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EXPERIMENTS

Testing Hypotheses about Herbivore Responses to Plant Vigor and Herbivore Impact on Plant Reproduction

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Apical rosette gall on "tall goldenrod", *Solidago altissima* (Asteraceae) formed by a parasitic fly *Rhopalomyia solidaginis* (Diptera: Cecidomyiidae)

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CITATION

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ABSTRACT

In this one to two week lab, students examine hypotheses about the interactions between herbivores and plants; these interactions could be studied in the context of a course segment on symbioses or trophic relations. This lab involves the study of the relationship between tall goldenrod and a gall-forming herbivorous insect; the presence or absence of the gall formed by the insect is easy to assess and plant reproductive output can be measured after first frost, making this system useful as a field-lab well into the fall semester. The purpose of the lab is to: determine if plants with particular attributes differ in susceptibility to attack by herbivores, and if herbivores act as parasites, commensals, or possibly mutualists when they feed on plants by having a negative, neutral, or positive effect on plant reproduction. First, students measure plant traits to test two competing hypotheses to explain variation in abundance of herbivorous insects among plants. The *Plant Stress Hypothesis* and the *Plant Vigor Hypothesis* both argue that plant physiological status and growth will impact the success of herbivores, but one proposes that stressed plants will be vulnerable to attack because they provide better food with higher amino acid content for herbivores, while the other proposes that vigorously growing plants will provide better food. Second, students measure plant reproductive traits to test competing hypotheses about the effect of herbivores on plant reproduction. These hypotheses are the *Negative Impact*, the *Plant Tolerance*, and the *Overcompensation* hypotheses; the hypotheses suggest, respectively, that plants attacked by herbivores will produce a number and quality of seeds that is less than, similar to, or greater than the number and quality produced by plants not attacked by herbivores. In this lab, students will discuss the nature of the interaction of herbivores with plants, collect and share data, analyze data using statistical tests, examine two primary sources from the research literature to address ideas in the lab, and draw conclusions and communicate their findings by writing a Brief Communication.

Class Time
One week (3 hour lab) if system is chosen by instructor. Two weeks if students are required to find a plant-herbivore interaction to study that is different from the goldenrod-gall system.

Outside of Class Time
Five to six hours to analyze data, to collect and interpret two literature sources, and to write a Brief Communication.

Student Products
Formative Assessment of student understanding of concepts to be studied: Students will be asked to discuss, as a class, some of the "Questions for Further Thought and Discussion" at the beginning of lab. A one-minute paper can be assigned before going to the field in which students address 'misconceptions' they had about plant-herbivore interactions before the lab discussion.

Brief Communication: this is a short report such as the Brief Communications found in many journals, in which students present a short but complete report on the results of the two or three comparative tests that they will perform.

Setting
This lab can be conducted in any field environment with plants of a single species that are attacked by one species of herbivore that leaves distinctive evidence of the damage it has caused. The plant-herbivore system that is most useful for this lab is the tall goldenrod (*Solidago altissima*) and one of the gall-forming herbivore species that attack this plant. Using the goldenrod-galling insect system, the lab can be conducted in late summer through late fall; plant attributes can be measured after the first frost in fall, since the goldenrod retains its inflorescences and indicators of seed production into November. All measurements can be recorded in the field.

Other plant-herbivore systems that can be used include many species of willows that are attacked by different species of stem galling insects and also produce evidence of their reproductive potential (enlarged reproductive buds) in the presence or absence of damage caused by a gall-forming herbivore (see *TIEE* lab by Ernest (2005) for one example of such a plant-herbivore system).

Course Context
This lab is taught during the fall semester in a general ecology laboratory class for biology majors. The class typically includes 24 students. Students work cooperatively in groups of three or four.

Institution
This course is taught to undergraduate Biology majors at a regional public four year university of 8000 students.

Transferability
This lab would work well with general ecology or upper division ecology courses at institutions of all sizes. The lab is simple in design and requires no special technical skills so could be transferred to non-majors general biology classes, but access to suitable field sites might be difficult for multiple-section non-majors classes. A suitable field site for the lab, as written, would include any area that includes a stand of goldenrod with more than a few hundred stems; such sites could include an old field, a prairie, an unmowed road edge, or perhaps an abandoned lot or an unmowed area in a park in an urban area.

Acknowledgements
The ideas presented in this lab exercise were developed largely as a result of the work and inspiration of colleagues in the lab of Peter W. Price at Northern Arizona University (NAU), where I completed my doctoral studies and later through interactions with students and colleagues, particularly Ed Connor, at the University of Virginia's Blandy Experimental Farm (UVa). Through discussions, seminars, work in the field, and critical review of the work of colleagues in the Price lab at NAU, my understanding of the rudiments of the Plant Vigor Hypothesis evolved. As part of my dissertation at NAU and later with input from Ed Connor at UVa, I studied the impact of herbivores on plant reproduction and developed familiarity with some of the ideas tested in this exercise. Colleagues who should be mentioned for their influence on my perspectives on the ideas in this lab include Tim Craig, Joanne Itami, Ken Paige, Mike Kearsley, Mike Wise, Tom Whitham, and both Peter Price and Ed Connor. Colleagues in the Biology Department at Kutztown University, including Carol Mapes, Ron Rhein, Wendy Ryan, Will Towne, and Anne Zayaitz have influenced my awareness of the pedagogical approaches employed in this lab exercise. This laboratory exercise was completed with support from the National Science Foundation's Division of Undergraduate Education, Course Curriculum and Laboratory Improvement (CCLI) program (Award # 0126817) to C.F. Sacchi, C.C. Mapes, W.L. Ryan, and A.E. Zayaitz. I would also acknowledge the efforts of Charlene D'Avanzo, Bruce W. Grant, and Kathy Winnet-Murray for helping authors to develop TIEE lab exercises and two anonymous reviewers whose comments have helped in the development of this lab.

SYNOPSIS

Principal Ecological Question Addressed

Does host plant vigor or physiological condition (as measured by plant size) influence the oviposition decision of an ovipositing gall-forming herbivorous insect? What are the effects of these oviposition decisions and gall formation on subsequent host plant growth and reproduction? Why might the physiological condition (as measured by plant size) of a host plant matter to an herbivore?

What Happens

Students test competing hypotheses to explain: 1) why galling herbivores choose the goldenrods they do, and 2) the effects of herbivores on plant reproduction. Students conduct a field study involving goldenrod plants and galls formed by one of two common gall-forming insect species. After measuring plant size and reproductive attributes of plants with and without galls in the field, students analyze the results using statistical tests to evaluate the competing hypotheses regarding plant susceptibility to attack and herbivore effects on plant reproduction. Students then communicate the concepts they have tested, the results of their tests, and their conclusions for the comparisons they performed.

Experiment Objectives

1. To learn to identify tall goldenrod and the galls formed by herbivores that oviposit on and feed on this plant.
2. To use a transect technique to survey the frequency of herbivores on plants in the plant population studied and to practice measurement and data collection skills.
3. To understand primary concepts of herbivore responses to plant vigor and plant reproductive responses to herbivory.
4. To test hypotheses about herbivore responses to plant vigor and plant reproductive responses to herbivores using appropriate statistical methods.
5. To effectively communicate the purpose, results, and conclusions of this study by writing a Brief Communication.
6. To examine the biology of the plant and insect species studied and the adequacy of the hypotheses tested to explain the attack of plants by herbivores and the reproductive response of plants following herbivory.

Equipment/Logistics Required

Tape measures (35m or 50m), meter sticks, 150cm rulers, tally meters (hand-held 'clicker' used for counting), and wire flags or flagging tape are all needed. A 0.5m x 0.5m or a 1m x 1m PVC square sample quadrat frame may be useful.

Summary of What is Due

At the end of the first lab period, I conduct a brief formative assessment in which students write a one minute paper to address misconceptions about plant-herbivore interactions that they had prior to the classroom discussion.

In a timely way, each group of students must prepare and submit a spreadsheet that contains the raw data they collected in the field. This task may be completed upon return to the lab if time permits; if not completed in the lab, students must e-mail the spreadsheet with their data to the instructor and to other students in the class.

Students will produce a Brief Communication; which is a short report that includes all sections of a traditional lab report except no abstract is expected. Students must include at least two citations to support the ideas in their Introduction or Discussion, and a figure and table of statistical results for each test they perform.

Keyword Descriptors

Ecological Topic Keywords: coevolution, galls, herbivores, parasites, plant ecology, plant-herbivore interactions, plant vigor hypothesis, plant stress hypothesis, oviposition decisions, habitat selection, foraging decisions, herbivore impact, plant tolerance, plant overcompensation

Science Methodological Skills Developed: collecting and presenting data, data analysis, evaluating alternative hypotheses, field work, graphing, scientific writing, statistics, use of primary literature

Pedagogical Methods Keywords: formative evaluation, guided inquiry, misconceptions, minute paper, rubric (for Brief Communication)

DESCRIPTION

Introduction

General Background about Plant-Herbivore Interactions

As primary producers, plants form the foundation of terrestrial trophic pyramids in terrestrial ecosystems providing a source of nutrition, first, for primary consumers. These primary consumers called herbivores include invertebrates, primarily insects such as the larvae of butterflies, as well as some vertebrates, including mammalian herbivores such as white tailed deer.

The question of whether all plants provide food that is equally accessible to herbivores could be studied in light of the observation that plants produce a variety of secondary chemical compounds that cause plants to differ in their palatability to herbivores. An example of a secondary compound is capsaicin, the chemical that gives hot peppers their pungency. These compounds were called secondary compounds because they seem to play no role in primary metabolic pathways, e.g. photosynthetic pathways. Fraenkel (1959) suggested that these secondary compounds could serve a defensive role in plants, allowing plants to escape attack, to some extent, by herbivores. Ehrlich and Raven (1964) advanced the theory of coevolution in which they proposed that the great diversity of plants and herbivorous insects was a result of a process of stepwise evolution. This coevolutionary theory proposed that plants producing novel defensive chemical compounds could escape from herbivores for some time allowing the plants to diversify in species richness under conditions of reduced attack by herbivores, but some herbivore would then evolve the ability to detoxify the novel compounds of plants that had temporarily escaped from their herbivores. The ability to detoxify novel compounds would then allow herbivores to diversify in species number on the plants that had earlier escaped attack. This process of change in defensive chemistry in plants followed by change in the ability of herbivores to feed on these plants would continue in a co-evolutionary "arms race."

Many plants also have evolved physical defensive traits, aside from secondary compounds, that allow them to escape from herbivory. Such traits include spines and thorns, trichomes or hairs on leaves, and tough tissues that include highly indigestible compounds. With both chemical and physical defenses, plants can differ in their suitability as food for herbivores.

Herbivores have diversified to feed on plants and the different tissues produced by plants. Free-feeding insects live and feed on the exterior surface of plants. Typical free-feeding insects include defoliating insects that feed by chewing on plant tissue such as leaves; examples include larvae of butterflies and larvae and adults of many beetles, such as the Colorado potato beetle. Phloem feeders, such as aphids, use a mouthpart, adapted as a stylet, to tap into the sugar-rich fluid that flows through the plant’s phloem. Xylem feeders, such as spittle bugs, feed successfully on the nutritionally poor fluids that flow through a plant's xylem.

Endophytic insects, which are not free-feeders, are confined to, and develop within, a particular plant structure; such species include gall-forming insects and leaf miners. Gall-forming insects lay their eggs in plant tissue and induce the plant to produce a conspicuous protective structure, the gall, within which the juvenile insect feeds and develops. Gall-forming insects include species that chew on tissue within the gall as well as species of phloem-feeding aphids. Galls can be formed by different species of insects on leaves, petioles, buds, stems, or on flowers of particular plant species. Leaf miners are insects that produce larvae that develop within a leaf; the juvenile insects feed on the parenchyma cells within the leaf, creating a blotch or sinuous pattern characteristic of each mining insect species, where tissue has been removed. The mine can be seen on the surface of the leaf.

The diversity of herbivores, their feeding habits, the tissues that they feed on, and other elements of their biology result from the complex interactions between plants and the herbivores, as well as the enemies of the herbivores. Within a population of a given plant species there is variation in attack by herbivorous insects which has led to a variety of hypotheses to explain why some plants are more readily attacked by herbivores while other individuals in the same population experience lower levels of, or even escape, herbivore attack.

Goldenrod and Gall-Forming Insects

The tall goldenrod, *Solidago altissima* (Asteraceae), is a widely distributed fall-flowering species found in successional fields, prairies, or woodland edges throughout eastern and central north-America (synonymous with *Solidago canadensis var. scabra* (http://plants.usda.gov/ 2005)). The plant flowers in late summer into fall and produces many small seeds in each inflorescence that are evident on plants in early to late fall (Figure 1A and 1B).

Figure 1A - late summer flowering tall goldenrod, *Solidago altissima* (Asteraceae)

Figure 1B - Fall seed set by tall goldenrod, *Solidago altissima* (Asteraceae)

Stems of tall goldenrod are attacked by three different species of gall-forming insects. The gall forming insects and the names for the gall they form are: 1) *Rhopalomyia solidaginis* (Diptera: Cecidomyiidae) that forms the apical rosette gall (Figure 2A and 2B), 2) *Eurosta solidaginis* (Diptera: Tephritidae) that forms the goldenrod ball gall (Figure 2C) (Abrahamson et al. 1989), and 3) *Gnorimoschema gallaesolidaginis* (Lepidoptera: Gelechiidae) that forms the goldenrod spindle gall (Figure 2D) (for other images of these galls visit: http://www.cals.ncsu.edu/course/ent525/close/gallpix/, 2005). Either the rosette gall or the ball gall has been abundant enough to serve as the subject of this lab exercise. Following oviposition by the maternal insect in late spring, the ball gall and the rosette gall both begin to develop and become evident in early to mid-summer.

Figure 2A - Apical rosette gall formed by *Rhopalomyia solidaginis* (Diptera: Cecidomyiidae) on tall goldenrod, Solidago altissima (Asteraceae)

Figure 2B - Apical rosette gall as in Figure 1A, except in fall

Figure 2C - Ball gall formed by *Eurosta solidaginis* (Diptera: Tephritidae)

Figure 2D - Spindle gall formed by *Gnorimoschema gallaesolidaginis* (Lepidoptera: Gelechiidae)

Conceptual Background

Part 1: Gall-forming herbivores may improve their progeny's growth and potential for reproductive success by choosing to oviposit on individual plants of high nutritional quality. One hypothesis, the *Plant Stress Hypothesis*, proposed the idea that plants that were physiologically stressed as a result of lack of water would provide the best food for herbivores because these plants produced tissue that contained higher nutrient content, particularly nitrogenous compounds in the form of free amino acids (White 1993). This hypothesis suggested that plants exposed to drought might be more susceptible to herbivore attack; further, this susceptibility to herbivore attack might account for observed outbreaks of herbivorous insects often following periods of drought.

A second hypothesis, the *Plant Vigor Hypothesis*, proposed that herbivores should prefer healthy, vigorously growing plants that provide abundant nutrition to allow insects to feed and grow more rapidly (Price 1991). Plants growing in environments that provide abundant water and mineral nutrients and that provide access to appropriate levels of sunlight may grow more rapidly than plants that are deficient in any of these resources making such plants high in nutritional quality. There have been studies that have supported both hypotheses; some have shown that water-stressed plants are attacked most heavily by herbivores offering support for the *Plant Stress Hypothesis* (White 1984). Other studies have shown that plants of great vigor may support the highest survival and most vigorous growth of herbivores, lending support for the *Plant Vigor Hypothesis* (Craig et al. 1986).

Price (1991) suggested that the *Plant Stress* and *Plant Vigor Hypotheses* should not be seen as strict alternatives that would lead to one hypothesis winning out over the other, but rather as ends of a spectrum to account for the diversity of responses of herbivores to plants found in diverse natural systems.

Gall-forming herbivorous insects provide an interesting model for testing the response of insects to plants that differ in vigor. Insect-induced galls, or plant tumors, are structures on plants that are produced following oviposition by a female insect of a gall-forming species. In galling insects, it is hypothesized that each female chooses to oviposit on the plant tissue in which her offspring will feed and develop best; therefore the offspring, which develops to become a feeding individual, has virtually no choice about where it will feed. Following oviposition by galling insects, the plant produces a structure, a gall, within which the larval insect will feed and develop. Depending on the species of galling insect, the developing offspring will either leave the gall and pupate and complete its development outside of the gall, or it will pupate within the gall and will emerge from the gall as an adult and will mate after emergence, leading to continuation of the life cycle. It should be noted that the gall provides not only nutrition but also protection from enemies for the developing insect.

Part 2: In traditional theories about the evolution of plant defenses in response to herbivores, a central assumption was that herbivores would reduce the fitness, the reproductive success, of plants they feed on (Strong et al. 1984). The *Negative Impact Hypothesis* states that herbivores will lead to reduced reproductive success of plants that are fed on by insects. In recent years, researchers have suggested the *Plant Tolerance Hypothesis*, the idea that plants faced with herbivory may have evolved the ability to tolerate the loss of tissue that is either consumed by herbivores or allocated to galls. With tolerance, plants fed upon by herbivores will produce a similar number of seeds compared with plants that have not been attacked by herbivores (Strauss and Zangerl 2002). Finally, a third hypothesis about plant response to herbivores has been advanced. The *Overcompensation Hypothesis* proposes that plants attacked by herbivores are able to produce more flowers, fruits, and seeds than plants that have not been attacked by herbivores (Paige and Whitham 1987). The reproductive response of tolerant plants or of those that overcompensate may result from increased photosynthesis in plants attacked by herbivores, release of dormant meristems, reallocation of stored resources, or increased growth rate (Fornoni et al. 2003).

An hypothesis that predicts a full range of reproductive response by plants to herbivory is the *Continuum of Response* hypothesis (or *Compensatory Continuum* hypothesis), which predicts that depending on their access to needed resources as influenced by soil nutrients, water, or interspecific competition, plants may exhibit a negative, neutral, or overcompensatory response to herbivores (Maschinksi and Whitham 1989). Wise and Abrahamson (2005) presented the *Limiting Resource Model* as an alternative to the *Compensatory Continuum* hypothesis. This model contends that it more reliably predicts the range of observed effects of resource levels on tolerance by considering the resources that limit plant reproduction and the resources that are affected by particular herbivores.

Further, *Plant Tolerance* theory considers the range of reproductive responses of plants to herbivores, from negative, to neutral, to positive as indicators of differing levels of tolerance. This theory uses the term: a) incomplete tolerance when the effect of herbivores on plants is negative, b) complete tolerance when herbivores have no effect on plant fitness, and c) overcompensating tolerance when plants have higher reproduction following herbivory (Fornoni et al. 2003). For this lab, however, we will use the names for the three competing hypotheses that were introduced in the first paragraph, namely, *Negative Impact*, *Plant Tolerance*, and *Overcompensation* hypotheses.

Researchers today are actively engaged in studies of herbivore effects on plant reproduction to determine which of these three hypotheses is best supported by available data, or under what environmental conditions a particular hypothesis, and plant response, might be most appropriate.

Materials and Methods

Study Site

You will visit an old-field site, a prairie site, a disturbed plot of land, or an infrequently mowed road edge or a park where a species of goldenrod, *Solidago* sp., grows (Figure 1A, 1B). In this site, you will locate a group of plants that support rosette galls (Figure 2A, 2B) or ball galls (Figure 2C), each of which can be abundant enough to allow this lab to be completed. The rosette gall is formed following oviposition by *Rhopalomyia solidaginis*, a Cecidomyiid fly, and the ball gall is formed after oviposition by *Eurosta solidaginis*, a Tephritid fly. In this lab, students should study plants that possess either rosette galls or ball galls, but not both. More information on goldenrod galls and gall identification can be found in some of the published papers and several of the websites devoted to study of goldenrod and gall formers; these papers and websites are cited in the ‘References and Links’ section below.

The upper portion of the stem of tall goldenrod produces shoots that bear inflorescences that eventually produce seeds (Figure 1A, 1B). The galls you will study, or encounter, in this study include the rosette gall, (Figure 2A, 2B), the ball gall (Figure 2C), and the spindle gall (Figure 2D). However, the spindle gall has never been abundant enough in the goldenrod populations my students and I have worked in to use it as a subject of study in this lab exercise.

Overview of Data Collection and Analysis Methods

Overview: First, you will survey the goldenrod population to document the proportion of stems within the population that are galled. This will suggest whether herbivores are abundant and have the potential to be an important ecological force within this system. Second, students will then make a preliminary test of the *Plant Vigor Hypothesis*, which predicts that vigorously growing plants will be galled at a higher rate than more stressed, less vigorous plants. Attack of less vigorous plant parts would lend support to the *Plant Stress Hypothesis*. In this exercise, the size of the plant part attacked by the gall-forming insect is considered as an indicator of vigor. Last, you will test the hypothesis that the rosette gall or the ball gall has a negative, positive, or no impact on goldenrod reproduction.

Procedure 1: Proportion of plants galled. In order to objectively assess the proportion of goldenrod stems that are attacked by rosette or ball galls within the goldenrod plant population, you will (as a class) use a belt transect method to survey approximately 1000 stems and to classify them as either galled (stem possesses the gall of interest) or ungalled. (Be aware that there are at least three species of stem gall formers on goldenrod; if you encounter any odd looking galls, you may want to note that the plant is galled but you should note the identity of the other type of gall). You will only count and record the number of stems that are galled or not galled by the gall-former species you have chosen for study. Your instructor will help with identifying the rosette gall, the ball gall, or a third gall, the spindle gall. Students may want to create a reference collection that includes one named sample of each type of gall taped to an index card, to aid in identification of galls along the transect. This reference collection can be used to help in identification of any galls that are not fully developed or to be certain of consistency of identification among all student groups.

1) Belt Transect (Figure 3): Each group should run a 30 meter transect in the field selected for study; place a flag, or tie flagging tape to a stem, at every 5m increment along the meter tape; you can lay the 30m portion of the tape on the ground to mark one boundary of your belt transect. You will sample from within a 0.5m wide belt that runs alongside the meter tape; you can use two additional flags to mark the corners of each 5m x 0.5m sampling increment along the belt transect. You can flip a coin to determine if you will run the belt sample along the left side or the right side of the meter tape. In the belt of 0.5m on one side of the transect, for each five meter increment on the transect, you will count and classify the first 40 stems that you encounter in that 5m x 0.5m belt. Then, survey the next 5m belt. In so doing, each group should survey 240 stems; each group can typically find 240 stems to survey within the 30m belt transect. With at least four groups collecting and sharing data, you will have data on the proportion of stems attacked for 960 stems. You can plot these data as proportion of stems attacked or not attacked on a graph; no statistical test is performed using these data.

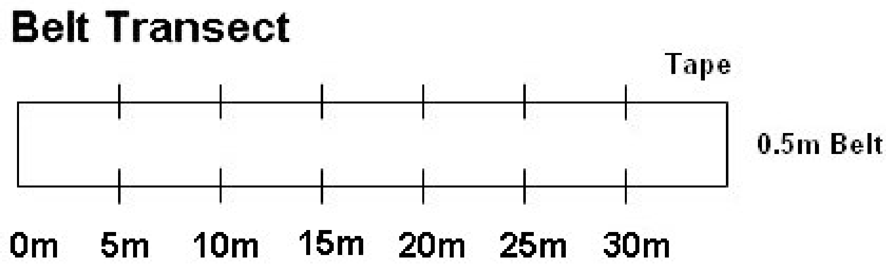

Figure 3. Illustration of belt transect that is 30m in length and 0.5m in width. Goldenrod will be sampled for the presence or absence of galls and for size and reproductive attributes within each 5m x 0.5m segment of the belt transect.

Procedure 2: Plant vigor hypothesis and herbivore impact on plant hypothesis: Within each lab group, while you are surveying plants for the proportion of stems with galls along the transect, mark 15 galled stems for later measurement. For each of these galled stems, mark the nearest ungalled stem. An objective way to sample galled stems is to choose the first three galled stems that you encounter in each 5m x 0.5m portion of the belt transect. (Can you think of other ways to select galled stems in an objective or randomized manner?) You should strive to select galled stems, and the paired ungalled stem for each galled stem, from along the length of the entire belt transect, e.g. choose about three galled stems per 5m portion of the belt transect. These galled and ungalled stems can be anywhere within the belt transect. A flag or flagging tape can be used to mark the matched galled and ungalled stems.

Plant Vigor vs Plant Stress: For each marked stem, note if it is galled or ungalled, measure the total height, in cm, of the stem with the gall on it (to the tip of the tallest branch); straighten out the longest stem if it is drooping. You will use the height data to examine the *Plant Vigor Hypothesis* and the *Plant Stress Hypothesis* by comparing galled and ungalled stems. With four groups, each measuring 15 pairs of stems, the group should have data on 60 pairs of stems; allowing for a fairly rigorous statistical test. The mean value for the height of the galled and ungalled stems should be plotted; consider the most appropriate type of statistical test that you might use for data that has been collected from two nearby stems, i.e. paired samples (data for stem height is collected from pairs of galled and ungalled stems) (see 'Statistics Appendix').

Procedure 3: Plant Reproduction: Herbivore Impact vs Tolerance vs Overcompensation: For each marked stem, note if it is galled or ungalled. For each marked galled and ungalled stem, count the number of every inflorescence-bearing shoot. You will then measure the length, in cm, of every inflorescence-bearing shoot at the end of each galled or ungalled stem on the plant. You should measure the portion of the stem from the location of the first inflorescence to the end of the stem where the last inflorescence is located. Provide the sum of the lengths of all stems with inflorescences at the end of the galled or ungalled stem. We will assume that the length of the inflorescence is correlated with seed production. Is this a reasonable assumption? Is this a testable assumption? You can plot the total mean length of galled and ungalled stems bearing inflorescences after summing all the lengths of all the stems that had inflorescences on them for each galled and ungalled plant. You can test whether the ungalled stems differed from the galled stems in the "number" of inflorescences or seeds they produced as measured by the length of the inflorescence bearing stems. You can perform a statistical test for paired samples to compare the length of inflorescences on galled vs. ungalled plants (see 'Statistics Appendix').

Measuring Reproduction: Optional Study Method: In addition to the simple method for measuring reproduction described above, a more rigorous method could be used to sample reproductive output by a goldenrod plant. One student group could be selected to test the assumption that length of inflorescence-bearing stems is correlated with reproductive output. This group could measure the length of 25 inflorescence-bearing shoots and collect each of these shoots and place them individually in an envelope or sandwich bag. Upon return to the lab, this group of students could remove all seeds from an inflorescence-bearing stem and weigh the seeds and associated floral structures from inflorescences. The students in the group could repeat this for each of the inflorescence-bearing stems they collected. They could then run a regression analysis to determine if the mass of seeds can be predicted by knowing the length of the inflorescence-bearing stem; students would plot the relationship between inflorescence-bearing stem length and reproductive biomass. Ultimately, there could be an even more elaborate method for determining total seed production that would involve counting individual seeds after weighing the reproductive structures, but it would be impractical to complete this confirmatory study in a lab of this duration.

Questions for Further Thought and Discussion

Questions on basic lab concepts for pre-lab discussion and formative evaluation

1. What is herbivory? Name three types of animals that act as herbivores and the plant tissues that they feed on. Are all plants and all plant tissues equally available as sources of nutrition to herbivores?
2. What are the predictions made by the *Plant Vigor* and *Plant Stress Hypotheses*? How will we test each of these hypotheses? For information about these competing hypotheses see Price (1991).
3. What are the predictions made by the *Herbivore Impact*, *Herbivore Tolerance*, and *Overcompensation* hypotheses? How will we test each of these hypotheses? The three possible plant reproductive responses are often considered as part of a *Continuum of Response* (Maschinski and Whitham 1989) or within the broader framework of theories about *Plant Tolerance* to herbivory (Fornoni et al. 2003).
4. Develop a timeline that illustrates the sequence of events that must occur if the assumptions made by this study are correct, i.e. A. differences in plant growth due to environmental factors, B. selection of oviposition site based on plant size, C. gall formation, and D. development of inflorescences and seeds that is influenced by gall formation.

Questions to consider in writing Brief Communication upon completion of the field portion of the lab (Instructor may want to specify which question(s) students will focus on in the Brief Communication)

1. Plant Vigor versus Plant Stress Hypothesis:
    a. Do herbivores affect plant growth in a way that might confound the results of your test of these two competing hypotheses? Consider the time of year when you are measuring stem height and the biology of this system; when did the herbivore make its oviposition decision? In light of this, are you making your measurement of plant size at the most appropriate time in the season to fully test these two competing hypotheses?

       Link to Literature: Examples of plants that exhibit a changed growth form following herbivory can be found in Weis and Kapelinski (1984) and Paige and Whitham (1987). How did a galling herbivore (Weis and Kapelinski 1984) and a mammalian herbivore (Paige and Whitham 1987) affect the growth of the plants these authors studied?
    b. Can you plan an experiment that would allow you to control or influence plant vigor and monitor or control herbivore attack, and thereby test these competing hypotheses?

       Link to Literature: As an example of such an experiment, Daane and Williams (2003) manipulated irrigation levels in a vineyard and asked how the differing hydric conditions might affect herbivore density of a major pest species (a leaf hopper) in California vineyards. According to the *Plant Vigor Hypothesis* what do you predict will be the effect of watering on leafhopper pest density? What do you predict according to the *Plant Stress Hypothesis*? What did the authors find? What did they say was the significance of their findings to the challenges of pest management in agricultural ecosystems?

c. Would you predict that herbivores with different modes of feeding, e.g. foliage feeders, and with greater mobility, compared with gall-forming insects, might choose to feed on plants that are more vigorous or more stressed? Would the habit of living and developing within a gall influence the type of plant a female might choose to oviposit on, compared with the choice that might be made by free-feeding insects such as leaf-chewing insects or phloem feeding insects, e.g. aphids? Explain your reasoning.

   Link to Literature: Huberty and Denno (2004) analyzed the evidence for support of the *Plant Stress Hypothesis* in light of empirical studies of herbivores of diverse feeding modes on plants that varied in physiological condition. Does the study by Huberty and Denno (2004) suggest that the *Plant Stress Hypothesis* is applicable to all herbivores regardless of feeding mode and the plant tissue they feed on? For example, do Huberty and Denno find that phloem feeders respond to plant stress in the same way that gall formers or free feeders do? Explain your reasoning.

2. Herbivore Impact, Tolerance, and Overcompensation:
   a. Are there environmental conditions encountered by plants that might influence their response to having tissue consumed by herbivores? Consider plants that are exposed to differing levels of needed resources including water, sunlight, and mineral nutrients and how they might respond to feeding by herbivores.

      Link to Literature: Could the reproductive response of plants following herbivory be influenced by the levels of available resources, as proposed in the *Continuum of Response Hypothesis* (Maschinski and Whitham 1989)? How does the Plant Tolerance model, in its broadest sense, explain or account for variation in plant response to herbivory (Wise and Abrahamson 2005; Fornoni et al. 2003)?
   b. *Gall Effect on Growth*: Does the presence of a gall influence the subsequent growth of a goldenrod stem? Further questions that might be addressed with regard to gall effects on subsequent growth include: A) do galls affect the ultimate height of the goldenrod stem, B) do galls affect apical dominance and lead to the production of more shoots through the release of dormant meristems? And C) do changes in growth caused by galls affect plant reproduction?

      Link to Literature: As cited in question 1A. above, Weis and Kapelinksi (1984) and Paige and Whitham (1987) studied plants that exhibited a changed growth form following herbivory. Did attack by herbivores in these two different systems affect both plant growth form and also reproductive output? Further, McCrea et al. (1985) studied the effect of ball galls on carbon allocation in goldenrod. What conclusions did they make concerning gall effects on carbon allocation and plant growth?
3. Overarching Conceptual Questions:
   a. Do you think it is realistic to predict that either the *Plant Stress Hypothesis* or the *Plant Vigor Hypothesis* can explain the totality of the ecological interaction between plants and their herbivores?

      Link to Literature: (A) Briefly summarize the major findings of the following three papers: (1) Hull-Sanders and Eubanks (2005), (2) Koricheva et al. (1998), and (3), Huberty and Denno (2004). (B) Critique and reflect upon what these papers tell us about the value and contribution of these simple hypotheses to explain complex ecological phenomena.

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Tools for Assessment of Student Learning Outcomes

A **pre-lab formative assessment** is conducted to prepare students to participate fully in the lab exercise. Students must read the Abstract, Introduction, and Methods sections of the lab exercise before coming to lab. A brief discussion with students, of approximately 10-15 minutes, should lead to review of basic ecology of herbivory and plant-herbivore interactions and the major conceptual issues associated with this lab. Upon conclusion of the discussion, students should write a one minute paper on misconceptions they had, before the discussion, concerning the interactions between plants and herbivores.

Next, students are assessed for this exercise based on prompt sharing of data. If the field portion of the lab is completed promptly, students can return to lab and type their results into a spreadsheet that has been prepared for the entire lab group. If the field portion of the lab runs long and data can't be logged back in the lab, students in each group have the responsibility to send to the instructor a copy of their data in a pre-defined format in an MS Excel spreadsheet by the end of the day (e.g. 5PM for a morning lab). I then forward the entire data set to all students in the class. I assign a small number of points to the members of each group for Data Sharing; if a group fails to send data in a timely way, all members of that group are penalized for failure to complete this part of the assignment.

Students must turn in a lab report in the form of a *Brief Communication*, not unlike short communications or brief communications in several journals. My intention with this lab report is to encourage students to write succinctly and clearly and to encourage them to produce a complete scientific report. In the report, students are asked to clearly present: 1) the goals of the study, 2) the methods used, 3) the results, including verbal description of the statistical analyses and the figures through which they test their hypotheses, 4) an interpretation of the results in light of the concept they tested in light of the current literature.

The Brief Communication consists of all parts of a traditional lab report, except without an abstract. Students must have a brief but descriptive title for the lab exercise. Students must write a brief Introduction, typically two paragraphs in length with one paragraph focused on the primary concepts in ecology that they will study, and the second paragraph presenting the hypotheses they planned to test. The Methods section is generally fairly brief; students may refer to the lab handout for the methods section, but the lab handout does not count as one of the two citations required for the assignment. The Results section includes figures (at least three for this lab exercise), statistical tests presented in tables (at least two for this exercise), and a verbal description of the study in which they refer to the figures, statistical tests, and tables. Students present a Discussion in which they interpret the outcome of the study and interpret why they obtained the particular results they did. In the Discussion, students must address one or more of the **Questions for Further Thought** (as recommended by the Instructor); these questions involve the student in higher order thinking, in which they must address issues or concerns about the biology of the organisms involved in this study or the basic ecological theory that is addressed through this lab exercise. The paper must include a Literature Cited section with at least two citations from the primary literature; citations must be used in the Introduction and or Discussion. Students use the citation format for articles from the primary literature using the format used in the **References and Links** section.

Point allocation totaling 10 points, for the different parts of the lab exercise are as follows (see 'Appendix 2: Scoring Rubric') for suggested point distributions for each section in the report):

Title	0.5 points	
Introduction	2.5 points	
Methods	1.0 points	
Results	3.0 points	(1.25 points for verbal description, 1.25 points for tables/figures, 0.50 points for statistical tests)
Discussion	2.5 points	(1.25 points for conclusions regarding tests of hypotheses, 1.25 points for use of literature to address Questions for Further Thought)
Lit Cited	0.5 points	

NOTES TO FACULTY

Challenges to Anticipate and Solve

Challenge 1. *Goldenrod and Gall Identification*: Students must learn to identify the goldenrod that they choose to study and the types of galls on plants in their study system; in SE Pennsylvania, I commonly find both the ball gall and the rosette gall, so I have asked classes to study the hypotheses related to plant vigor and herbivore impact in response to a different herbivore in different years, e.g. in year 1 study the ball gall and in year 2 study the rosette gall. In some years, you must choose a particular gall type due to its greater abundance while the second species may be less abundant in that same year. Carefully dissect a ball gall to show students the larvae that can be found within the gall (Figure 4).

Figure 4. A ball gall formed by Eurosta solidaginis that has been dissected to show the larva in the interior of the gall. A ruler is included to provide scale.

Challenge 2. *Estimating Goldenrod Shoot Height*: Students often ask exactly which part of the plant stem to measure. I encourage students to measure from the ground to the apical bud on the tallest stem of the plant. Frequently, there are many lateral stems on plants that are attacked by the rosette gall, which appears to affect the apical dominance of the main stem of the plant; I still encourage students to measure from the ground to the height of the tallest stem, even if it the tallest stem is a branch formed by release of an axillary bud.

An important assumption of this study is that the gall former does not appreciably alter the growth of the stem of the plant. This assumption is likely violated in this study system since we are measuring the stems long after herbivores have attacked the goldenrod stems, and you will probably find that growth of the stem is altered by the presence of the gall. To more carefully test the *Plant Vigor Hypothesis* and the *Plant Stress Hypothesis*, it would be best to measure plant size at, or near, the time when the female gall forming insect oviposits and the gall is initiated. In the goldenrod system, stem height on galled and ungalled stems should be measured in late June or early July. I ask students to consider factors that may have influenced the results they obtain. The time of year when we conduct this study and the effect of the gall on plant growth are among the complicating factors. The results of the test of the *Plant Vigor* and *Plant Stress Hypotheses* may be influenced profoundly by the effect of the gall on plant growth and the time of year when the students record plant height on galled and ungalled stems.

Challenge 3. *Estimating Goldenrod Reproduction*: Students often ask which part of the stems that bear inflorescences should be measured. I encourage students to measure the portion of the stem from the location of the first inflorescence to the end of the stem where the last inflorescence is located. Measurement of the number of seeds produced by goldenrod could be a real challenge; as a member of the plant family Asteraceae, each 'flower' is a composite inflorescence with many flowers and many seeds. Further, there can be many inflorescences on a single stem and there can be few to many stems with inflorescences on an individual plant. So, for expediency, we make the grand assumption that cumulative length of stems bearing inflorescences is a proxy for seed production and fitness. I am unaware of studies that have verified the assumption that length of stems bearing composite inflorescences is correlated with total seed production. However, the use of biomass data that is correlated with goldenrod seed production, rather than counts of seeds themselves, has been used in past studies of the effect of galls on goldenrod reproduction (Hartnett and Abrahamson 1979).

Students should be aware that measurement of length of stems bearing inflorescences may be a very good estimator of seed output or it may, in reality, be an inadequate predictor of seed number and ultimately plant fitness. In the discussion, students may speculate about the methods they might use to determine if length alone is a good indicator of seed output. One or two student groups may focus on specifically addressing this issue by measuring the relationship between length of stems bearing inflorescences with some other trait associated with reproduction such as biomass.

For their analysis of herbivore impact, students may ask how to deal with plants on which no inflorescences were found. I encourage students to count stems with 0 inflorescences, using a measure of 0 cm length, in making their estimates of plant reproductive output. In their spreadsheet, every galled plant and ungalled plant for which they recorded length of stems with inflorescences should be represented, even if the value is a zero when the plant has produced no inflorescences.

Challenge 4. *Statistical Analyses*: Students may need guidance about which statistic to use in comparing galled vs. ungalled plants for both the *Plant Vigor/Plant Stress Hypothesis* and for the *Herbivore Impact Hypotheses*. Students should be aware that they should use a paired t-test, since the ungalled and galled stems that were studied were selected due to their proximity to one another, so they should be treated as non-independent samples for purpose of statistical testing. Instructions on how to run a paired t-test are provided in the 'Statistics Appendix'.

Experiment Description

Within the framework of a General Ecology course, this lab has been taught under the heading of: 1) *Coevolution* and *Mutualism* in Ricklef's, *The Economy of Nature*, Fifth Edition and 2) Predation in Smith and Smith, *Elements of Ecology*, Fifth Edition, and might also be taught in the context of Trophic Relations.

Introducing the Lab to Your Students: There are several published studies that demonstrate that in some insects, females oviposit or insects feed on the most vigorously growing plants or plant parts (Craig et al. 1988, Fritz et al. 2003, Hull-Sanders and Eubanks 2005). For other herbivorous insect species, there is support for the idea that herbivores attack stressed plants (White 1984, Hull-Sanders and Eubanks 2005). Finally, some authors report that they can support neither the *Plant Vigor* nor the *Plant Stress Hypotheses* (Williams and Cronin 2004). Huberty and Denno (2004) survey the literature on tests of the *Plant Stress Hypothesis* and they report that herbivore-feeding mode can play an important role in determining whether the herbivore responds to plant stress.

Plant Vigor: Types of herbivory and applying ideas to other systems

Different species of herbivores obtain nutrition by feeding on a variety of plant tissues, using mouthparts that are adapted to allow the insect to obtain food from the particular tissues on which they feed. Types of herbivorous insects include: 1) free–feeders which include a) foliage feeders such as Monarch caterpillar larvae or potato beetle larvae or b) sap feeders, such as aphids that feed on phloem fluids, 2) gall formers whose offspring feed on gall tissue or on plant sap obtained from a location within the gall, and 3) leaf miners where insects feed on plant tissue located between the epidermal layers of the leaf. Students could study how herbivores in different feeding guilds respond to plant vigor or plant stress by comparing the size of plant parts attacked by herbivores and the sizes of those same parts that are not attacked.

If the instructor would like to conduct this study using another type of herbivore, or if students are given the chance to independently choose a plant and herbivore for study, ***some or all*** ideas in this lab might be suitably tested using foliage feeders, leaf miners, or other herbivores whose presence on a plant or plant part, e.g. a mine or chewing damage, can be documented. *The Plant Vigor* and the *Plant Stress Hypotheses* can be tested once the presence of an herbivore (or signs of the damage an herbivore causes, e.g. chewing) is documented. By measuring the size of the plant part the herbivore has attacked, e.g. the area of a leaf attacked by a leaf miner or a foliage feeder, and comparing this size measure to similar measures taken from unattacked plant parts, one can test these hypotheses. In many parts of North America, galls on oak leaves would permit study of the *Plant Vigor* and *Plant Stress Hypotheses*.

The hypotheses about the reproductive response of plants to herbivores can only be tested if flowers or fruits are present on the plant at the time of year when the study is conducted. Not every plant species will bear indicators of plant reproduction during the fall, when this lab exercise is most often conducted.

Other gall formers that might be suitable for this lab exercise include the different types of galls formed on many species of willows, particularly stem galls. Stem galls are retained into the winter, simplifying identification and measurement of galled and ungalled stems after the growing season. Further, many (but not all) willows produce enlarged reproductive buds in fall that will produce catkins the next spring; these reproductive buds can be counted on galled and ungalled stems and are indicators of future reproduction (Sacchi et al. 1988). Since willows are dioecious, with separate male and female plants, one can only conclude that reproductive output is or is not affected by herbivores. With willows attacked by stem gall-forming insects, both the *Plant Vigor* and the *Herbivore Impact Hypotheses* can be studied. See the *TIEE* laboratory experiment by Ernest (2005) that includes a thorough description of leaf galls and provides mention of the stem galls formed on willows.

An additional study system in eastern North America, particularly in the mid-Atlantic and southeastern US, is the flowering dogwood, *Cornus florida* and the dogwood club gall*, Reselliela clavula*. Sacchi and Connor (1998) studied reproductive effects of the club gall on flowering dogwood; since the dogwood produces inflorescence buds in the fall, one can survey the length of stems attacked by the club gall to test the *Plant Vigor Hypothesis* and the presence or absence of inflorescence buds on galled and ungalled shoots allows one to test the effect of herbivores on plant reproduction. Thus, all questions discussed in this lab exercise could be successfully studied using the dogwood-club gall system.

Herbivore Impact

In coevolutionary theory relating to the evolution of herbivores and plant defenses, it was traditionally assumed that an herbivore would need to reduce plant fitness (Strong et al. 1984). In several study systems, the negative effects of herbivores on plant reproductive structures have been documented (Sacchi et al. 1988; Wise and Sacchi 1996).

Tolerance is a strategy whereby a plant can be damaged by herbivores but the plant exhibits little or no reduction in plant fitness (Strauss and Zangerl 2002, pp. 88-89). Levels of plant tolerance to herbivory can range from little loss in fitness, to no loss, to overcompensation in fitness (Strauss and Zangerl 2002, p. 89).

The case for overcompensation following plant damage by an herbivore has been an area that has been controversial. Paige and Whitham (1987) studied a particular plant-herbivore interaction, between elk and the monocarpic *Ipomopsis aggregata*, and they documented an instance of *Overcompensation*, in which plants fed on by elk produced more seeds than plants that were not fed on by elk. The work by Paige and Whitham has stimulated a vigorous debate (see Crawley 1997, p. 443) about overcompensation and whether this could be an infrequent or a common phenomenon.

Constructivist Approach and Inquiry

Some faculty members may prefer to lead students to study the hypotheses posed in this lab exercise in the context of a constructivist and basic inquiry approach to the exercise. In this case, students could be told that they will study plants and herbivores, to list all factors that they think might influence plants and herbivores, and students could visit field sites where they would look for plant-herbivore systems to study. Students would be guided to measure any attributes of plants or herbivores that they consider to be important to explain the abundance of herbivores on plants. Students would analyze data upon return to the lab, they would be asked to look for patterns in the data, and they would then be asked to define questions and hypotheses they would like to study to account for the patterns they have observed. The students would then plan a study they would conduct to test their hypotheses and would return to the field to conduct this study. The instructor would discuss with students their findings along the way and could guide students to appropriate citations in the plant-herbivore literature.

Activities in the Lab

The information in this lab should be fairly straightforward to present to students. The non-technical nature of the data collection should allow the data collection portion of the lab to proceed relatively quickly.

A blank data sheet is provided to help students to structure their data collection; you may choose to give the blank data sheet appended to this exercise to your students.

This lab can be done after the first frost in the fall, since the goldenrod seems to retain its inflorescences and seeds into November. This lab works well when you are looking to conduct a lab that does not involve study of actively growing plants or animals.

Faculty members interested in the relationship between the *Plant Stress Hypothesis* and herbivores in different feeding guilds, e.g. phloem feeders, foliage feeders, gall formers, are encouraged to read the careful review and analysis of the many studies on insects in diverse feeding guilds by Huberty and Denno (2004).

See the section on **Introducing the Lab to Your Students** for ideas about other study systems that can be used to test the ideas in this lab exercise.

Questions for Further Thought

The questions for further thought are useful in several contexts. First, there are Formative Assessment questions that should be discussed in the lab with students before they conduct the exercise to assure that students have a basic understanding of the concepts that will be studied in this lab. Second, there are a variety of questions that would be helpful to students in interpreting the data they have analyzed. Further, the questions also provide students information that they might consider in writing the discussion of their Brief Communication; all questions provide students with a link to the literature so that they may use not only their personal experience and impressions in addressing the questions but also a primary literature source. As this lab exercise is written, there are probably more **Questions for Further Thought** than any one student would have time to research and consider in a one week lab assignment. The instructor for the course will probably want to choose one or two of the **Questions for Further Thought** for students to consider as they write their Brief Communication.

Students are often surprised to learn that plants differ, both between and within plant species, in their suitability as food for herbivores. The idea that plants can actively defend themselves is a concept that many students are only vaguely familiar with, but they can easily grasp this concept with some discussion about structural and chemical defenses in the pre-lab introduction. Further discussion will also clarify the idea that plant physiological status may affect plant vulnerability to herbivores leading to the contrasting ideas represented by the *Plant Vigor* and *Plant Stress Hypotheses*. Many students assume that plants will be damaged if fed upon by herbivores based on the common perception that in gardens and on farms herbivores reduce yield of crop plants. Many students are often surprised to learn that native plants may be tolerant of damage caused by herbivores and that in some cases plants may overcompensate in their reproductive output following reproduction compared with plants that have not been attacked by herbivores.

Assessment of Student Learning Outcomes

Student learning outcomes are assessed based on: 1) the pre-lab formative evaluation represented by the one minute paper about misconceptions students had before the pre-lab discussion, and 2) the Brief Communication. A detailed rubric is provided for instructor use in assessment of the Brief Communication the students have written. Lab objectives are assessed through the combination of the formative evaluation and the Brief Communication. The expectation that students will use higher level thinking in completing this exercise is addressed by student responses to **Questions for Further Thought** either submitted separately or embedded in their Brief Communication.

Evaluation of the Lab Activity

Three different formats for a post-lab formative evaluation will be suggested. Each is intended to provide the instructor with information about student perceptions of the lab and about student learning so that the lab might be improved in the future. It is assumed that you will choose one of these three formative evaluation formats that best suits your needs.

A simple type of formative evaluation of the lab activity that may be given to students when the lab has been completed could consist of two simple questions: what was the part of the lab you liked the best, and what part or parts of the lab should we change for the future to make things work better? By asking these questions, we get an idea of whether students have identified the goals that we've established for the exercise and with appropriate feedback, we can plan to adapt the lab in the future. This is not a highly structured formative evaluation but it provides rapid feedback from students about the lab exercise.

A second format for a formative evaluation of the lab activity is to ask students what they thought of the lab as suggested by the author. Questions in this survey might include: 1) What aspect of the lab helped you learn the most, and 2) what part of the lab was most confusing? Responses to these questions could provide some indication of how to improve student learning.

A third format for a formative evaluation of the lab activity would be to use a directed evaluation in which you ask students to rate each activity on a 1-5 scale on the degree to which the activity helped with student understanding of the topics studied. Students could rate: introductory lecture, discussion questions, data collection, data analysis, Brief Communication assignment, use of scientific literature, discussions with lab partners, etc. The information provided in such a survey could provide some indication of how to improve the lab exercise in the future.

Extensive notes on how to conduct formative evaluation are in the Teaching Resources sector of *TIEE* in an ESSAY ON EVALUATION.

Translating the Activity to Other Institutional Scales or Locations

This lab involves non-destructive study of plants in old fields using simple supplies such as rulers and tape meters. Multiple lab groups in a larger multi-section ecology course at a larger university could do this lab on separate days or in separate areas of the same field, as long as there is a large enough stand of goldenrod available.

In the section, **Introducing the Lab to Your Students**, there are ideas for several alternative study systems for conducting this lab including gall formers on willows (different willow species and gall formers can be found throughout North America), dogwood and the dogwood club gall found in eastern North America, and oaks and their gall formers (cynipid wasps cause galls on leaves and other plant parts in oaks). In urban areas, the *Plant Vigor* and *Plant Stress Hypotheses* may be studied on oaks in parks or in botanical gardens. Further, the lab may be conducted in abandoned lots or unmowed areas in parks that harbor goldenrod or weeds such as lamb's quarters (*Chenopodium album*); lamb's quarters are likely to have leaf miners that would allow testing of the *Plant Vigor* and *Plant Stress Hypotheses*. Community gardens may hold plants that have insects on them that would allow tests of the *Plant Vigor* or *Plant Stress Hypotheses*, e.g. *Brassica* including cabbage, broccoli, or brussel sprouts and the larvae of *Pieris rapae*.

Additionally, there are ideas in **Introducing the Lab to Your Students** that describe how the instructor might guide students to be more actively involved in selecting the study systems that they choose or that would allow students to use a constructivist and inquiry approach in formulating the questions that they would study.

This lab could be easily used in the pre-college setting; t-statistics might be eliminated for younger students, but interpretation of effects could be based on plots of averages for data collected to test both of the major hypotheses presented in the exercise.

STUDENT COLLECTED DATA

Appendix 3: Excel data sheet. Access all three worksheets. Each worksheet provides data for graphs that students were asked to analyze using statistical tests, and to plot using graphing software. The statistical tests are completed on the spreadsheet.

Teaching Issues and Experiments in Ecology (TIEE) is a project of the Education and Human Resources Committee of the Ecological Society of America (http://tiee.ecoed.net).