TIEE

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EXPERIMENTS

Testing Hypotheses on Plant-Herbivore Interactions Using Sawfly Galls on Willows

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Sawfly galls (*Pontania* sp.) as they appear on the under surface of willow (*Salix lasiandra*) leaves in central Washington. Photo by K. Ernest.

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ABSTRACT

Due to differences in genetic makeup and exposure to environmental factors (such as soil moisture and nutrient levels and exposure to plant pathogens and herbivores), plants vary in their chemical and physical traits. This can cause differences in susceptibility to herbivory or differences in nutritional quality that attract herbivores. Therefore, one might expect to find differences among plants in the number of herbivores that feed on them, the ways herbivores select feeding and oviposition sites, and the success of these herbivores. In this observational experiment, students will conduct investigations of sawfly galls (Hymenoptera) on willow (*Salix*) trees to examine some of the ecological and possible evolutionary consequences of plant-herbivore relationships to each of the interacting species. Galls make great sampling units for investigating herbivory because they are discrete (each gall contains one herbivorous larva), quantifiable (easy to see and count), and indicative of insect preference (since each gall represents one successful oviposition by a female sawfly) and performance (successful larval development is indicated by an emergence hole). During a single lab period, students will become familiar with the plant-herbivore system and work in teams to collect data to test a general hypothesis proposed by the instructor. Teams then choose a second instructor-generated hypothesis or develop their own to test. Examples of these are (1) The level of herbivory by sawflies varies among willow trees, and (2) Galls on leaves with competing galls are less successful than single galls on leaves. Outside of lab time, students will analyze their data statistically, and prepare a formal oral report on their investigation.

KEYWORD DESCRIPTORS

- **Principal Ecological Question Addressed:** What are the ecological and possible evolutionary consequences of this plant-herbivore relationship to each of the organisms involved? In particular: does herbivory vary among individual plants and among leaves within plants? do oviposition choices affect larval success?
- **Ecological Topic Keywords:** herbivory, galls, plant-animal interactions, parasite-host interactions, willow, *Salix*, sawfly, *Pontania*, hypothesis testing, statistical tests, field studies
- **Science Methodological Skills Developed:** field observation skills, hypothesis testing, random sampling, sample size, statistical tests, graphing data, oral presentation
- **Pedagogical Methods Used:** guided inquiry, group data collection, cooperative learning, peer evaluation

CLASS TIME

One 3-hour lab period, plus approximately an hour of another lab or lecture period for oral reports. Additional lab time for data entry into a spreadsheet (requires computer access), and statistical analysis.

OUTSIDE OF CLASS TIME

Two to three hours for statistical analysis and preparation of oral report.

STUDENT PRODUCTS

1. data set for hypothesis 1,
2. data set for additional hypothesis,
3. formal oral report based on investigation,
4. peer evaluation of other oral reports.

SETTING

The field work is conducted at any site with several willow trees that have galls on the leaves. This investigation works only in the fall, when galls are fully developed and easily visible on the leaves.

COURSE CONTEXT

I use this lab activity in a junior-level general ecology course for all biology majors. I have 20-24 students per lab section.

INSTITUTION

Public, primarily undergraduate university of 8500 students, with a small master's program.

TRANSFERABILITY

This lab should be transferable to other types of institutions. It may be used for sophomore to senior levels, primarily for biology majors. Both plant and herbivore have broad distributions and are speciose. More than 200 species of sawflies form galls on willow (*Salix*) species (Nyman et al. 1999). *Salix* occurs in every state of the U.S. (see USDA map for *Salix*: http://plants.usda.gov/cgi_bin/topics.cgi?earl=plant_profile.cgi&symbol=SALIX), and *Pontania* sawflies have a broad distribution in North America as well as Europe and Asia. However, *Pontania* galls may not be present or common at all sites where willows grow. *Euura* is another sawfly genus that forms galls on willow, but on petioles or stems rather than leaves. The hypotheses could be adjusted to address the distribution of galls on stems or branches. Alternate host plant-herbivore systems are available in most sites, but it may take a bit of searching to find an appropriate system. Consider galls on maple (*Acer*) leaves (commonly caused by eriophyid mites), goldenrod (*Solidago*) stems (caused by *Epiblema* caterpillars or the tephritid fruit fly, *Eurosta*) or leaves (*Asteromyia* gall midges), oak (*Quercus*) stems and leaves (primarily caused by cynipid wasps), hackberry (*Celtis*) leaves (by jumping plant lice, psyllids), or creosotebush (*Larrea*) stems and buds (20+ species of the cecidomyid genus *Asphondylia*)..

SYNOPSIS OF THE EXPERIMENT

What Happens

Students observe galls on willow leaves, and begin their investigations in teams by collecting data to test the instructor-posed hypothesis that the number of galls per leaf varies among willow trees. This step gives students more guidance, allows them to practice sampling on a question they will discuss in class but won't include in their graded assignment, and gives them a chance to view a number of galls and leaves to get a better sense of the study system and the typical pattern that herbivory varies among leaves and among plants (due to differences in plant chemistry, physical traits, environmental traits, etc.). Student teams then choose among several instructor-directed questions (such as whether female sawflies oviposit independently of other oviposition events, whether galls on leaves with other galls are less successful than single galls on leaves, and whether leaf-chewing herbivores select leaves independently of galls), or pose their own hypothesis. Instructors may assign these randomly to ensure that each hypothesis is tested by at least one team, or briefly discuss why each hypothesis might be interesting to test. More motivated teams might be challenged to formulate their own question based on their preliminary observations at the site. For example, they may notice that trees vary in size/age, or in distance to surface water, or that not all galls are the same size. Instructors can capitalize on these observations by encouraging students to ask how these variations might influence gall distribution or success. Once teams select a hypothesis, they then collect data to test the hypothesis, analyze their data, and prepare a formal oral report on their investigation.

Lab Objectives

At the conclusion of this lab, students will be able to:

- discuss in what ways and why herbivory varies among plants and among units (e.g., leaves) within plants,
- articulate several ecological and possible evolutionary consequences of this plant-herbivore relationship to each of the organisms involved,
- recognize sawfly galls on willows,
- use common statistical tests to analyze data on the distribution of galls on leaves,
- work collaboratively to collect and analyze data, find appropriate scientific literature, and organize a formal oral report using PowerPoint.

Equipment/Logistics Required

Equipment:

- data sheets
- clipboards
- random number table
- pocket knives to cut open galls
- hand lens or portable dissecting scope to view sawfly larvae
- digital camera (optional, but nice) to take photos for oral reports

Logistic Requirements:

- finding a site with willow trees that have sawfly galls, where leaves are easily accessible to students
- arranging transportation, if necessary

Summary of What is Due

1. Proposal — Student groups are assessed on either an oral or written presentation of their hypothesis and investigative design.
2. Oral presentation — Each group is evaluated on the analysis and interpretation of data as presented to the class in a PowerPoint format.
3. Paper(s) — The results of each group's study is assessed based on one or two papers (ranging between 5-10 pages in total length), including figures, tables, and bibliography. Papers are formatted following standard journal style.

DESCRIPTION OF THE EXPERIMENT

Introduction (written for students)

The interactions between herbivores and their host plants are often complex, involving plant chemical and physical defenses, herbivore foraging behaviors, and many other factors. Most plants are attacked by several to many different types of herbivores. Each herbivore may feed in a different manner or on different plant tissues, causing different types of feeding damage. One of the more unique plant-herbivore interactions is the formation of galls. Galls are modified plant tissue stimulated by the oviposition and feeding activities of certain insects and spider mites. They result when the cells around the damaged area grow larger or divide more often than normal cells. As the insect feeds on the plant, it becomes surrounded by this abnormal plant growth. The insect continues to feed from within the gall, which protects it from many (but not all!) of its natural enemies. Other organisms, including viruses, bacteria, fungi, nematodes, and mites, may induce plant galls, but insects are the most common gall formers.

Galls can be used to test a number of interesting ecological and evolutionary questions about plant-herbivore interactions. The hypothesis that host plant quality affects herbivore densities and community structure was tested by Fritz *et al*. (1987b). As predicted, both densities of individual sawfly species and the relative abundances of these species varied among clones of arroyo willow. Additional data showed that shoot size is an important plant trait affecting gall densities: larger shoots have higher sawfly densities (Fritz *et al*. 1987a.) Since galls act as nutrient sinks (Nakamura, et al. 2003, Price *et al*. 1987), larger galls should provide more nutrients and therefore increase the success rate of the galling insect. Investigating the relationship between gall size and gall success (*e.g.*, percent emergence) would provide a test of the generally supported hypothesis that plant galls are adaptive for the galling insect. The mechanisms through which habitat affects the density of galling insects were investigated by Fernandes and Price (1992). Lower rates of parasitism and fungal attack of galls may be at least partly responsible for higher gall densities in xeric (dry) environments compared with mesic (moist) habitats.

Willow trees (genus *Salix*) are attacked by several gall-forming herbivores. Gall midges form galls on buds, and sawflies form galls on leaves and shoots. Studies for this lab will be conducted at Engelhorn Pond on the Central Washington University campus where many of the willows have leaves with elongate, reddish capsules emerging from the leaf surface (gall – upper surface [left] and lower surface [right] – photos © K.A. Ernest).

These galls are caused by sawflies of the genus *Pontania*. Sawflies are not actually flies but relatives of bees and wasps (Order Hymenoptera). Adult females oviposit (lay eggs) into the leaf tissue. The egg hatches into a larva, which feeds on the leaf tissue while enclosed in the gall. When the larva has completed its development, it chews a in the gall and departs. See weblink in References to "Forest and Timber Insects in New Zealand" for pictures of egg, larva, pupa, and adult *Pontania*.

Willows are also eaten by a variety of free-feeding invertebrate herbivores. Lace bugs suck sap from leaves, spider mites chew leaves, and flea weevils chew on leaves and new shoots. You may find other insects feeding on the willows at the study site.

During this lab, you will (collectively) test a number of hypotheses about the gall-forming sawflies on willows. Particular questions chosen by student groups, in consultation with the instructor, may include:

- Do some trees have more galls than others?
- Do female sawflies avoid ovipositing on leaves that already have galls?
- Are galls on leaves with several other galls less successful that single galls?
- Do chewing herbivores avoid leaves with galls?

In the process, perhaps you will become expert cecidologists (students of plant galls)!

Materials and Methods (written for faculty)

Study Site(s):

We are fortunate in being able to walk to our study site, a very small reserve (<1 ha) across the street from the Biology Building at the western edge of Central Washington University's campus. Engelhorn Pond formed as a borrow pit in the 1920's when gravel was excavated for use in the construction of the Interstate 90. The pit filled with water from runoff and groundwater and vegetation (including willows) colonized the site. Although very small, and nearly surrounded by university buildings, this site offers an urban refuge for ducks and other wildlife. As a wetland it garnered the attention of The Nature Conservancy, which purchased the site and donated it to the Biology Department. The pond is the dominant feature of the site and is surrounded by willow trees (Pacific Willow, *Salix lasiandra*). Gall densities vary from year to year, but usually galls are fairly abundant on the leaves. Numerous other sites are possible inside the city limits where willows grow along streams and irrigation canals. At your location, any site where willows grow and you can easily find galls would be appropriate. You might also consider sites with other plant species that harbor galls (such as poplars, goldenrod, maple trees).

Overview of Data Collection and Analysis Methods:

Introduction to the Study System

When we first get to the field site, I show students galls on the willows. Have a few students carefully open the galls (with a pocket knife or thumbnails) to find a sawfly larva. You could collect a few larvae in advance of the lab, and let students view them under a microscope during the lab introduction, or take a portable dissecting scope or simple hand lens with you to the field.

Hypothesis 1: all teams

- *Background:* Plants vary in their chemical and physical traits as a result of differences in genetic makeup as well as varying environmental factors such as soil moisture and nutrient levels, amount of sunshine, ambient temperature, and exposure to plant pathogens and herbivores. Therefore, one might expect to find differences among plants in the number of herbivores that feed on them (either because of differences in susceptibility to herbivory or differences in nutritional quality that attracts herbivores).
- *Hypothesis:* Level of herbivory by sawflies varies among willow trees (i.e., some trees have more galls than others).
- *Method:* Randomly sample 20 leaves from each of 2 trees. Record the number of galls on each leaf. Do not include aborted galls (substantially smaller than the "typical" galls).
- *Analysis:* For each tree, calculate the mean, and standard deviation of the mean, of the number of galls per leaf. Keep data for each individual tree separate. Then add data for the individual trees you sampled to a class list of means and standard deviations for each tree sampled. Compare the mean and standard deviation of number of galls/leaf among the various trees. Do trees vary significantly in the extent of herbivory by sawflies?

Additional Hypotheses

In addition, each team will choose one of the other hypotheses described below, or can design a new hypothesis, to test. Read through these hypotheses to see which interests you most, or discuss ideas with your instructor. You may come up with interesting hypotheses based on your initial observations of the trees and galls, or even by reading the titles of some of the journal articles in the References section. Alternatively, your instructor may assign a hypothesis to each team to be sure each hypothesis gets tested by your class. See the *Appendix* for suggestions on random sampling, descriptive statistics, and statistical tests. When you "sample" leaves, please avoid removing them from the branch so that other teams may sample the same plants and we leave the willows as undisturbed as possible.

Hypothesis 2

- *Background:* If sawflies compete for plant nutrients (sugars, proteins, lipids), there might be selective pressure for the evolution of behaviors to avoid competition. Females might avoid ovipositing on leaves that already have galls, so that leaves with one gall are more common than leaves with multiple galls. On the other hand, if some leaves are better food sources than others, galls may be clumped on the good leaves. We can test these ideas against the alternative hypothesis that females oviposit independently of other oviposition events.
- *Hypothesis:* Females select leaves for oviposition independently of whether the leaf has other galls.
- *Method:* Count the number of galls per leaf on 20 randomly chosen leaves on each of 5 trees. Fill in the following table ("# leaves" column) with tick marks as you observe each leaf. Combine your data with the class data from question #1 to get the total number observed (f) for each row in the table.

# Galls/leaf	# Leaves	Class Data from Q #1	Total = Observed (f)	Expected ($\hat{f}$)	Deviation from expected ($\lvert f - \hat{f} \rvert$)
0					
1					
2					
3					
4					
5					
6					
7+					
Total	100		n =		

Analysis: This test is sensitive to small expected frequencies in a cell (ˆf < 5), so you may need to group together cells (e.g., 5-6 galls/leaf) to make sure ˆf ≥ 5. Then see how closely f matches ˆf. Are there more or fewer leaves with just one gall than expected? Are the galls distributed independently of one another on leaves? See Appendix.

Hypothesis 3

- *Background:* When sawflies form galls on willow leaves, plant nutrients become more concentrated in the galls. If several galls are formed on a leaf, the sawflies might compete for these plant nutrients, and each sawfly might get fewer nutrients than if there were no other galls on the same leaf.
- *Hypothesis:* Galls on leaves with competing galls are less successful than galls on leaves with only one gall.
- *Method:* Determine for 50 single galls (only 1 gall/leaf) and 50 "multiple" galls (share their leaf with other galls) whether the sawfly was successful (an exit hole indicates success) or not (no exit hole). Enter the number of galls that fit into each category in the following table (you can use tick marks):

Number of galls/leaf	**Successful Emergence?**		
	Yes	**No**	**Total**
Single			50
Multiple			50
Total			100

Analysis: G-test of independence. See *Appendix*. Are single galls more successful?

Hypothesis 4

- *Background:* Willows have leaf-chewing herbivores as well as gall-formers. Do these different types of herbivores feed on the same leaves (because the selected leaves have low chemical or high nutrient concentrations), avoid each other (to avoid competition), or feed independently of each other?
- *Hypothesis:* Leaves with galls are more likely to have leaf damage from chewing herbivores than are leaves without galls (leaf chewers do not select leaves independently of galls).
- *Method:* Randomly sample 5 leaves with galls and 5 leaves without galls from each of 10 trees. Record whether each leaf has damage from chewing insects.

Galls present?	**Chewing Damage Present?**		
	Yes	**No**	**Total**
Yes			50
No			50
Total			100

Analysis: G-test of independence. See *Appendix*. Is gall presence independent of chewing damage?

Hypothesis 5

- *Background:* Construct your own hypothesis to test. Discuss your ideas with the instructor before you begin; your instructor can give you advice on methods and analysis.

Questions for Further Thought and Discussion

Conclusions about hypothesis 1:

- What did we find as a class?
- Do trees vary much in the level of herbivory by sawflies? Justify your answer by referring to the class data.
- Give at least one explanation for our result.

For additional hypotheses tested by individual teams:

- Did you accept or reject your null hypothesis?
- How did the results of your statistical test lead to this conclusion?
- What did you conclude about sawfly herbivory on willows based on your data?
- Give at least one ecological explanation for the result you obtained, and the possible ecological and evolutionary consequences of that result to the plant and to the animal.
- What have other investigators concluded about this hypothesis? Use your library's research databases or Google Scholar (http://scholar.google.com) to find **and read** at least 2 journal articles to support your statement.

References and Links

- Boecklen; W. J., P. W. Price, and S. Mopper. 1990. Sex and drugs and herbivores: sex-biased herbivory in arroyo willow (*Salix lasiolepis*). *Ecology* 71:581-588.
- Boecklen, W. J., S. Mopper, and P. W. Price. 1991. Size and shape analysis of mineral resources in arroyo willow and their relation to sawfly densities. *Ecological Research* 6:317-331.
- Clancy, K. M., P. W. Price, and C. M Sacchi. 1993. Is leaf size important for a leaf-galling sawfly (Hymenoptera: Tenthredinidae)? Environmental Entomology 22:116-126.
- Clancy, K. M., and P. W. Price. 1989. Effect of plant resistance, competition, and enemies on a leaf-galling sawfly (Hymenoptera: Tenthredinidae). Environmental Entomology 18:284-290.
- Clancy, K. M., P. W. Price and T. P. Craig. 1986. Life history and natural enemies of an undescribed sawfly near *Pontania pacifica* (Hymenoptera: Tenthredinidae) that forms leaf galls on the arroyo willow, *Salix lasiolepis*. *Annals of the Entomological Society of America* 79:884-892.

- Craig, T. P., J. K. Itami, and P. W. Price. 1990. The window of vulnerability of a shoot-galling sawfly to attack by a parasitoid. *Ecology* 71:1471-1482.
- Craig, T. P., J. K. Itami, and P. W. Price. 1989. A strong relationship between oviposition preference and larval performance in a shoot-galling sawfly. *Ecology* 70:1691-1699.
- Craig, T. P., P. W. Price, J. K. Itami. 1986. Resource regulation by a stem-galling sawfly on the arroyo willow. *Ecology* 67:419-425.
- Felt, E. P. 1965. Plant galls and gall makers. Hafner Pub. Co., New York, New York. (SB767 F4 1965)
- Fernandes, G. W., and P. W. Price. 1992. The adaptive significance of insect gall distribution: survivorship of species in xeric and mesic habitats. *Oecologia* 90:14-20
- Fritz, R. S., W. S. Gaud, C. F. Sacchi, and P. W. Price. 1987a. Patterns of intra- and interspecific association of gall-forming sawflies in relation to shoot size on their willow host plant. *Oecologia* 73: 159-169.
- Fritz, R. S., W. S. Gaud, C. F. Sacchi, and P. W. Price. 1987b. Variation in herbivore density among host plants and its consequences for community structure. *Oecologia* 72: 577-588.
- Hartley, S. E. 1998. The chemical composition of plant galls: are levels of nutrients and secondary compounds controlled by the gall-former? *Oecologia* 113:492-501.
- Johnson, W. T., and Lyon, H. H. 1988. *Insects that feed on trees and shrubs*. Comstock Publ. Assoc., Ithaca, New York, USA.
- Kause, A. E., E. Haukioja, and S. Hanhimaki. 1999. Phenotypic plasticity in foraging behavior of sawfly larvae. *Ecology* 80:1230-1241.
- Kokkonen, K. 2000. Mixed significance of plant vigor: two species of galling *Pontania* in a hybridizing willow complex. *Oikos* 90:97-106.
- Kopelke, J. P. 2003. Natural enemies of gall-forming sawflies on willows (*Salix spp.*) (Hymenoptera : *Tenthredinidae*: *Euura*, *Phyllocolpa*, *Pontania*). *Entomologia Generalis* 26: 277-312.
- Larew, H., and J. Capizzi. 1983. *Common insect and mite galls of the Pacific Northwest*. Oregon State University Press, Corvallis, Oregon, USA.
- Mani, M. S. 1964. *Ecology of plant galls*. W. Junk, The Hague, Netherlands.
- Nakamura, M., Y. Miyamoto, and T. Ohgushi. 2003. Gall initiation enhances the availability of food resources for herbivorous insects. *Functional Ecology* 17:851-857.

- Nyman, T., H. Roininen, and A. Widmer. 1999. Evolution of gall morphology and host-plant relationships in willow-feeding sawflies (Hymenoptera: Tenthredinidae). *Evolution* 64:526-533.
- Price, P. W., and K. M. Clancy. 1986. Interactions among three trophic levels: gall size and parasitoid attack. *Ecology* 67:1593-1600.
- Price, P. W., G. W. Fernandes, and G. L. Waring. 1987. Adaptive nature of insect galls. *Environmental Entomology* 16:15-24.
- Price, P.W., T. Ohgushi, H. Roininen, M. Ishihara, T. P. Craig, J. Tahvanainen, and S. M. Ferrier. 2004. Release of phylogenetic constraints through low resource heterogeneity: the case of gall-inducing sawflies. *Ecological Entomology* 29: 467-481.
- Roininen, H. and J. Tahvanainen. 1989. Host selection and larval performance of two willow-feeding sawflies. *Ecology* 70:129-136.
- Wagner, M. R., and K. F. Raffa, eds. 1993. Sawfly life history adaptations to woody plants. Academic Press, San Diego, California. (SB945.S3 S29 1993)
- Whitham, T. G. 1983. Host manipulation of parasites: within-plant variation as a defense against rapidly evolving pests. Pages 15-41, in R. F. Denno and M. S. McClure, editors. Variable plants and herbivores in natural and managed systems. Academic Press, New York, New York, USA.
- Woodman, R. L., and P. W. Price 1992. Differential larval predation by ants can influence willow sawfly community structure. Ecology 73:1028-1037.

Web Links

- The Solidago Eurosta Gall Homepage: A Resource for Teaching and Research. Useful information, photographs, illustrations, clips of an educational video (Goldenrod and the Gallfly), maintained by Dr. Warren Abrahamson. http://www.facstaff.bucknell.edu/abrahmsn/solidago/main.html
- Forest and Timber Insects in New Zealand, No. 45: Willow Gall Sawfly. Nice photographs of Pontania galls on willow leaves, and egg, larva, pupa, and adult stages of sawfly. http://www.forestresearch.co.nz/PDF/Ent45Pontaniaproxima.pdf
- USDA, Agricultural Research Service: List of Plant Galls Web Sites http://www.wcrl.ars.usda.gov/cec/teaching/galls.htm
- North Carolina State University: Galls and Gall Makers. http://www.cals.ncsu.edu/course/ent591k/galls.html
- Colorado State University Cooperative Extension: Insect and Mite Galls http://www.ext.colostate.edu/pubs/insect/05557.html

Tools for Assessment of Student Learning Outcomes

Informal Assessment

While students are deciding which hypothesis to test, I wander among groups and ask them some simple questions to assess whether they are grasping the main concepts and hypotheses.

- Does this hypothesis make sense?
- What other factors might influence the distribution of galls besides the one hypothesized?
- What factors should you try to control in your study?

While they are collecting and analyzing data, I informally walk among teams of students and ask them simple questions such as:

- Do you have enough data? If not, should you increase your sample size? How would you know if your sample size is sufficient to answer the question?
- What did your statistical tests tell you?
- How confident are you in your data and conclusions? Explain why.

We discuss the results of hypothesis 1 as a class, after putting the means and standard deviations on the board. I ask students whether trees vary in the extent of herbivory by galling sawflies.

Formal Assessment

I assess student learning primarily by requiring a formal oral report (similar in style to paper presentations at ESA meetings). I give students a guideline (below) for what material they should include in the talk, and give them the grade sheet I use to grade their oral report (see Oral Report Grade Sheet). Assessment of their data analysis is via written data summary and analysis they hand in at the time of the oral report. Students also evaluate oral presentations by other teams, providing me an opportunity to see if they understand what other teams found.

ORAL PRESENTATION

Your team will present a short, concise (5-10 min.), well organized oral presentation (in PowerPoint) based on the additional hypothesis you tested.

Use the following outline to prepare for your talk:

- Introduction/Background — sufficient information from the literature to lead up to your hypothesis
- Hypothesis — stated clearly; give both null and alternative hypothesis; what predictions follow logically from this hypothesis?

- Methods of data collection — overview of how you collected data to test your hypothesis, including sample size (number of leaves, trees, galls, as appropriate), how you selected units to sample; what you recorded
- Statistical test — which test did you use, and what does it test? (e.g., t-test tests for difference in mean between 2 groups)
- Results — spend some time thinking about the best way to present your data. Graphs are typically better than tables. Make sure the axes are labeled with large lettering that can be easily read. You want your audience to be able to quickly see any patterns in your data. Give results of statistical tests, and whether you accepted or rejected your null hypothesis.
- Interpretation — what do your data mean? Did they support the hypothesis or not? Very briefly, how confident are you in your data and conclusions? (large enough sample size? Errors minimized?) How do your results square with what is known about herbivory/galls from the literature? Cite at least 2 journal articles that you have read.

Your grade will be based on the criteria listed in the Oral Report Grade Sheet.

Tools for Formative Evaluation of this Experiment

NOTE: An extensive discussion on Evaluation appears in the Teaching section of TIEE.

NOTES TO FACULTY

Challenges to Anticipate and Solve

1. **Low gall density:** Gall densities fluctuate from year to year. One to several weeks before the lab is scheduled, check the willow leaves at your site. If gall densities are quite low (students will have to sample many leaves to get any with galls), then adjust sample sizes to include more leaves or look for alternative sites with more galls.

2. **Early leaf drop:** The date of leaf drop varies from year to year. You may need to monitor your study site and adjust the date of the lab so that it can be done before most leaves fall. If you do get caught by early leaf drop, you might be able to adjust the hypotheses tested. If trees are close together, hypothesis 1 can't be tested. Some hypotheses can be tested with leaves on the ground. If your lab occurs partway through leaf drop, you might test the hypothesis that leaves with galls will be abscised earlier than leaves without galls (trees getting rid of herbivores) against the hypothesis that leaves with galls are retained longer (galls are carbohydrate sinks, so trees have more invested in them).

3. **Choice of hypothesis to test:** Students may not be particularly motivated here. Sometimes they just choose the first one, or the one that looks easiest (fewest samples required). A bit of discussion about these hypotheses when you introduce the lab should help motivate students. Encourage students to formulate their own hypothesis based on what they have learned of plant interactions in lecture, the introductory material in the lab handout, or initial observations at the study site. Be sure they have some time in lab to make this decision, and discuss briefly with each team why they chose that hypothesis.

4. **Statistical literacy:** Depending on how much exposure your students have had to statistical testing, they may not be comfortable with data analysis and testing. The examples should help, but you may need to go through some examples on the board after the students collect their data. Ensure them that the calculations are really quite simple, and emphasize the importance of statistical testing (e.g., how big would the differences have to be before you would be confident saying there are differences?). Walk around among the teams and discuss their results with them to be sure they understand whether they should accept or reject their hypothesis, and why.

5. **Production of graphs for oral reports:** Students often have trouble with 2 aspects of producing graphs. The first is deciding upon an appropriate kind of graph for their data. You could discuss this with each group in lab after they have collected their data, or discuss more generally in lecture. You could give examples of the best kinds of graph for each hypothesis. The second issue is actual graph production. Many students are familiar with Excel, and some have used it to graph data before. But they often use Excel's defaults, and don't format graphs appropriately. You might want to give some guidelines about graphing, or post an example on a course webpage (or give a handout), with large enough axis labels, a descriptive figure legend, etc.

6. **Oral presentations:** The quality of oral presentations may depend on whether students have done this before in a biology class. If you have time in lecture, you might want to give them an example. Present an oral presentation yourself, perhaps based on a previous lab students did, so they see what level of detail and professional conduct you expect. Be sure to adhere to the time limits!

Comments on the Lab Description

Introducing the Lab to Your Students:

I usually try to introduce herbivory as a type of species interaction in the lecture part of the course. I cover the various ways plants defend themselves from herbivores, and how herbivores both respond to and are affected by chemical and physical defenses. During the lab, I give a brief overview of galls as a particular type of herbivory, and show some different types of galls. The Detailed Description of the Experiment provides introductory information.

Activities in the Lab:

- I give a very brief introduction to the 1st hypothesis, which all students will address. I ask students to form teams of 2-3 members.

- For descriptions of aborted galls, successful galls, and parasitized galls, see Clancy et al. (1987). Aborted galls are usually quite smaller than active galls, and tissues are nearly solid (seen when the gall is cut open: (aborted galls at left) rather than organized into a hollow structure: (opened galls at right).

- Successful sawfly larvae chew emergence holes (mean diameter 1.3 mm for *Pontania* sp. in Arizona) that are larger than holes made by parasitoids. To detect parasitized galls, cut open and observe under a dissecting microscope. If the larva has been parasitized, you will typically see an egg or other stage of the parasite (see parasite at right).

Potential modifications of this lab activity:

- Rather than discussing the class results of Hypothesis 1 in lab, you could have each group include a very brief summary of what the class found, and how this provides relevant background information leading to the specific hypothesis their team tested.
- Students could be asked to hand in the literature citations they used for the oral report.
- Students can submit a formal written lab report (similar in style to manuscripts submitted to *Ecology*) instead of the oral report. See *Written Report Gradesheet*.
- For students with more experience in analyzing data, you might give more general comments on methods (e.g., for hypothesis 2, randomly sample leaves with galls and leaves without galls, recording whether chewing damage is present or not. How many leaves should you sample?), or not give any hints and have students propose methods and types of analyses. You would need more time in lab to inspect their proposals before they collect data.
- This activity can easily be extended to 2 weeks, with more student-based inquiry. During the first week, introduce the students to the study system, have them make their own, unguided observations about galls on willows, and collect data to test hypothesis 1. After summarizing and discussing class data, have each group think about an additional question to ask about the distribution of galls on willow leaves. (Do not provide them with the list of additional hypotheses.) They can propose their own hypothesis based on this question, which you can discuss with them. By the end of the first lab period, teams will hand in a written version of the additional hypothesis they will test, the types of data they will collect, sample sizes, and methods for selecting sample units. The instructor should review these, and provide comments to ensure a viable hypothesis, sufficient sample size, and guidance as to appropriate statistical tests. During the second lab period, teams will collect and analyze data on their hypothesis, and begin preparing their oral reports. An example of a student-generated hypothesis is: Gall densities are higher on willows in close proximity to a water source (e.g., a pond) than on willows farther from surface water.

Questions for Further Thought

Conclusions about hypothesis 1:

- You will probably find that trees do vary considerably in the level of herbivory by sawflies (means differ; but look for large standard deviations; you might mention that statistical testing helps sort out whether these apparent differences in the mean values are real, i.e., statistically significant).
- Potential explanations include that individual trees vary genetically as well as in their ecological conditions, age, etc. These differences could result in differences in nutrient levels, concentrations of chemical defenses, etc. There may also be differences among trees in how many predators or parasitoids are around.
- If willow trees preferentially drop leaves with galls, then trees may not vary much in how many galls are on intact leaves. But you may find higher numbers of galls on leaves on the ground under some trees.

For additional hypotheses tested by individual teams:

- Ecological explanations can be found in the background information supplied with each hypothesis, as well as in the literature cited.

Assessment of Student Learning Outcomes

I find that students often have difficulty with statistical tests. I try to make sure I visit each team several times while they are analyzing their data to make sure they properly summarize their data before beginning analysis, understand the basic idea of what the statistic is testing, and understand how to interpret P-values and test statistic values. Preparation for the oral report should be pretty straight forward if the students read through the *Oral Report Gradesheet* in advance.

Translating the Activity to Other Scales

1. **Translating this experiment to larger scales:** you may want to form larger teams (5-6 students), and assign each team a hypothesis to test rather than taking time to have them select a hypothesis. Larger teams should be able to collect more data, so increase the sample size suggestions.
2. **Translating this experiment to pre-college settings:** you could omit the statistical tests, and revise the hypotheses to simpler terms. Focus on having students understand the basic concept that herbivory is everywhere, and some plants are more susceptible to herbivores than others. Herbivores also have choices in where they place their offspring, and this affects how well their offspring do.

STUDENT COLLECTED DATA FROM THIS EXPERIMENT

Hypothesis 2: Females select leaves for oviposition independently of whether the leaf has other galls. Sample data provided in the *Appendix*.

Hypothesis 4: Leaves with galls are more likely to have leaf damage from chewing herbivores than are leaves without galls. Sample data provided in the attached *Hypothesis 4 Data* document and are reproduced below.

Example of student-collected data for hypothesis 4:

Galls present?	Chewing Damage Present?		Total
	Yes	No	
Yes	16	4	20
No	13	7	20
Total	29	11	40

G-test of independence:

1) a = 16 ln 16 + 4 ln 4 + 13 ln 13 + 7 ln 7 = 96.87
2) b = 20 ln 20 + 20 ln 20 + 29 ln 29 + 11 ln 11 = 243.86
3) c = 40 ln 40 = 147.56
4) G = 2(a-b+c) = 1.14

Compare with critical value (alpha .05) of 3.841

Conclusion: do not reject null hypothesis that galls occur independently of chewing damage

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