTION

Private, small liberal arts college.

TRANSFERABILITY

This activity could be used at any institution with lab sections small enough for field work (10–25). It is suitable for biology majors in early stages of their college careers, nonmajors, and high school students. For more advanced courses, complexity could be increased. This lab could easily be adapted to any landscape with forests where an understory species— any native or invasive tree, shrub or herb—has the potential to affect tree regeneration. The native understory shrub pawpaw is common in moist forests across much of central eastern North America (range map: Flora of North America 2014).

ACKNOWLEDGEMENTS

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SYNOPSIS OF THE EXPERIMENT

Principal Ecological Question Addressed

How do pawpaws affect tree regeneration?

What Happens

In this study, students investigate whether and how much the native understory shrub pawpaw (*Asimina triloba*) affects tree regeneration in a forested habitat. During one lab period in the field, they collect data on the densities of tree seedlings and saplings under varying densities of pawpaws. They may enter the data outside of class. During a second lab period in a classroom, they learn fundamental principles of statistics and practice them by testing whether tree densities are lower under pawpaws, and whether plots with higher densities of pawpaws have lower densities of trees. They use descriptive statistics, bar charts, histograms, paired t-tests, scatterplots and correlations. After discussing a guide to writing lab reports, students participate in a "writing workshop" in which they read examples of student lab reports and discuss how to improve them. Students then report their findings in a brief lab report.

Experiment Objectives

1. Begin to understand species interactions and community structure
2. Explore a local forested habitat and learn to identify a key plant species
3. Gain familiarity with transect and plot sampling and paired designs
4. Practice and report graphs and statistical analyses such as descriptive statistics, bar charts, histograms, paired t-tests, scatterplots and correlations
5. Improve scientific writing skills

Equipment/ Logistics Required

- Meter tapes (e.g. 50-m, one for each team of 2–4 students)
- Pins to anchor the meter tapes (one for each team of 2–4 students)
- Compasses (one for each team of 2–4 students)
- Meter sticks (three for each team of 2–4 students)
- Clipboards (one for each team of 2–4 students)
- Datasheets (one for each team of 2–4 students; Pawpaw datasheet.pdf)
- Any available statistical software

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Summary of What is Due

Participation; entered data; graphs and statistical analyses (Pawpaw stats.pdf); a brief (2-3 page) lab report—i.e., a miniature scientific paper without references.

<u>DETAILED DESCRIPTION OF THE EXPERIMENT</u>

Introduction

Forests maintain themselves through regeneration—the germination, establishment and growth of tree seedlings and saplings. To reach the canopy, trees must successfully compete with other plants for light, water and soil nutrients. Many factors can influence these competitive interactions. For example, when a mature tree dies and falls over, it creates a canopy gap where the seedlings and saplings below suddenly receive more light and may be able to increase their growth rates. In some forests, small trees, shrubs or herbaceous plants form a dense understory layer that can also influence canopy tree regeneration (Royo and Carson 2006). If the understory layer becomes dense enough, it can prevent many trees from reaching the canopy. Conditions under the understory layer could also favor particular species of trees. In these ways, understory layers can change the structure, diversity and composition of the forest. They can also become an obstacle to the production of valuable timber.

Some invasive shrubs such as buckthorn (*Rhamnus frangula*) and honeysuckle (*Lonicera maackii*) have caused problems in forests by interfering with tree regeneration (Gorchov and Trisel 2003, Fagan and Peart 2003). Native species such as hayscented fern (*Dennstaedtia punctilobula*) can have similar effects (George and Bazzaz 1999, De la Cretaz and Kelty 2002). One native shrub that has the potential to affect tree regeneration is the pawpaw, *Asimina triloba* (Baumer and Runkle 2010). Common in moist forests across much of central eastern North America, this plant spreads clonally and can grow up to about 10 m tall. Pawpaw belongs to the plant family Annonaceae, a large, mostly tropical family that includes custard apple, cherimoya and ylang-ylang. In May, the maroon flowers produce a fetid or yeasty scent that attracts flies and beetles (Willson and Schemske 1980). Flowers are perfect—with both male and female parts— and protogynous—first female, then male (Willson and Schemske 1980). The fruits are delicious—many liken the taste to bananas. Supposedly, the pawpaw is the largest edible fruit native to the United States. Pawpaw leaves have a lot of secondary compounds that people have tried to use as pesticides or medicines. Deer and many insects find pawpaw relatively unpalatable (Asnani et al. 2006, McGarvey et al. 2013), but larvae of the zebra swallowtail butterfly feed exclusively on young *Asimina* leaves; chemicals in the leaves make the butterflies unpalatable to predators (Martin et al. 1999)

Some have suggested that native shrub understories in eastern North American forests have increased over the past century due to logging, chestnut blight, fire suppression and deer overpopulation (Baumer and Runkle 2010). In the case of pawpaw, the hypothesis that pawpaw patches have expanded due to deer overpopulation makes sense since deer tend not to eat it. Does this (potentially increasing) pawpaw understory affect tree regeneration, and if so, how much? We will conduct a study to find out, by collecting data on the densities of tree seedlings and saplings under varying densities of pawpaws and testing whether tree densities are lower under pawpaws, and whether plots with higher densities of pawpaws have lower densities of trees.

Materials and Methods

Study Site(s): We will conduct this study in a mature oak-hickory stand in Susquehannock State Park in southeastern Pennsylvania. This park consists of a forested plateau overlooking the Susquehanna River. The stand we will sample has impressive, mature trees and a diversity of tree species that is broadly representative of the historical vegetation of our region (Keever 1973). This stand also has a very healthy population of pawpaws. In fact, Lancaster County has the largest pawpaw patch north of the 39th parallel (the latitude of Washington, DC and Cincinnati, Ohio), and this one is similar. To my knowledge, no one has been measuring whether the pawpaw population has been increasing. However, this area has likely been affected by factors thought to lead to denser shrub understories, including logging, chestnut blight, fire suppression and deer overpopulation.

Overview of Data Collection and Analysis Methods: During one lab period in the field, we will collect data on the densities of tree seedlings and saplings under varying densities of pawpaws (Pawpaw protocol.pdf). One way to do this would be to sample a number of randomly located plots with pawpaws and a number of randomly located plots without pawpaws, and to compare the numbers of trees in each group. Imagine, however, that our study site had a slope, and that both pawpaws and tree seedlings were denser toward the bottom of the slope. Randomly located plots with pawpaws would tend to occur toward the bottom of the slope, and they would tend to have more trees, even if tree densities were not affected by pawpaws, but by the slope. In this case, it would be better to compare plots with pawpaws to plots without pawpaws at the same slope position. This would hold slope position constant while varying only the presence or absence of pawpaws, so that any difference between plots with pawpaws and plots without pawpaws would be more likely to be due to the pawpaws themselves. For this reason, we will use a paired design, in which we compare

plots with pawpaws to adjacent plots without pawpaws. The goal of this design is to control for any other factors besides pawpaws that could influence tree densities.

Our study site does indeed have a slope. Upon arriving, we will form groups of 2–4 students, and each group will start at the top of the slope, 20 m away from the next group. Each group will then establish a transect perpendicular to the slope (i.e. downhill—why is this a good idea?) and sample plots according to the instructions in the protocol (Pawpaw protocol.pdf). Use a compass to maintain a straight transect. Each group should try to sample about 25–30 pairs of plots. Before the next lab period, you will enter your data into a Google spreadsheet.

During a second lab period in a classroom, we will test whether tree densities are lower under pawpaws, and whether plots with higher densities of pawpaws have lower densities of trees (Pawpaw stats.pdf). You will then report your findings in a brief lab report (Writing lab reports.pdf).

Questions for Further Thought and Discussion:

Note: These discussion questions are intended for use after students obtain their results. They are thus based on the results my students obtained, in which plots without pawpaws had tree densities about twice as high as plots with pawpaws, but in plots with pawpaws, tree density did not vary with pawpaw density (see results in **Student Collected Data from this Experiment**). The questions can be adapted as suggested to the results your students obtain.

1. Explain all the possible mechanisms you can think of that might cause tree density to be lower (similar, higher) under pawpaws.

2. Which mechanism do you think is most likely to be operating and why?

3. How would you test this hypothesis?

4. Besides pawpaws, what other factors influence the distribution of tree seedlings and saplings? Do you think the paired design adequately controlled for these factors?

5. How could tree density be lower under pawpaws, and yet not vary with pawpaw density (or how could other apparently counterintuitive results have occurred)?

6. If tree density is lower (similar, higher) under pawpaws, how might this affect the future structure, diversity and composition of the forest?

7. Propose a manipulative experiment that would build on your findings.

8. Propose one short-term (one afternoon to several weeks) and one long-term (Ph.D. to career-long) observational study that would build on your findings.

9. Find another study of whether an understory species interfered with tree regeneration. How did its design and findings compare to ours? Why do you think the findings were similar or different?

10. Do you think dense understories influence all tree species equally? Find and present evidence that some species are influenced more than others, and find and present evidence that shows WHY some species might be influenced more than others.

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Tools for Assessment of Student Learning Outcomes:

Pawpaw protocol.pdf
Pawpaw stats.pdf

Writing lab reports.pdf

NOTES TO FACULTY

Challenges to Anticipate and Solve

This activity is very straightforward, and I have not encountered significant challenges in the field portion. However, the data analysis and lab report still present challenges to students with no background in statistics or scientific writing.

Challenge #1. Students vary in their statistical preparation and facility with statistical software. Some students speed through the analyses, while others need a lot of help. I address this challenge by providing a detailed, step-by-step handout that students can work through at their own pace (Pawpaw stats.pdf).

Challenge #2. Scientific writing. For some students, this will be their first lab report in full, scientific-paper format, and many more experienced students still struggle with scientific writing. I address this challenge by providing a detailed handout on how to write a lab report (Writing lab reports.pdf; I expect every instructor has a similar handout specific to their goals and preferences) and by referring students to the scientific writing guides in Anderson (2012) and Karban et al. (2014). I also devote the latter part of the second lab period to a "writing workshop" in which we read examples of student lab reports and discuss how to improve them. Many student papers that serve this purpose well can be found in Juniata College's Journal of Ecological Research (http://departments.juniata.edu/biology/eco/jer.htm). I find that if you present students with "model" examples, they may imitate them to an undesirable degree, but if you present students with examples that could use some improvement and discuss how to improve them, then students learn how to improve their own papers.

Comments on Introducing the Experiment to Your Students:

Upon arriving at the site, I introduce students to the forest community and the focal species. I describe the natural history of pawpaw, including its clonal growth form, edaphic associations, ecological roles in forest communities, affinity with tropical plants, flowering and fruiting phenology, pollination biology, and

edibility—students will then enthusiastically search for fruits during the sampling (see details in **Introduction**). Students can practice identifying the species on the hike to the sampling location, and they typically have no trouble.

I find the ecological question simple and intuitive enough that a theoretical background in species interactions is not necessary. I simply discuss the ecological and practical implications of interference with tree regeneration (see details in **Introduction**). Then I provide the sampling protocol (Pawpaw protocol.pdf), or provide only the question and let students develop a sampling protocol.

Comments on the Data Collection and Analysis Methods:

This activity is extremely simple and easy to adapt. I have kept it as simple as possible because I have used it as a first or early experience with quantitative sampling, statistics and scientific writing. Its complexity could easily be increased for more advanced courses. Students could participate in developing the sampling protocol. They could design and/or conduct follow-up studies. For example, they could compare light or soil moisture levels between plots with and without pawpaw. They could compare the height and age of tree seedlings and/or estimate their biomass.

One important issue to bring up during the data analysis is correlation versus causation. If tree density is lower under pawpaws, does this mean that the pawpaws led to the lower tree densities? This discussion is likely to grow naturally out of discussion questions 1–4. Another potential issue of interest to more advanced students is the independence of the plot data. Data from plots along transects are commonly analyzed as if the plots were independent, as in the correlation analysis presented here. However, you may want to have students discuss whether this analysis is in fact appropriate. This could lead to a productive discussion of sampling bias and statistical assumptions. For introductory courses, you may simply want to omit the correlation analysis.

In the lab report, I prefer to challenge students to use their own minds before adding the skill of integrating the research of others, but for more advanced students, the lab report could be expanded to include literature review.

Comments on Questions for Further Thought:

Questions 1–8 could guide a discussion during the second lab period, serve as brief in-class or outside writing assignments, or help structure the Discussion section of the lab report. Questions 9–10 ask students to engage with the

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literature and could form separate in-class or outside presentation or writing assignments.

Note: These discussion questions are intended for use after students obtain their results. They are thus based on the results my students obtained, in which plots without pawpaws had tree densities about twice as high as plots with pawpaws, but in plots with pawpaws, tree density did not vary with pawpaw density (see results in **Student Collected Data from this Experiment**). The questions can be adapted as suggested to the results your students obtain.

1. Explain all the possible mechanisms you can think of that might cause tree density to be lower (similar, higher) under pawpaws.

Students should propose the mechanisms of competition for light, water and soil nutrients.

2. Which mechanism do you think is most likely to be operating and why?

This question gets students to think critically about their hypotheses. It doesn't matter which mechanism they choose.

3. How would you test this hypothesis?

You should encourage students to think of both experimental and observational studies to test their hypotheses. For example, to test the hypothesis that competition for light causes tree density to be lower under pawpaws, you would probably want to measure light levels at different heights in plots with and without pawpaws, and then create different light levels with shade cloth and follow tree seedling and sapling performance.

4. Besides pawpaws, what other factors influence the distribution of tree seedlings and saplings? Do you think the paired design adequately controlled for these factors?

Many other factors could influence the distribution of tree seedlings and saplings, including ecological interactions with soils, canopy gaps, deer herbivory, fires or other disturbances, or insect or disease outbreaks. The point is to brainstorm. As designed, the paired sampling accounted for any factors associated with the slope. Since we don't know much about the spatial and temporal distributions of these other factors, we don't really know if the paired design adequately controlled for them. However, students should recognize that it's a good start,

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and perhaps the best we can do until we learn more about which factors are most important and how they vary in space and time.

5. How could tree density be lower under pawpaws, and yet not vary with pawpaw density (or how could other apparently counterintuitive results have occurred)?

If this result occurs, it can be counterintuitive for students. They should suggest that maybe there is some threshold pawpaw density above which greater densities of pawpaws have little additional effect.

6. If tree density is lower (similar, higher) under pawpaws, how might this affect the future structure, diversity and composition of the forest?

Students should recognize the extreme possibility that pawpaws could take over and the forest could convert into a pawpaw thicket or shrubland, and the less extreme possibility that stem density or basal area may decrease. They may also suggest that the pawpaw understory could favor particular species of trees, shifting species composition toward more shade-tolerant species, for example. If the pawpaw understory excludes only some tree species, this could reduce diversity.

7. Propose a manipulative experiment that would build on your findings.

Students may propose some of the same experiments they talked about for Question 3 or get more creative. They may want to do competition experiments in which they remove pawpaws or plant different densities of pawpaws and tree seedlings together and separately. I would press students a little on the design of these experiments—how exactly they would set them up, what they would measure? What are the pros and cons of field versus pot experiments? etc.

8. Propose one short-term (one afternoon to several weeks) and one long-term (Ph.D. to career-long) observational study that would build on your findings.

Again, students may propose studies of mechanisms as in Question 3. Ph.D. or career-long studies would allow them to get a sense of how pawpaw and tree densities are changing over time. Why would this be valuable? etc.

9. Find another study of whether an understory species interfered with tree regeneration. How did its design and findings compare to ours? Why do you think the findings were similar or different?

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Part of the point of this question is to have students realize that researchers are actually doing and publishing studies not that different from their own, that forest regeneration is a larger concern, and that other understory species might have similar effects. It might inspire students to think about how lines sometimes blur between "invasive" and aggressive native species. This question also asks students to compare and critically evaluate different sampling designs and results.

10. Do you think dense understories influence all tree species equally? Find and present evidence that some species are influenced more than others, and find and present evidence that shows WHY some species might be influenced more than others.

Obviously, no. If students don't easily find papers about the effects of dense understories specifically, they could expand their search to include different canopy conditions in general. The second part of the question should introduce students to the long-established and extensive literature on shade tolerance, etc.

Comments on the Assessment of Student Learning Outcomes:

In the field, I circulate to check that students are following the protocol and collecting data correctly. During data analysis, I have a lot of interaction with students as they progress independently, so that I ensure that every student has correctly completed, understood and reported the analyses as per the handout (Pawpaw stats.pdf). Because practicing scientific writing is a principal goal, formal assessment is based on a written lab report, which instructors should adapt for their specific goals and preferences. If feasible, instructors can make substantial learning gains by allowing students to revise the lab report based on their comments. A peer review process could also be implemented.

Comments on Formative Evaluation of this Experiment:

Student-written lab reports provide a very sensitive and informative gauge of how well students grasped the ecological question and the principles of statistics and scientific writing. I use them to continually improve my introduction to the lab and my guides to the statistical tests (Pawpaw stats.pdf) and to writing lab reports (Writing lab reports.pdf).

When I used this lab near the beginning of a general ecology course, thirteen of fourteen students made positive comments about the field projects on course evaluations, and eleven of fourteen students made positive comments

about how the writing assignments helped them improve their writing and thinking skills.

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

This activity could be used at any institution with lab sections small enough for field work (10–25). It is suitable for biology majors in early stages of their college careers, nonmajors, and high school students. Less advanced students could focus on graphing rather than statistics. For more advanced courses, complexity could be increased (see suggestions in **Comments on the Data Collection and Analysis Methods Used in the Experiment**).

This activity could easily be adapted to any landscape with forests where an understory species— any native or invasive tree, shrub or herb—has the potential to affect tree regeneration. The native understory shrub pawpaw is common in moist forests across much of central eastern North America (range map: Flora of North America 2014). Besides pawpaw, good candidates include invasive trees such as *Acer platanoides* and *Ailanthus altissima*, invasive shrubs such as *Berberis thunbergii, Lonicera maackii, Rhamnus cathartica* and *Rosa multiflora*, other native shrubs such as *Kalmia latifolia* and *Rhododendron maximum*, and native ferns such as *Dennstaedtia punctilobula*. This activity works best if the focal species is abundant but patchily distributed—not a monospecific thicket—so that plots without it can be easily located as well.

For five groups of 2–4 students to sample transects 20 m apart from each other, you need at least a hectare. In my experience, student transects that start only 10 m apart tend to intersect by about 100 m.

STUDENT COLLECTED DATA FROM THIS EXPERIMENT

Pawpaw data.xlsx

In these data, plots without pawpaws had tree densities about twice as high as plots with pawpaws (paired t-test: t = -6.019, df = 133, P < 0.001), but in plots with pawpaws, tree density did not vary with pawpaw density (Pearson correlation coefficient = -0.018, P = 0.834, N = 134).
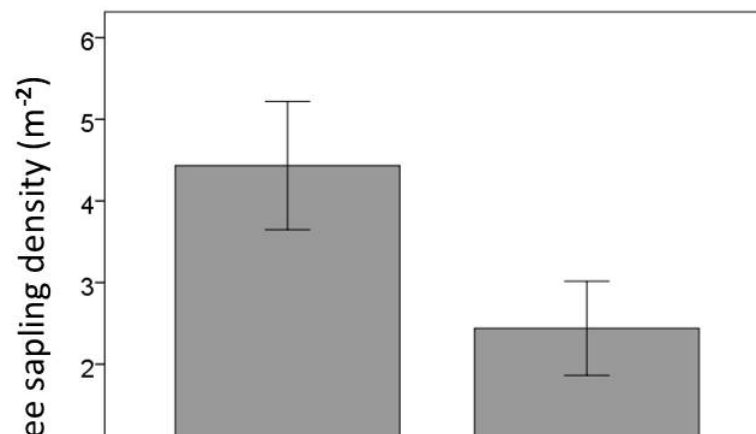
Figure 1. Tree sapling density (mean ± se) in 134 pairs of 1-m2 plots with and without pawpaws (Asimina triloba) in a mature oak-hickory stand in Susquehannock State Park in southeastern Pennsylvania.

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