Learning in An Inquiry-Driven Plant Physiology Laboratory

Robert J. Joly,* Michelle L. Jones, Sven Verlinden, David Rhodes, and William R. Woodson

ABSTRACT

Plant physiology is best explored through participatory activities, where students are closely engaged in the process of discovery. A two-stage approach to instruction was implemented to enrich the learning in an undergraduate plant physiology laboratory. First, a core of 10 experiments was developed to provide structured, hands-on experience and to teach the principles and skills of modern physiological analyses. During Week 11 of a 15-wk semester, students observe a demonstration of a plant response, where the underlying cause of the phenomenon is not evident. Working together in small groups, students hypothesize on the mechanisms that may be involved. They choose among alternative lines of inquiry and decide which variables will be most informative in revealing the underlying nature of the problem. Each research team presents a study plan, and hypotheses and experimental approaches are defended. Results of team experiments are presented during Week 15. To date, inquiry-based projects have focused on the roles of (i) ethylene in seedling development in mouse-ear cress [*Arabidopsis thaliana* L. (Heynh.)] and tomato (*Lycopersicon esculentum* Mill.), (ii) glycinebetaine in drought tolerance of maize (*Zea mays* L.), (iii) catalase in chilling sensitivity of tomato, and (iv) ascorbic acid in oxidative stress in tomato. The *Chilling-Injury Problem* utilized in 1996 and 1997 is described, and pedagogical advantages as well as challenges to implementation are discussed. The approach appears to foster a capacity for connective, integrative thinking. Students develop competency in judging the reliability and significance of experimental results, and in evaluating whether their conclusions are justified by the data.

TEACHERS of plant physiology face a dilemma in their dual role as disseminators of disciplinary knowledge and teachers of the scientific process itself. The laboratory should be a place where students learn not only the fundamental methodologies of plant physiology but also experience the process of discovery in a direct and personal way. Students should experience the process of science, not simply the content.

The concept of inquiry-based laboratories is not novel (Stedman, 1986), and there are numerous reports of its use, especially in the physical and chemical sciences (e.g., Allen et al., 1986; Arons, 1993; Lee and Jones, 1993). There are few reports of its adoption within a plant sciences curriculum, however, and we are not aware of any published reports describing the merits and challenges of guided inquiry in a plant physiology laboratory. Nevertheless, both the rapid response of plants to their environment and the multifaceted nature of the response (molecular, biochemical, physiological, anatomical, morphological) make these organisms ideally suited for inquiry-oriented student projects.

A typical experiment in a college science laboratory is often really only an exercise designed to illustrate a well-established principle (Arons, 1993; Igelsrud and Leonard, 1988). Many students never understand what hypothesis, if any, is actually being tested. They typically are not involved in the design of the experiment, nor do they understand how the results they obtain relate to any hypothesis (Allen et al., 1986; Janners, 1988; Tinnesand and Chan, 1987). Such an approach may be useful in illustrating the concepts and processes under discussion, but it does little to convey the sense of excitement that comes from probing a complex system and making a discovery.

Our premise has been that teachers should strive to maintain an appropriate balance between presenting factual, disciplinary knowledge and allowing students to frame questions and be personally engaged in the process of inquiry (Leonard 1989a, 1989b). This tenet formed the basis of our plan to restructure plant physiology instruction at Purdue University. During the period from 1994 to 1998, we shifted the focus of the 15-wk laboratory associated with the class Plant Physiology (HORT 301) to a more active, participatory experience. Our goal has been to provide an instructional structure in the undergraduate plant physiology laboratory where each student is actively and personally engaged in the process of inquiry. A two-stage approach to achieving this goal has been implemented and is described below.

Learning Objectives

Before implementation of the inquiry-based approach, we established four primary objectives of the project. First, students will participate directly in the process of inquiry by conducting experiments they have, in large part, designed themselves. Second, students' capacities for analytical thinking and problem solving will be enhanced. Students will make informed decisions, choose among alternative lines of inquiry, and decide which physiological variables will be most informative in revealing the underlying nature of the problem. Third, improvement of students' written and oral communication skills will be fostered by the need to present their ideas in small groups, to defend their approach before their peers and professors, and to explain the outcome and significance of their results in writing. Finally, the inquiry-based approach will develop the capacity for connective, integrative thinking. Students will develop competency in assessing the methods used to answer a question, in judging the reliability and significance of the reported results, and in evaluating whether their conclusions are justified by the data.

Course Background

The Plant Physiology class (HORT 301) is a 4-credit-hour course at Purdue University. The class surveys the basic phys-

---

R.J. Joly, and D. Rhodes, Dep. of Horticulture and Landscape Architecture, 1165 Horticulture Bldg., Purdue Univ., West Lafayette, IN 47907-1165; M.L. Jones, Dep. of Horticulture and Landscape Architecture, Colorado State Univ., Fort Collins, CO 80523; S. Verlinden, Div. of Plant and Soil Sciences, West Virginia Univ., Morgantown, WV 26506; and W.R. Woodson, Agric. Res. Programs, Agric. Administration Bldg., Purdue Univ., West Lafayette, IN 47907-1165. Publ. no. 16,034 of the Purdue Univ. Office of Agric. Res. Programs. Received 6 July 1999. *Corresponding author (joly@purdue.edu).

Published in J. Nat. Resour. Life Sci. Educ. 29:31–35 (2000).
http://www.JNRLSE.org

iological processes of higher plants, particularly as related to the influence of environmental factors on growth, metabolism, and reproduction. The typical enrollment is 60 third- and fourth-year undergraduate students. The laboratory sections are 2-h periods, and approximately 20 students are assigned to each of three sections, all held on 1 d. Prerequisites include undergraduate courses in plant biology and organic chemistry. The HORT 301 class is required of all horticulture majors. In addition, approximately 25% of the students are from biological sciences, agronomy, botany and plant pathology, and forestry and natural resources. These students are enrolled either on an elective or required basis, depending on their departmental curricula.

LABORATORY OVERVIEW AND INSTRUCTIONAL APPROACH

A core of 10 laboratory experiments was designed to complement the concepts and processes introduced in lectures. The objectives of the core labs are to (i) provide structured, hands-on experience with the study of plant physiology, (ii) teach the principles and skills used in modern techniques of measurement, and (iii) reinforce the idea that numerous unresolved questions exist in plant physiology. Students are introduced to a variety of analytical methods, including spectrophotometry, electrophoresis, thin-layer and gas chromatography, as well as measurements of growth, photosynthetic oxygen evolution, leaf water relations, leaf gas exchange, and chlorophyll fluorescence. Lab topics include: spectrophotometric analysis of chlorophyll, enzyme extraction and kinetics of alpha-amylase, thin-layer chromatographic separation of anthocyanins, light reactions of photosynthesis as measured via oxygen evolution from isolated chloroplasts, drought and salt effects on leaf water potential and transpiration, estimation of osmotic potential and turgor pressure, the influence of environmental variables on CO_2 and water vapor exchange rates in intact leaves, and the effects of plant hormones on growth and development.

The second stage of the laboratory provides students with opportunities for active, personal participation in the process of inquiry. During the 11th wk of the semester, students are asked to observe a demonstration of a plant response or behavior, where the underlying cause of the phenomenon is not evident (or indeed may not be known). They are challenged to develop hypotheses regarding how and why plants treated in a particular manner responded as they did. The instructors must select problems for which substantive answers to focused questions can be obtained within 3 to 4 wk. The inquiry-driven component of this laboratory has the following characteristics.

1. The problem may focus on physiological changes that occur during development, responses to environmental factors (water, light, temperature, humidity), transition between vegetative and reproductive phases, or on physiological differences arising from specific mutations.
2. Students work together in teams of three or four. Specific assignments are shared among group members, maximizing individual participation. Three faculty instructors and two graduate teaching assistants serve as advisors and information resources during the hypothesis formation and experimental design phases of the project.
3. Each team presents a plan of study to a laboratory instructor and defends their hypotheses and experimental approaches. Criticisms by the instructor at this stage may result in the formulation of new experiments or reconfiguration of the original plan.
4. A team may choose to pursue several experimental approaches simultaneously or to conduct a logical sequence of experiments, where the direction of inquiry is dictated by what has been learned at each step. In short, the structure permits open-ended inquiry.
5. Each student prepares a final written report, and each research team presents a 20-min oral summary of their project to students in their laboratory section during the final week of the semester. Students, led by the instructors, discuss and evaluate the strengths, weaknesses, and significance of the results.

We employed an inquiry-based approach to teaching the HORT 301 laboratory during each year from 1994 to 1998. The problem chosen by the instructors varied from year to year, and was developed from recent observations in our own laboratories or from current literature. To date, inquiry-based projects have focused on the roles of (i) ethylene in seedling development in mouse-ear cress [*Arabidopsis thaliana* (L.) Heynh.] and tomato (*Lycopersicon esculentum* Mill.), (ii) glycinebetaine in drought tolerance of maize, (iii) catalase in chilling sensitivity of tomato, and (iv) ascorbic acid in oxidative stress in tomato. We describe below the chilling-injury problem utilized in 1996 and 1997.

The Chilling-Injury Laboratory “Problem”

The problem chosen for student-directed research in 1996 and 1997 focused on chilling injury in tomato plants and on the possible role of catalase in ameliorating the damage. Students were asked to observe seedlings that had been chilled at 4°C in darkness for 4 h and returned to room temperature for at least 4 h. They observed the extent of physical damage relative to unchilled controls and to seedlings that had been sprayed with H_2O_2 4 h before chilling.

Students were provided in advance with background information on chill-induced metabolic and physiological dysfunctions in plants of subtropical origin. In addition, chilling injury in plants was the topic of a 50-min lecture during the week before the initial laboratory. A full-text version of the “HORT 301 Chilling-Injury Laboratory Problem” was made available to students, and can be obtained via the Internet (http://www.hort.purdue.edu/hort/people/faculty/joly.html).

Research teams were challenged to hypothesize on the physiological mechanisms that may have been involved in the chilling injury they observed. After formalizing their hypotheses, students were expected to choose among alternative lines of inquiry and to decide which variables would be most informative in revealing the underlying nature of the injury. At the conclusion of the 2-h laboratory session, students were expected to have: (i) clearly articulated their hypothesis, (ii) designed one or more experiments and decided upon treatments, (iii) provided an outline of the experiment(s) to the lab instructor, and (iv) provided a list of equipment, materials, and growth chamber requirements needed to conduct the study. Each research team was provided with a fixed number of seedlings. Teams could choose one or more age classes of seedlings. In addition, transgenic tomato plants expressing low catalase activity and their corresponding nontransformed, wild type controls were each made available for experimentation.

RESULTS AND DISCUSSION

Student Learning from the Chilling-Injury Problem

For most students, the chilling-injury project was their first direct experience with the scientific process; this was confirmed through numerous unsolicited comments. Although no formal comparison of the inquiry-driven laboratory vs. a corresponding verification-type experiment was performed, the following positive outcomes were observed by the instructors.

First, students gained an appreciation for the nature of scientific inquiry as a *process*. They learned that if their data did not support their hypothesis, that outcome was not a failure. Rather, it could lead to other questions that may deepen their understanding of a biological phenomenon. Students came to appreciate the complexity of a genuine physiological problem, and they learned how to apply knowledge acquired from one experiment to help them hypothesize and design the next experiment. They learned that results often are inconclusive. Importantly, students developed a clearer understanding of the concept of *hypothesis* itself; they learned that science is not merely techniques and description, but rather a seeking of knowledge and understanding.

Secondly, students learned firsthand of the practical difficulties that arise during the conduct of experiments and of the importance of minimizing experimental errors. They faced, often for the first time, the necessity of randomization and replication and the need to decide how best to evaluate and display their results. Further, because they had to manage their own experiments, students learned how to plan and utilize resources and to work cooperatively to maximize their efforts. Inquiry-driven student projects in HORT 301 require substantive effort over a sustained period of time. Working intensively within a small peer group for a month simulates, at least partially, the experience of working scientists interacting with each other within a contemporary university or industrial laboratory.

We observed that students developed new competency in judging the reliability and significance of experimental results. This began with a consideration of their own team's data. Students were forced to grapple with the interpretation of their findings and to consider whether their results were truly reliable and repeatable. They became adept at spotting the weaknesses and limitations of their own experiments. This thinking process was intensified during the final week of the projects, when students were asked to evaluate whether the conclusions drawn by other teams were justified by their data. Student questions to one another during this phase provided evidence that they had developed higher standards of proof. They challenged each other, and they learned to ask questions like "What is the evidence for your conclusion?" and "What would happen if …?"

An impartial observer visiting these inquiry-based laboratories would likely record a higher degree of intensity among students than is typically seen in an undergraduate science laboratory. Most students invested themselves in the problem to a much greater degree than in a typical laboratory exercise. They were challenged by the problem, and many were genuinely excited about their discoveries. A small group of students have actively sought additional independent-study coursework, following their experience with this and other inquiry projects.

Although student response to the inquiry-based approach was largely positive, it is apparent each time the laboratory is taught that some students are less comfortable in this relatively unstructured environment. Spears and Zollman (1977) discussed a similar observation made in a first-year undergraduate physics course. The authors concluded that students in an unstructured laboratory were not intellectually prepared to hypothesize and predict and that these individuals learned more effectively in a structured environment. Although students in HORT 301 typically are third- and fourth-year undergraduates, most have had little experience with self-sustained inquiry. We have found that it is essential to gradually introduce the idea of science as a process. The lecture portion of the class can be used to provide some focus on how scientists think and work rather than exclusively on the subject matter. Questions like "What kinds of experimentation would have been necessary for us to come to such a conclusion?" or questions that are inherently open-ended or that call for interpretation help prepare students intellectually for the inquiry laboratory.

Results of the Chilling-Injury Experiments

In addition to the observations reported above, the results obtained by students during their experimentation (Table 1) provide an indication of the breadth and sophistication of the studies undertaken. Students recognized that their team's approach was not the *only* valid way to seek to understand the problem. This was especially apparent during the oral reporting phase, where it was evident that multiple approaches each revealed something (positive, negative, or inconclusive) about the underlying nature of the injury. Multiple experiments had moved the class toward a consensus that chilling injury in tomato (i) is age-dependent, (ii) is manifested after, not during, the low temperature exposure, (iii) involves disruption to membrane integrity of Photosystem II, (iv) leads to significant alterations of leaf water relations and loss of the ability of stomata to retain leaf water, and (v) may be ameliorated in vivo by catalase activity.

CONCLUSIONS AND RECOMMENDATIONS

The positive outcomes described above validate the power of this approach in fostering student learning. However, the need for instructors to manage the student research on several levels (pedagogical and logistical) creates potent challenges for the adoption of inquiry-driven laboratories, especially where class size exceeds about 50 students.

Instructor Management of Student Research

Although the inquiry-driven laboratory emphasizes student-initiated planning and experimentation, the approach requires extensive preparation and intensive involvement by the instructors. Teachers who contemplate adopting an inquiry-based approach should consider the following.

1. The inquiry-driven approach provides a strong focusing goal (i.e., to understand the problem), yet minimal direc-

Table 1. Hypotheses developed and summary of results obtained by each of 11 teams of students in inquiry-driven studies of the physiological basis of chilling injury in tomato seedlings.

Research team members	Hypothesis	Results
Christa Mike Melisa	Plants exposed to chilling stress during the light period will sustain less membrane damage than plants chilled during the dark period. The extent of membrane damage will increase in proportion to the duration of chilling stress.	No differences were observed in leaf disk electrolyte leakage between dark- and light-chilled seedlings. Results were inconclusive, however, due to large experimental errors.
Eric Laura Katie Brent	Transgenic tomato plants with reduced catalase activity will be more susceptible to chilling injury, relative to wild type controls. Loss of catalase activity will reduce the ability of leaf tissue to degrade hydrogen peroxide, and this will result in greater tissue damage.	The hypothesis was supported by the results. Transgenic plants displayed lower catalase activity relative to wild type controls and were severely injured by either H_2O_2 pretreatment or by chilling. Catalase activity increased with H_2O_2 pretreatment in both chilled and nonchilled wild type seedlings.
Clay Melanie Christy	Chilling will decrease water uptake by roots and decrease transpiration from leaves. Leaf turgor will decrease as cell membranes become leaky.	Hypothesis no. 1 is accepted; a significant reduction in transpiration was measured in chilled seedlings compared with nonchilled controls. The effect was strongly age dependent, with 4-wk-old plants being more susceptible than 8-wk-old plants. Hypothesis no. 2 is accepted. Leaf turgor decreased in chilled plants, apparently as a result of loss of membrane integrity.
Scott Brian Cherie Amy	Leaf cell membranes will be damaged when seedlings are subjected to chilling. The quantum yield of Photosystem II will be reduced, and cellular membranes will become leaky to ions. Chilling during the light period will be more injurious than chilling during the dark period.	Eight-wk-old seedlings chilled in darkness exhibited significant reductions in both Fv/Fm and quantum yield of PSII, compared with nonchilled controls. Chilling in light was less injurious, and fluorescence variables were unaffected. Electrolyte leakage increased to a similar extent in both light- and dark-chilled plants.
James Clint Heather Kirk	Susceptibility to chilling injury is related to seedling age. Leaves and stems of younger plants (4 wk old) will sustain greater cellular membrane injury than older plants (8 wk old).	The hypothesis is supported by the results. Visual observations revealed that 4-wk-old plants were much more susceptible to chilling injury than 8-wk-old plants. A large increase in solute concentration of leaf sap was observed in chilled 4-wk-old seedlings, suggesting loss of membrane semipermeability and compartmentation. Age is a significant factor in the extent of injury sustained.
Justin Bryan Kelly Erica	Catalase activity will be induced by pretreatment of seedlings with hydrogen peroxide. This treatment will reduce the susceptibility of tomato plants to chilling injury. Transgenic tomato plants with low catalase activity will be more susceptible to chilling injury than nontransformed wild-type controls.	The data do not conclusively indicate a role for catalase in ameliorating chilling injury. Transgenic tomato plants expressed very low catalase activity, and these plants were killed by spraying with 3% H_2O_2. Therefore, the second hypothesis could not be tested. A new experiment using lower concentrations of H_2O_2 and greater replication is required.
Kate Linda Michelle Brian	Chilling injury affects the rate of net photosynthesis. The greater the injury, the lower the rate of photosynthesis and the slower the rate of recovery.	The results support the hypothesis. Net CO_2 assimilation rate was significantly reduced by chilling, and the degree of reduction was correlated with the length of treatment (1, 3, or 5 d). Visual symptoms of injury did not occur until seedlings were returned to nonchilling temperatures. This transfer was associated with a large increase in stomatal conductance to water vapor.
Megan Colleen Leah Tim	The seedling root system is more susceptible to chilling injury than the aerial portion of the plant. Warming the roots while the shoot is chilled will lessen the injury to the shoot.	Warming the root zone while seedling shoots were chilled reduced the chilling injury to the leaves, as assessed by visual scoring. Both leaf water potential and leaf turgor were higher in seedlings with warm roots, suggesting that roots may be an important site of chilling injury.
Colleen Phillip Tim Anne	Electrolyte leakage from leaves of chilled seedlings will increase in proportion to the duration of the chilling stress. Chlorophyll degradation will increase as the duration of chilling increases.	The hypotheses are supported by the data. Concentrations of both Chl *a* and Chl *b* decreased significantly following 1 d exposure to 4°C and then subsequently increased each day until, on Day 4, it was not different from controls. Ion leakage from leaf samples increased slightly as the duration of chilling stress increased up to 3 d. Then a large increase in leakage occurred on Day 4.
Patricia Elaine Luke	Net photosynthetic rate and chlorophyll fluorescence can be used as predictive indicators of the extent of chill-induced tissue damage.	The results were inconclusive. Both net photosynthesis and chlorophyll fluorescence parameters exhibited substantial experimental variability. No correlation to visual scores of injury were evident.
Rick Dwain Jason	Transgenic tomato plants with low catalase activity will be more susceptible to chilling injury, compared with nontransformed controls. Membrane injury, assessed by ion leakage from leaf samples, will be greater in seedlings with low catalase activity. Pretreatment with hydrogen peroxide before chilling will have a protective effect in wild type seedlings, but not in catalase-deficient transgenic seedlings.	Four-wk-old transgenic seedlings with low catalase activity exhibited greater ion leakage from leaf samples than did wild-type individuals. Further, visual scores of injury revealed that they were much more susceptible than wild type plants. The effect of H_2O_2 pretreatment was inconclusive, due to the toxic effect of the concentration used.

tions are provided. Thus, students must develop their own lines of inquiry and select from an array of methodologies they have learned during the semester. In this activity, the instructor may need to subtly guide the research team discussions. The nature of the guidance provided is critical to the quality of the learning attained. The challenge is to provide sufficient guidance to lead students into productive areas of thinking. If we give away too much, however, we risk defeating the most important intellectual component of the experience. In short, student thinking and insight must be cultivated with restraint.

2. Logistical considerations dictate that the overall array of projects be managed, at least to some extent. Not all student hypotheses can be tested expeditiously, and the lack of special instrumentation and environmental controls may preclude some areas of study. Further, instructors may need to limit the complexity of experiments that students design.

3. In the Chilling-Injury Problem described above, two graduate teaching assistants helped to establish and monitor the treatments chosen by the research teams. In this case, there were 11 discrete experiments, each with their own treatments and controls. This approach placed substantial demand on the teaching assistant's time.
4. Significant demands also occur before and during the conduct of the experiment. All equipment and supplies must be made available, and instruments must be ready to use. Students must be well informed of operational and safety considerations before using sophisticated equipment. We have provided an instructor/student ratio of approximately 1:4 to facilitate the conduct of experiments.

Guided Inquiry vs. Authentic Inquiry

In 5 yr of experience with this approach, we have learned that the nature of instructor guidance of the inquiry is critical to its vitality and success. Providing guidance for projects where the general nature of the response is already well understood by the instructors may lead to a high-quality learning experience for students, but it may fail to provide intellectual stimulation to the teacher-scientist. We have found that authentic investigations, where neither student nor teacher knows the outcome, provide a more compelling involvement. The heightened curiosity of the teacher becomes transparently obvious to students and helps to establish a collaborative environment in the laboratory. Thus, although the Chilling Injury Problem described here leads to effective learning, we are unlikely to repeat it in future unless new insights lead us into novel areas of experimentation.

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation Instrumentation and Laboratory Improvement Program (grant DUE-9451170) to R.J. Joly and W.R. Woodson. Transgenic tomato plants were developed and generously shared with the plant physiology class by Dr. Kanogwan Kerdnaimongkol during her Ph.D. studies in the Department of Horticulture and Landscape Architecture, Purdue University.

REFERENCES

Allen, J.B., L.N. Barker, and J.H. Ramsden. 1986. Guided inquiry laboratory. J. Chem. Educ. 63:533–534.

Arons, A.B. 1993. Guiding insight and inquiry in the introductory physics laboratory. Phys. Teacher 31:278–282.

Igelsrud, D., and W.H. Leonard. 1988. Labs: what research says about biology laboratory instruction. Am. Biol. Teach. 50:303–306.

Janners, M.Y. 1988. Inquiry, investigation, and communication in the student-directed laboratory. J. College Sci. Teach. 18:32–35.

Lee, J.A., and L.L. Jones. 1993. Teaching the process of science in geography courses. J. Geogr. 92:223–226.

Leonard, W.H. 1989a. An experimental test of an extended discretion laboratory approach for university general biology. J. Res. Sci. Teach. 26:79–91.

Leonard, W.H. 1989b. Research and teaching: ten years of research on investigative laboratory instruction strategies. J. College Sci. Teach. 18:304–306.

Spears, J., and D. Zollman. 1977. The influence of structured *versus* unstructured laboratory on students' understanding of the process of science. J. Res. Sci. Teach. 14:33–38.

Stedman, C.H. 1986. An examination of science texts from early America to the 1900s for evidence of inquiry oriented presentations. Doc. ED-271291. U.S. Dep. of Education. Educational Resources Inf. Center (ERIC). Austin Peay State Univ., Clarksville, TN.

Tinnesand, M., and A. Chan. 1987. Step 1: Throw out the instructions. Sci. Teach. 54:43–45.