Chapter 10

Crops of the Future: A Problem-Based Learning Exercise for the Laboratory

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Introduction

In problem-based learning (PBL), students identify, research, and analyze information they need to resolve the issues they encounter as they work through a complex problem, using processes that resemble those of scholarly inquiry in general. In an inquiry laboratory setting students design and carry out experiments to answer their own questions, using processes that more resemble those used to conduct scientific research. The exercise presented here is designed to integrate elements of these two pedagogical approaches—by adding a real-world context for students to frame their laboratory investigations, and providing students with the opportunity to ask and answer the questions they pose as they work through a PBL problem.

What is PBL?

The PBL cycle begins with complex problems that motivate interest in learning. As students work in small groups to reach initial understandings of the problem, they identify issues that need clarification, called "learning issues." The students assign responsibility for researching learning issues outside of class and discuss the best resources (e.g., textbooks, journals, newspapers, personal contacts, government agencies, online databases, etc.) for finding the information. When students reconvene, they give informal oral presentations to their groups on the results of out-of-class research, and reformulate their understandings based on this new information. Students continue to define and prioritize new learning issues as they home in on their group's resolution of the problem.

The PBL cycle challenges students to learn concepts in the context of their applications, think critically, communicate effectively, find and process new information, and become influential members of productive teams, therefore connecting with many of the basic skills needed to sustain habits of life-long learning. Instructors who incorporate PBL activities into their classroom are also striving to change the face of biology (in students' eyes) from abstract concepts that appear distant from real life to authentic problems that motivate the need for sound understandings of biological principles.

Who Owns the Geritol Solution?, which is published along with teaching notes in the *PBL Clearinghouse* (Allen, 2001) provides an example of a premise for a PBL in biology. The Geritol solution is based on the premise that iron availability limits primary productivity in the high-nutrient, low-productivity zones of certain ocean waters. John Martin, then from the Moss Landing Marine Biological Laboratory, proposed that by dosing these waters with an iron tonic, we could harness the latent primary productivity of marine phytoplankton to lessen the impact of excess carbon dioxide emissions on global warming. In the context of this "Geritol solution," students encounter and make connections between major concepts related to global biogeochemical cycles, cellular energy transformations, and marine ecosystems. They engage in environmental decision-making concerning use of the Geritol solution, particularly as it relates to its patenting and exclusive commercial use (for carbon sequestration and fish farming) by an environmental engineering firm. A synopsis of activities of students and instructors as this problem unfolds in an introductory biology course can be found in Allen and Tanner (2003); a more expanded version is included in the teaching notes in the PBL Clearinghouse (Allen, 2001).

Implementing PBL in the Laboratory

As is the case for any other instructional method, PBL has potential deficiencies. One of these is that although PBL problems strive to connect to the real world outside the classroom, they can sometimes fail to get beyond being paper-and-pencil activities that connect only with a student's “mind's eye” of experience in the real world. They may not require engagement in the types of concrete activities that are needed by some students to reach deeper understandings of the underlying biology concepts. Also, while the PBL cycle models procedural skills that resemble the general processes of scholarly inquiry, it may not explicitly model the "ways of knowing" specific to scientific inquiry. Connecting PBL problems to the laboratory setting is one way to address these potential deficiencies, and in turn, help to resolve issues that may arise with use of inquiry laboratories with novice investigators in introductory courses. These include failure to develop the background behind the hypothesis that is being tested (even when this is a required component of a subsequent write-up or report), or to see how an experiment they are doing could provide useful information to the world outside of a research laboratory. PBL also provides a structured cycle of learning activities to a guided inquiry setting, giving students a sense of how to engage in a problem-solving process in a laboratory context.

There are several ways in which implementation of PBL could play out in a laboratory setting. In the context of the PBL laboratory problem we describe in this chapter, these implementation alternatives could serve as an accompaniment to either a PBL or non-PBL “lecture” course. For example, in a PBL “lecture” course in which a problem about photosynthesis or global carbon cycles is being featured, a PBL lab exercise could either expand students’ conceptual understandings in these areas or give them opportunities to test hypotheses about the problem’s resolution. A specific example is the previously described “Who Owns the Geritol Solution” problem, which motivates students to explore the carbon cycle in the context of biochemical processes in a marine ecosystem, but does not extend their understandings about environmental factors that can alter the relative rates at which these processes occur in either marine or terrestrial ecosystems. Problem resolution depends on an understanding of net primary productivity, a concept that students often neglect if they hold the common misconception that plants do not respire. Both of these conceptual areas could form the basis of a PBL laboratory problem, while at the same time reinforcing the underlying concepts from the “lecture” problem and extending students’ views on the nature of scientific inquiry. These objectives for PBL laboratory implementation could also connect well to a “lecture” class using other instructional strategies—the instructor, for example, could lecture and/or guide class discussions about photosynthesis, then implement the laboratory exercise we present here in lieu of lecture coverage of connections to the global carbon cycle and role of environmental factors.

This is in fact what the co-authors have done. One co-author has used this laboratory PBL problem in the course of students' encounter with the Geritol solution problem in a two-semester introductory biology course for majors, while the other has used it as an accompaniment to a lecture- and discussion-based coverage of photosynthesis in another section of the same course. In the latter course, the students had little or no prior experience with PBL.

PBL problems ideally connect to real-world events, some of which can involve messy, ill-structured dilemmas that stretch the abilities of even the expert to resolve. However, the ideal PBL problem is written with multiple entry and exit points that allow for both novice and expert learners to reach resolutions that demonstrate varying degrees of insight. Therefore we have used and recommend this problem for introductory biology students, but also think it is suitable for intermediate and advanced courses in plant and environmental science.

This investigation as it is written requires special instruction in use of electronic equipment and data analysis software. Our introductory biology students have two prior encounters with this electronic data acquisition and analysis system before working on the investigation presented here. We do not feel that this prior exposure is essential if time is available to familiarize the students with how the system can be set-up and used. Alternatively, the problem could be rewritten such that its protagonists are hoping to fund the purchase of an electronic system through the grant they are seeking to obtain (see Student Outline section that follows), and such that the protagonists are conducting their pilot experiments using other methods to estimate photosynthetic rate (for example, by oxygen evolution or starch production).

In this chapter, we have reversed the typical ordering of its sections by placing the "Student Outline" before the "Notes for the Instructor"—we think that the Notes will be clearer if the Outline is read through first. In order to save space in this volume, the various stages of the problem are not separated out in the "Student Outline" section. However, in using the problem, we recommend a classic PBL "progressive disclosure" approach. That is, students receive the problem stages one by one, completing each stage before moving on to the next (exception: the "Problem Background" and "Stage 1" are designed to be distributed to the students at the same time). The "Notes for the Instructor" section includes a possible scheme for classroom management of the problem as it unfolds, including sequential timing of each stage of the problem.

Student Outline

Problem Background

Predictions of global warming resulting from an increase in atmospheric carbon dioxide – the so-called "greenhouse effect" – is in the news. However the reality of global warming is controversial, and if it is indeed real the magnitude over a period of time, say 100 years, is uncertain (Walker, 1992). A predicted increase in atmospheric carbon dioxide concentration, however, is not controversial and not small; it could be 2- to 3-fold after a century of fossil fuel consumption at the current rate (Walker, 1992).

But what might be the effects of global warming and increased atmospheric carbon dioxide on ecosystems and agriculture? One might think that both factors would boost plant growth and crop yield. For agriculture this could be a good thing. Well, let’s not be too hasty here. There are many factors, both physical and biological, that could be affected by increases in these two components, and therefore prediction needs to be backed with experimentation; i.e. with some data.

One might be surprised by inferences drawn from experimental data. Consider the following quotation from an article that appeared in a recent issue of *Science News* (Perkins, 2003:260):

> “During a long-term research project in a Central American rain forest, mature trees grew more slowly in warm years than they did in cooler ones. This observation hints that tropical forests may become less efficient at removing planet-warming carbon dioxide from the atmosphere if global temperatures continue to rise.
>
> “From 1984 to 2000, scientists studied the old-growth forest at La Selva, Costa Rica. Annually, the team measured the diameter of all mature trees within a 2-square-kilometer area. They found that diameter growth varied significantly from year to year and was related to average daily temperature. The annual tree growth from 1984 to 1986, the coolest interval during the period, averaged 81 percent greater than the growth tallied during the record hot spell related to the El Nino that began late in 1997. The average daily temperature difference between the two periods was about 1.4°C.
>
> "Tree growth in the forest was also particularly slow during the El Niño year of 1987, says Deborah A. Clark, a biologist at University of Missouri-St. Louis. Clark and her colleagues presented their results in an upcoming *Proceedings of the National Academy of Sciences*.
>
> "Looking at global carbon dioxide measurements during the same period, the researchers noticed that quantities of the gas attributable to land plants in tropical regions increased during warm years. That phenomenon could stem from typical plant growth characteristics, the researchers say.
>
> “Plants use photosynthesis to convert sunlight, carbon dioxide, water, and nutrients into carbohydrates. When the plants tap into their stores of carbohydrates for chemical energy, however, they return carbon dioxide to the atmosphere—just as animals do—in the process called respiration. Although a plant's rate of photosynthesis begins to drop off above a temperature that's characteristic of its species, its rate of respiration, continues to rise with increasing temperatures, says Clark.
>
> “Most of the observed global spikes in carbon dioxide during warm years probably stemmed from the increased respiration of tropical land plants, but some may have been produced by other sources, such as forest fires or agricultural burning, says Stephen C. Piper, a biogeochemist at Scripps Institution of Oceanography in La Jolla, California, and a coauthor of the team's report.
>
> “The growth rate of mature trees can be a useful indicator of the climate's effect on the rest of an ecosystem, says David S. Schimel of the National Center for Atmospheric Research in Boulder, Colo. The link that Clark's team discovered between slow growth rates in Costa Rican trees and increases in the atmospheric carbon dioxide traceable to tropical plants is "an innovative result that's hard to argue with," he says.”

So you see, one’s first ideas might not be what actually will happen. There are just so many variables. In light of this, imagine the dilemma of a person in the following situation.

Stage 1: What's at Stake?

The Canadian government has requested that its Ministry of Agriculture prepare a position paper assessing which agricultural crops will be most important to the Canadian economy one hundred years from now. In particular, the government is wondering about the likelihood of cereal grains being replaced by corn or soybeans, both of which are currently grown more in lower latitudes. The reason for suggesting this is the projection that average yearly temperature may be increasing due to the greenhouse effect of increased carbon dioxide in the atmosphere. Projections indicate that the partial pressure of atmospheric carbon dioxide may increase by a factor of 2 to 3 in the next 100 years, from a current value of about 360 parts per million (ppm) to as much as 1000 ppm.

The problem, then, is for the Ministry of Agriculture to obtain information that will allow the position paper to be written with justification from verifiable scientific data. A staff assistant does an initial search to find these data and reports that the needed information does not seem to be available in printed form. The Ministry therefore decides to provide funding to several academic or industrial research teams so that they can conduct experiments to provide the crucial information need to write a report. In order to be considered for receipt of the funds, these teams will need to submit a grant proposal outlining the research questions they intend to explore, the rationale behind these questions, the way in which they intend to conduct the experiments to find answers to their questions, and preliminary or "pilot" data that will document that the team is qualified to conduct the research it proposes.

Your laboratory group decides that it wants to apply for some of the Ministry’s funds. Before deciding what experiment you will feature in your proposal, however, you decide to “brainstorm” and discuss the background issues that will be important to consider before going on to think about your research questions and methodology and about the preliminary studies you will need to conduct.

Issue 1. What biological and physical variables could be a factor in the response of plants to increases in average yearly temperature and atmospheric carbon dioxide concentration? [You think you should consider both plants in a laboratory setting and plants in nature.]

Issue 2. In consideration of these variables, what effects on plants can be expected if temperature and atmospheric carbon dioxide do increase as predicted?

Issue 3. Considering the effects on plants your team has proposed, what outcomes do you predict for plant metabolism in response to changes in carbon dioxide concentration?

Stage 2: Collecting and Analyzing Preliminary Data

Now that your team has thoroughly considered the background issues, it is faced with the task of identifying preliminary experiments that should be conducted. The data you obtain from these preliminary experiments will be an important factor in writing a persuasive proposal to obtain the funds needed to conduct a full and in-depth exploration of your research questions. For conducting these pilot studies, you have the following materials on hand. (You intend to request funds to buy additional equipment and materials in your proposal to the Ministry.)

Materials:

- Soybean (C_3) and corn (C_4) plants
- Cylinder of compressed air
- Photosynthesis package—gas bags, air pump, leaf chamber, LED light source, humidity sensor, temperature sensor, drying columns, infra-red gas analyzer (IRGA), connecting tubing, analog-to-digital converter, and software
- Computer

- Data capture and analysis software
- Spreadsheet and graphing software

A quick look at your bookshelf reveals that one of your laboratory assistants has conveniently compiled a Handbook for Using a Data Acquisition System that describes use of a computer data acquisition system for measuring parameters related to photosynthesis in plants. You use the index and find a diagram (labeled Figure 1) that shows how the photosynthesis package can be used to measure various indices related to photosynthesis in plants.

Your team determines that it is ready to plan an experiment. It uses the lab's standard Research Planning Guide for this purpose. The Guide was originally designed to help new investigators in the lab, but you find that even for an experienced investigator such as yourself, it helps to clarify your thinking about the preliminary experiments necessary for making a good case to a funding agency. The Planning Guide calls for provision of a statement of the hypothesis or research question and the rational behind it, a prediction of the results that would be obtained if the hypothesis were supported, and a step-by-step work plan that you will be able to follow easily when you first conduct the experiment.

You complete a first draft of your plan, and make some improvements after getting feedback from the other members of your team at your weekly laboratory meeting. You look forward to trying out your ideas at the first opportunity.

A few days later your team has collected preliminary data that need to be analyzed. Once again you are thankful for having hired the lab assistant who so nicely has provided a Microsoft Excel spreadsheet in the lab's handbook of commonly used procedures. The spreadsheet will be helpful in organizing the data, and for determining rates of photosynthesis and transpiration, among other variables.

Handbook for Using a Data Acquisition System

The system can be manipulated in the following ways:

- Gas flow rates of 0 to 500 mL per minute can be obtained.
- Carbon dioxide concentration in the range of 0 to 2000 ppm can be continuously measured.
- Red light fluxes can be produced in the range of zero to roughly half maximum sunlight (1000 microEinsteins per square m per second).
- Ambient temperature in the gas stream can be continuously measured.
- Relative humidity in the gas stream can be continuously measured

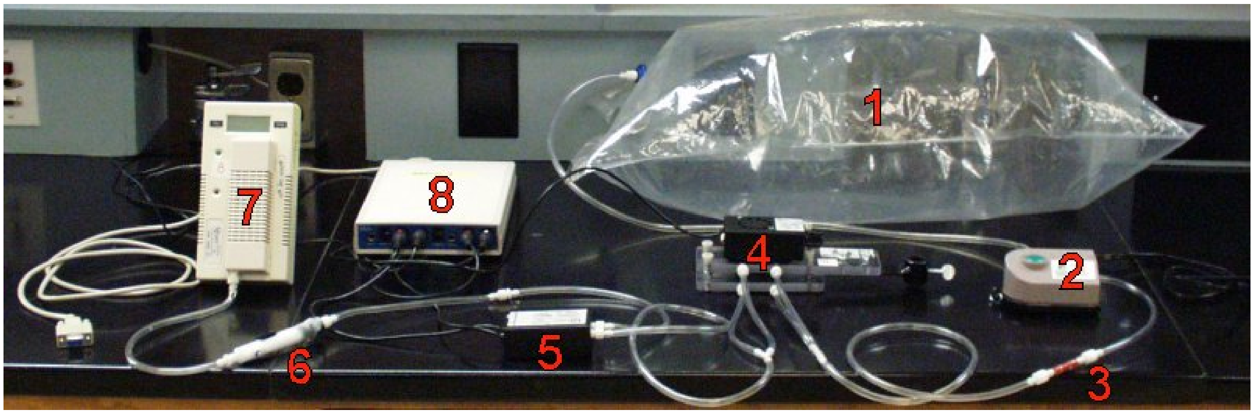

Figure 1. Arrangement of components for photosynthesis in order of use. Computer and electrical connections are omitted.

1	Gas bag	Supplies air with constant carbon dioxide concentration.
2	Air pump	Maximum output is about 1400 mL per minute.
3	Flow restrictor	Reduces air flow rate to increase differential carbon dioxide concentration entering and exiting leaf chamber and to protect IRGA from excessive pressure. Red is nominally for 200 mL per minute.
4	Leaf chamber and LED light source	Chamber for an attached or detached plant leaf. The area within the gaskets is 16 square mm.
5	Temperature and humidity sensor	Measures temperature and percent relative humidity in gas stream.
6	Magnesium perchlorate column	Removes water vapor from gas stream. The IRGA requires dry air.
7	Carbon dioxide sensor (IRGA)	Measures carbon dioxide by absorption of infra-red light. Model S151 has ranges of 0-500 and 0-2000 parts per million.
8	ULI	Universal Laboratory Interface. Converts signal from probes from analog (voltage) to digital and sends to computer (not shown).

Electronic Probe Apparatus

1. Connect all components in the order given in Fig. 1. Turn on the IRGA at least 24 hours before its use. This recharges the internal battery and stabilizes performance. Set the IRGA ppm range switch to 2000.

2. Check the IRGA zero setting (reading with CO_2-free air) by passing air through a syringe tube filled with granular soda lime upstream of the air pump. The reading should be close to zero. If it is not, adjust using the fine adjust screw. It is nearly impossible to get a reading of exactly zero; plus or minus 20 ppm is close enough.

3. Check the IRGA calibration. Pass normal air through it, assuming the carbon dioxide concentration is approximately 360 ppm, or use a purchased tank of 500 ppm calibrated air. The

Instructor's Guide from the IRGA supplier, Qubit Systems, Inc., has instructions for adjusting the IRGA calibration using the straight line method.

4. Inspect the magnesium perchlorate tube. If the substance appears crusted or wet it is time to be replaced. It is imperative that liquid water be kept out of the IRGA.
5. Check the air pump performance and apparatus for leaks. A flow rate of about 150 to 200 mL per minute downstream of the red flow restrictor tube is desired.
6. Air enriched with CO_2 can be produced by combining small amounts of human exhaled breath (usually at about 40,000 ppm CO_2) with compressed air in the supplied gas bag. The actual CO_2 concentration can be determined with the calibrated IRGA.
7. Use a large enough leaf to span the leaf chamber and stick out on all sides. This helps seal the chamber against gas leaks.
8. Equilibrate the leaf at maximum light flux until a steady rate of CO_2 uptake and water vapor loss is obtained. For plants that have been at normal room illumination this may take approximately 30-60 minutes.

Data Collection

1. Make sure the ULI (Universal Laboratory Interface) and computer are turned on. If the ULI is not turned on, the computer will not know the ULI is connected to a COM port when you execute the next step.
2. Double click on the *Logger Pro* software icon for a photosynthesis investigation. Qubit Systems provides a preset template called "S151co2.mbl.". The computer screen should show a window with four graphs: CO_2 Concentration vs Time (upper left), Relative Humidity vs Time (upper right), LED Irrad vs Time (lower left), and Air Temperature vs Time (lower right) (Fig. 2). At the bottom of the screen there are four little windows showing probe outputs in real time.
3. Associate the proper calibration file for the IRGA set to a maximum reading of 2000 ppm. Qubit Systems provides a calibration file called "CO2_2000.cal", and their Instructors Manual describes how to make the association.
4. Various parameters of the Logger Pro data collection window can be varied such as the default timeout and the graph X and Y axis scales. The Instructors Manual or manual from Vernier Software and Technology describes how to make these changes.
5. Start data collection by clicking the "Collect" button. Click on this button again (its name will have changed to "Stop") to stop data collection.
6. Plan to stop data collection periodically and save it on the computer as insurance against computer failure and for later analysis. IMPORTANT: If you attempt to exit the application without storing data in a file with a new name, you may get a dialog box asking if you want to save the setting. Always say no to prevent overwriting the calibration file.

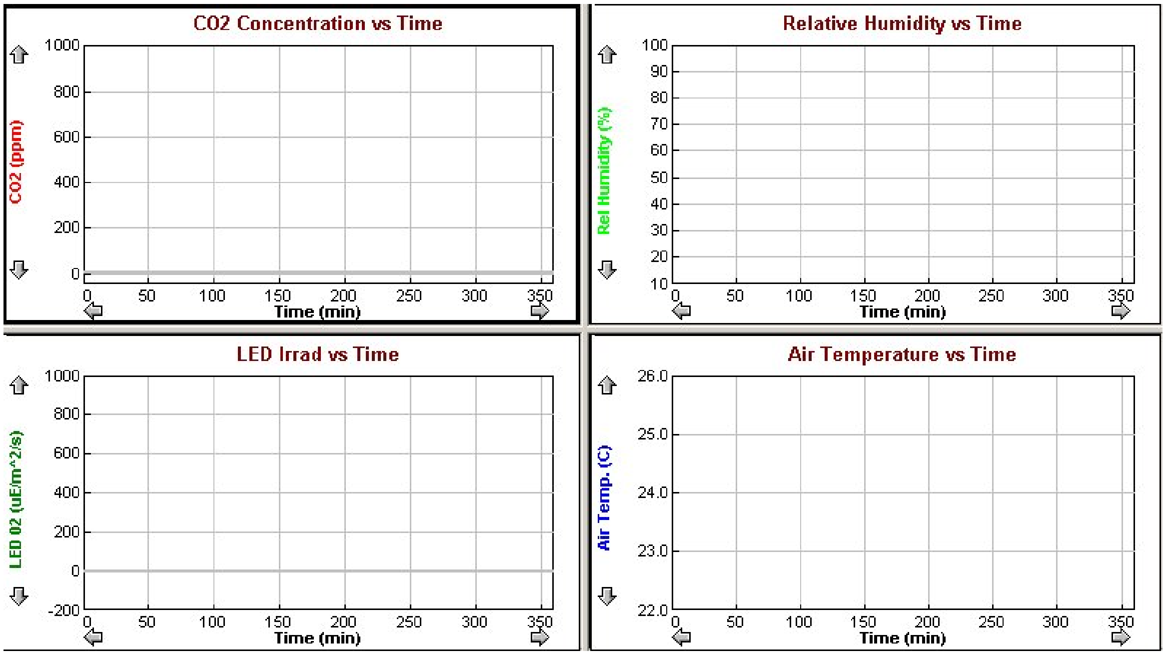

Figure 2. Sample screen from *Logger Pro* for photosynthesis data collection.

Data Analysis

1. Data analysis is performed with built-in functions of *Logger Pro*. Y values for any X are obtained by clicking "Analyze>Examine" and moving the mouse pointer along the X axis. Slopes are obtained from a selected range of X values. Click on any value of X and drag the mouse pointer to another value of X. Then click on "Analyze>Linear Fit" and a small window appears with slope and Y intercept values.
2. Further data analysis for determining rates of photosynthesis and transpiration is conveniently obtained with the calculation template "Calc Template XL PC.xls" available from Qubit Systems.

Stage 3: The Minister Wants a Progress Report

A year has passed since you were awarded a grant from the Ministry of Agriculture (congratulations!) to conduct your laboratory investigation of the effects of varying CO_2 concentrations on photosynthesis in C_3 versus C_4 plants. Your team refined your experimental protocol, and because the pilot studies were so carefully done went on to replicate the findings from them in a larger number of plants. The data from the replications were collectively consistent and otherwise reliable enough to allow for in-depth analysis.

Today you received a request from the Ministry to write a progress report on your project outcomes to date. Continuation of your grant funding for an additional year depends on the quality of what you are able to say in the report about the progress you have made. You prepare to write the report, following the specific guidelines required for all Ministry reports (Ministry of Agriculture Guidelines for Writing a Progress Report). It should not be difficult to write—you have been contacting the Ministry on an informal basis since your work began in order to share what you knew they would find to be intriguing results from your study.

The Ministry is looking forward to receiving yours plus the other teams' formal reports so that they can begin to think ahead towards making an informed recommendation about "crops of the future" in Canada. Following the advice of the science advisory committee, the Ministry had carefully selected the teams who would be collecting the information they need to encompass a variety of disciplinary perspectives and experimental approaches.

Final Issue for Reflection

What other data and experiments (in addition to yours) would the Ministry need to obtain a more complete analysis of plant responses to the temperature and carbon dioxide concentration variables?

Ministry of Agriculture Guidelines for Writing a Progress Report

Include the following sections in your progress report. All reports must be double-spaced, with 2.5 cm margins. The font size should be 12 point. Keep in mind that while the report will be read by scientists and science policy experts, it will also be read by government officials who are not scientists. For that reason, be sure to explain specific scientific terminology, and use language that can be understood by lay people as well as scientists.

Project Title. The title should be descriptive of your project and should not exceed 60 characters in length.

Project Summary (Abstract). This section should clearly and briefly summarize your project objectives, methods, results, and conclusions in 200 words or less. The importance of your findings to the goals of the Ministry of Agriculture with respect to crops of the future should also be clearly stated.

Aims and Rationale (Introduction). Provide a statement of your project aims, and their importance to the Ministry of Agriculture's overall goals related to "crops of the future." Supply sufficiently theoretical information to allow the reader to understand the pattern of response you expected, and to evaluate and understand the results of your study. Concisely summarize what has been found in previous studies on the same or related topics, and state what you intended to accomplish in your project to extend these previous observations. Cite literature you refer to in this section in a separate "References Cited" section at the end of the report.

Methods. Describe what experiments you did and how you did them. Provide enough detail so that a “competent worker” could repeat the experiment, and so that the readers can judge whether the methods you used are valid. State the names of specialized instruments and equipment that you used and the number of replicates that you conducted. Describe any unusual calculations and/or statistical techniques that you used to analyze the data.

Results. Provide easy-to-interpret data tables and/or graphs (accompanied by captions or figure legends) of your key findings. In addition provide a text narrative that guides the reader through your figures and tables in a logical and systematic way, pointing out patterns, trends, and significant differences that pertain to the hypothesis or question that you tested.

Conclusions (Discussion). Provide an explanation and interpretation of your results, and indicate whether they support or do not support the hypothesis you tested, or answer the scientific question you asked. Summarize the most important results that you found, and compare them to results of any previous studies on the same topic. Include possible explanations for similarities or differences between past studies and yours. Point out the potential impact of your findings on the decisions that the Ministry of Agriculture must make in considering what crops to grow in the future.

References Cited. Include here a full citation of any books, journal articles, electronic sources, and other material cited in the other sections of the paper. Use the CBE style for both the citations within the text and the listing of the corresponding references in this section.

Materials

Each group of two-four students will need the materials listed below. Figure 1 in the “Handbook for Use of a Data Acquisition System,” in the “Student Outline” shows how the photosynthesis package is set up. Additional information about suppliers is provided in Appendix A.

Soybean (C_3) and corn (C_4) plants
Cylinder of compressed air
Photosynthesis package—gas bags, air pump, leaf chamber, LED light source, humidity sensor, temperature sensor, drying columns, infra-red gas analyzer (IRGA), connecting tubing, analog-to-digital converter, and software
Computer
Data capture and analysis software
Spreadsheet and graphing software

Notes for the Instructor

These notes provide a general guide for how we have staged this PBL activity for students in a way that incorporates a guided inquiry approach. In our classes, in one section students worked in teams of three students each. In the other section that uses PBL in "lecture," students worked in their four-member PBL "lecture" groups for the discussion portions of the activity, then split into pairs to conduct the actual experiments; one pair worked with C_3 plants, and the other with C_4. They reconvened again in the original groups of four for discussions related to analysis of the combined data from both types of plants.

Problem Session 1 - Brainstorming the Issues (Up to 60 minutes; held two weeks prior to conducting the investigation)

This session can be held in either the time allotted for laboratory or during the lecture class. We chose to hold it during the last hour of the regularly scheduled lab session, after students finished another, unrelated activity. The purpose of this session is to open up students' thinking about the relationships between environmental factors that can influence the rates of respiration and photosynthesis and the global carbon cycle, and to put these factors in the context of more widespread and gradual changes in the global climate.

Step 1. Students Receive Handouts of the Problem background and of Stage 1. In small groups they discuss the issues posed at the end of Stage 1, which were designed to prompt them to define and consider the broad nature of the problem, including what variables that could be considered in addressing the problem.

Step 2. Whole class discussion led by the instructor. Groups are called on in turn to contribute their ideas about the issues posed. This provides a mechanism for identifying areas in which students' knowledge base is on firm ground, and areas in which they need to learn more. These areas of needed knowledge become the topics that students need to research in order to design an experiment that will address the questions in which the Ministry has an interest.

Step 3. Identifying the questions to research independently. Groups identify issues or topics (the so-called "learning issues" of PBL jargon) that they need to know more about, posing these as questions that guide their search for information. The instructor checks each group's list (to make sure its members' research will allow for the necessary progress) or leads a whole class discussion with the goal of producing a refined and prioritized class list of learning issues. Typically in PBL the students would assign one another one or two of the topics to research as individuals, rather than all of the issues—we shortcut this process (due to time constraints) by asking each student to research the entire list before the next class meeting.

Possible student responses to Issue 1 include the following:

- Type of photosynthesis (C_3, C_4)
- Environmental conditions during long-term plant growth
- Annual versus perennial life history
- Response of stomata to increased CO_2
- Response of respiration to increased temperature
- Response of photosynthesis to increased temperature
- Response of photosynthesis to increased CO_2 (enzyme substrate concentration)
- Response of photorespiration to increased CO_2 concentration
- Evapotranspiration rates in response to increased temperature.

Possible student responses to Issue 2 include the following.

- Increased temperature:
  - will speed up enzymatic reactions in respiration and photosynthesis
  - will decrease solubility of O_2 and carbon CO_2
- Elevated carbon dioxide:
  - will increase rate of photosynthesis in the light (substrate concentration on diffusion rate and enzyme activity) – more in C_3 than C_4 plants
  - will inhibit stomatal opening via effect on guard cells
  - will inhibit photorespiration in C_3 plants.

Possible student responses to Issue 3 include the following.

- Elevated carbon dioxide will cause stomatal closure, more in C_3 than C_4 plants
- Stomatal closure will decrease amount of water lost by transpiration
- Elevated carbon dioxide will steepen diffusion gradient into plant and increase diffusion rate, increasing rate of photosynthesis, with effect greater in C_3 than C_4 plants.
- Stomatal closure and increased diffusion gradient of CO_2 are opposites, and which will supercede is not predictable.

The extent to which student responses will align with these possible ones will vary widely, depending on their prior background in these areas. At the end of the whole class discussion in which students present their insights about each of these issues, the instructor could bring into the discussion any additionally important factors and explanations that the students overlooked – this is the strategy that we use.

Interval between First and Second PBL Sessions

In this interval, students do independent research into the areas of uncertainty identified in the discussion, and begin to formulate ideas for an experiment, which they are asked to put in writing for the next class.

PBL Session 2 - Designing the Investigation (60 minutes, approximately a week before conducting the investigation)

Again, this class session could take place during the time allotted for lecture or at the end of a laboratory in which students also worked on an unrelated activity. Students are presented with Stage

2 of the problem, and this stage prompts them to design an investigation. The Stage 2 problem statement includes a materials list to assist students to consider what equipment is available. A more detailed materials list for the instructor is also given in Appendix A. A Research Planning Guide is referred to in the problem statement. Its distribution is optional, since the problem statement itself describes its essential features. We use this guide as a worksheet for students to hand in so that we can give them feedback on their design prior to the actual investigation. The worksheet calls for students to state their hypothesis or question (along with a brief rationale for why they think the hypothesis is important, interesting, or relevant to the Ministry's goals), a prediction of major results in the form of a narrative with accompanying table(s) or graph(s), and a step-by-step plan for how they will conduct their investigation. The problem statement also contains a reference to a laboratory handbook—the handbook materials should be distributed to students along with Stage 2.

The way in which Stage 2 is constructed, as well as the nature of the additional handouts, clearly shapes students' thinking along certain lines. We made this decision for the usual reason—resource and time limitations constrain our willingness to provide any and all materials that students would need for an investigation that uses more of a free inquiry approach. The experimental designs were in many, but not all cases, very similar. We used this as an opportunity to suggest that teams communicate with one another to pool resources to either increase their collective power to include replicates, or to investigate a greater number of levels of the independent (manipulated) variable(s).

PBL Session 3 - Conducting the Investigation (2.5-3 hours)

Students typically take about 2.5 hours to conduct their investigations. Stage 3, along with the Ministry of Agriculture Guidelines for Writing a Progress Report, are distributed and discussed about midway through this session. At this point, students are collecting data, but can take their attention away for the necessary time. Stage 3 essentially calls for the writing of a progress report to the Ministry on the results of their investigations thus far. Data analysis is aided by a template provided by Qubit Systems, Inc. The template is in the form of a Microsoft Excel file and can be obtained from the authors or Qubit Systems, Inc. (Appendix A.). This is the spreadsheet referred to in the problem statement.

A sample progress report, written by a student in one of the introductory biology sections, is included in Appendix B.

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Appendix A – Annotated Materials List

1. Soybean (C_3) and corn (C_4) plants. Plants grown in a glasshouse with normal atmospheric carbon dioxide concentration and at 20 ^{o}C for 6-8 weeks respond well. The plants should be grown one to a pot for ease of manipulation with the leaf chamber.

2. Cylinder of compressed air. Cylinders are available from a local compressed gas supplier. The carbon dioxide concentration will be around 360 ppm. Calibrated gas mixtures are available at extra cost.

3. Photosynthesis package. The Advanced Photosynthesis Package is available from Qubit Systems, Inc (www.qubitsystems.com). It consists of gas bags, air pump, leaf chamber, LED light source, humidity sensor, temperature sensor, drying columns, infra-red gas analyzer (IRGA), connecting tubing, analog-to-digital converter, software, and instruction manuals.

4. Computer. *Logger Pro* software, included in the Advanced Photosynthesis Package, is available for both the PC and Mac platform.

5. Data capture and analysis software. The software, called *Logger Pro*, is produced by Vernier Software and Technology.

6. Spreadsheet and graphing software. Microsoft Excel is suitable.

Appendix B – Sample Technical Report

The Effect of Elevated CO_2 Concentration on C_3 and C_4 Plant Functions

Student in BISC207 Introductory Biology

Project Summary

Atmospheric carbon dioxide concentrations are expected to increase two- to three-fold in the next 100 years. To test the effect of increasing CO_2 concentration on C_3 and C_4 crops, soybean and corn plants were treated with varying CO_2 concentrations and the data from these treatments used to calculate and graph photosynthesis rate, transpiration rate, and water use efficiency as functions of CO_2 concentration. It was observed that in both corn and soybean plants, photosynthesis rates increased and transpiration rates decreased as CO_2 concentration increased. It was also observed that both soybean and corn plants became more efficient in using water, as the photosynthesis-to-transpiration ratio increased as CO_2 concentration increased. Because this experiment had few replications and much room for error, it was not advisable to take the information obtained at face value until conducting further experimentation. From these data, corn and soybean crops seem to benefit from higher atmospheric CO_2 concentrations, and might prove to be good crops to use in the coming century as atmospheric CO_2 concentration continues to rise.

Aims and Rationale

Atmospheric carbon dioxide concentration has varied throughout the life history of the earth. It has increased from about 280 ppm in pre-industrial times to 315 ppm in 1958 when the first careful continuous measurements were made in Hawaii (Allen et al 2003). Since then, atmospheric CO_2 concentration has continued to increase and is about 370 ppm currently. This increase has been due primarily to burning of fossil fuels and secondarily to deforestation and land-use changes (Allen et al 2003). Predictions indicate that after a century of fossil fuel consumption, atmospheric CO_2 concentration could increase two- to three-fold (Walker 1992). This increase of CO_2 is expected to cause global warming and other climate changes, including having a direct effect on plants.

Studies have implicated that crops grown in CO_2-enriched environments have been more productive, and therefore the technique of carbon dioxide fertilization has become more common (Walker 1992). Looking at plant photosynthetic rates in enriched CO_2 environments, it has been seen that C_4 plants are less responsive than C_3 plants to CO_2 enrichment because of their own built-in CO_2 concentrating mechanisms (Walker 1992). In studies of wetland plants, it has been seen that C_3 growth rate increases more than C4 growth rate in CO_2-enriched environments (Marsh 1999). When looking at C_3 versus C_4 transpiration rates, the effect of elevated atmospheric CO_2 on plant water use appears to be more uniform. As the size of the stomatal opening is controlled by CO_2 concentration and water vapor inside the leaf, elevated CO_2 concentrations therefore decrease the size of the stomatal opening, resulting in a decrease in transpiration (Marsh 1999). In a Maryland wetlands study, elevated CO_2 concentrations significantly decreased water loss in both C_3 and C_4 communities by as much as 30% (Marsh 1999). Many experiments have also shown that, with the decrease in transpiration and increase in photosynthesis, water use efficiency increases uniformly in both C_3 and C_4 plants (Walker 1992). For most plants, the transpiration-to-photosynthesis ratio is cited as 600:1, meaning that the plant transpires 600 grams of water for every gram of CO_2 that is incorporated into carbohydrate during photosynthesis. However, for C4 plants, the transpiration-to-photosynthesis ratio is 300:1 or less (Campbell and Reece 2002).

In order to test the potential effects of increased atmospheric CO_2 concentration, and the difference of its impact on C_3 and C_4 plants, it was determined that soybean (representing the C_3 pathway) and corn (representing the C_4 pathway) plants would be treated with CO_2 concentrations of 350 ppm (slightly below the atmospheric CO_2 concentration known today), 700 ppm (a two-fold increase), and 1050 ppm (a three-fold increase). Carbon dioxide concentration in the air exiting the leaf chamber with the leaf in the chamber (analysis CO_2) and humidity were the dependent variables of interest in the experiment. From these variables, calculations could be made to determine rate of photosynthesis, rate of transpiration, and water use efficiency. It was determined that the photosynthesis-to-transpiration ratio would be used to evaluate how efficiently the plants used water. It was predicted that photosynthesis rate of both corn and soybean plants would increase, however, soybean plants would be more responsive to the increased CO_2 concentration than corn plants. It was also predicted that transpiration rates of both plants would decrease due to stomatal closure in the presence of higher CO_2 levels. Finally, it was predicted that both plants would use water more efficiently as CO_2 concentration increased.

Methods

The apparatus was assembled using parts from Qubit Systems' "Photosynthesis Package" and "Plant CO_2 Analysis Package". The Vernier Logger Pro data analysis system was used in collecting data emitted by the Qubit Systems' sensors and organizing it graphically. Qubit Systems model numbers used included: infrared gas analyzer (IRGA) S151, LED light source A113, humidity sensor S161, and gas bag G122.

Custom carbon dioxide in air mixtures were created using a nomagraph. A gas bag was filled with 5% CO_2 concentration for the number of seconds predicted by the graph for the desired CO_2 concentration (i.e. the 80 second time point was predicted to create a 650 ppm mixture). T he remaining gas space in the bag was filled with normal air from a compressed gas cylinder. Two sets of gas mixtures were made for 350 ppm, 700 ppm, and 1050 ppm (the CO_2 concentrations in these bags varied slightly from these theoretical values). One set of bags was used in testing corn, and the other in testing soybean.

Three young corn plants and three young soybean plants were treated with varying CO_2 concentrations. Thus, there were three replicates of both corn and soybean. Protocol for treating the plants was as follows: light flux remained constant at about 1000 $\mu E/m^2*s$ (the LED was set at its maximum output), all plants were first treated with room air until steady CO_2 and humidity readings were obtained, then the plants were treated with each of the three custom CO_2 concentration bags, switching bags when steady readings were obtained.

To make calculations based on the data obtained from Logger Pro, a Qubit Systems template was used. F or this, it was necessary to measure the flow rate of the air pump. Key calculations included transpiration rate, CO_2 assimilation rate, and water use efficiency. These calculations are shown below.

CO_2 assimilation rate = (reference CO_2 – analysis CO_2)*[flow rate/(22.4*(273+IRGA temperature)/273)/60*10000/leaf area]
Transpiration rate = (analysis humidity-reference humidity)/(atmospheric pressure*1000-analysis humidity)*[flow rate/(22.4*(273+IRGA temperature)/273)/60*10000/leaf area]
Water use efficiency = CO_2 assimilation rate/transpiration rate

Results

The prediction was that photosynthesis rates of both corn and soybean plants would increase, but soybean plants would be more responsive to the increased CO_2 concentration. It was also predicted that transpiration rates of both plants would decrease at about the same rate due to stomatal closure and that both plants would use water more efficiently in the presence of higher CO_2 levels.

Figure 1 shows the apparatus used in the treatment of both soybean and corn plants. Figure 2 shows the results of the treatment of a single soybean plant. Photosynthetic rate increased linearly, but not by a very large amount, as CO_2 concentration increased. Transpiration rate showed a decreasing trend, but not by a very large amount. Water use efficiency showed a roughly linear increasing trend as CO_2 concentration increased. The plant was assimilating more grams of CO_2 during photosynthesis (rate of photosynthesis was increasing) per gram of water lost through transpiration (transpiration was decreasing) as CO_2 concentration increased. Figure 2 was chosen as representative of the trend observed in other corn analyses. Because the numerical data in the three corn analyses varied, it was determined that a graph of the averaged data would not be an accurate representation, as standard deviation bars would be large. Figure 3 shows an almost linear increase in photosynthetic rate, but once again, not a very large-scale increase. Transpiration rate decreased, while water use efficiency showed a roughly linear increasing trend as described above. Figure 4 is a graph combining the average data from two of the treated soybean plants. Experimental data from the third soybean plant reflected a similar trend, but differences in numerical values would have caused greater error bars had it been averaged and graphed in Fig. 3. Figure 3 shows a small increase in photosynthetic rate (with little variation), a larger decrease in transpiration rate (but with much variation), and a large linear increase in water use efficiency (with much variation) as CO_2 concentration increased as described previously.

Figure 1. Apparatus used in treatment of soybean plant. The same apparatus was used in treatment of corn plants.

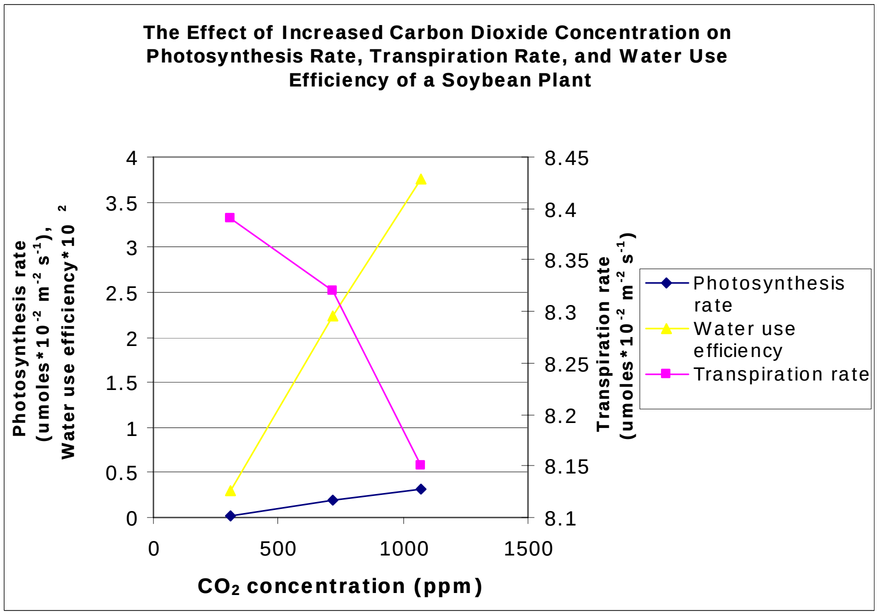

Figure 2. The effect of increasing carbon dioxide concentration on photosynthetic rate, transpiration rate, and water use efficiency of a single soybean plant as tested by Team 1.

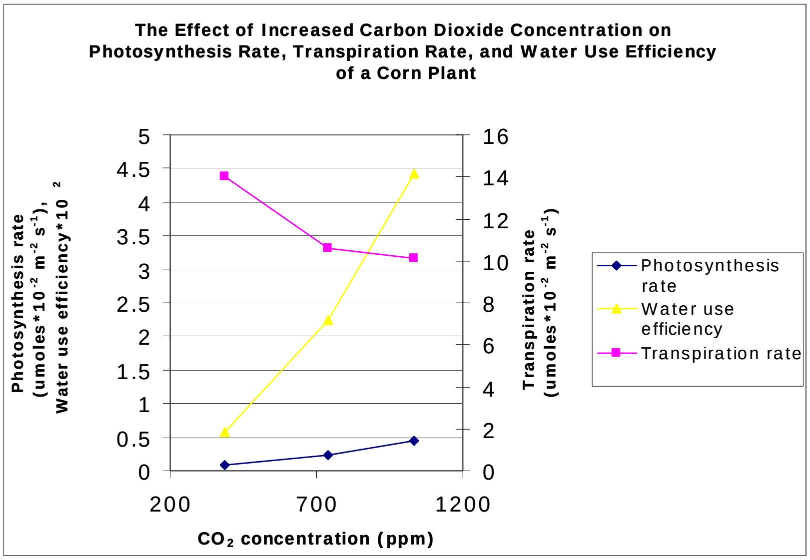

Figure 3. The effect of increasing carbon dioxide concentration on photosynthetic rate, transpiration rate, and water use efficiency of a single corn plant. This plant was chosen as being representative of the trend shown in further analyses.

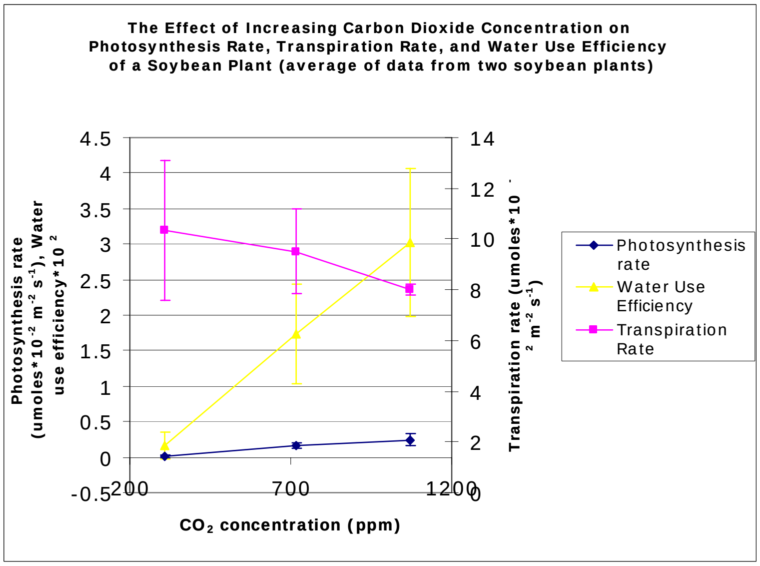

Figure 4. The effect of increasing carbon dioxide concentration on photosynthetic rate, transpiration rate, and water use efficiency of two soybean plants. The average of the data from two plants is shown with standard deviation error bars to represent variation in the data.

Conclusions

The results obtained support the prediction that photosynthesis rate would increase and transpiration rate would decrease as CO_2 concentration increased for both C_3 and C_4 plants. Even though in both corn and soybean, photosynthetic rate only increased slightly, there was still an increasing, roughly linear trend as CO_2 concentration increased. In both corn and soybean, transpiration rates decreased with increased CO_2 concentration.

Transpiration rates in both corn and soybean plants decreased by close to the same amount overall. This result is consistent with experimentation on wetland C_3 and C_4 plants which found that despite their differing types of metabolism, transpiration rates in C_3 and C_4 plants decrease uniformly when exposed to increasing CO_2 concentration (Marsh 1999). Thus, it is likely that the stomata of both types of plants open and close in similar manners when exposed to higher CO_2 concentrations, operating independently of their different photosynthetic processes. However, to conclude this, different experimentation would have to be designed to test this hypothesis.

The results obtained concerning the plants' water use efficiencies support the prediction that as CO_2 concentration increased, both corn and soybean plants would use water more efficiently. As seen in the graphs of water use efficiency for corn and soybean (Fig. 2 and 3), the photosynthesis-to-transpiration ratio increased as CO_2 concentration increased. This means that, as CO_2 concentration increased, the plant assimilated more grams of CO_2 per gram of water lost through transpiration. Inversely, the plant lost less water per gram of CO_2 assimilated. This makes sense because, in both sets of results for corn and soybeans, water lost due to transpiration was decreasing as photosynthesis rate (CO_2 assimilated) was increasing. Therefore, they used water more efficiently as CO_2 concentration increased.

Interestingly, the corn plant whose data was reported in this experiment was more responsive to CO_2 concentration than the soybean plants. Its photosynthetic rate increased more than those of the soybean plants averaged together. This contradicts the prediction that the soybean plants would be more responsive because of their C_3 metabolism, which would benefit from being saturated in more CO_2. The data obtained also contradict experimentation conducted on wetland C_3 and C_4 plants (Marsh 1999) and experimentation with CO_2 as fertilizer, which showed C_3 plants responding to changes in environment as though they were C_4 plants when supplied with enough CO_2 to suppress photorespiration and enhance photosynthesis (Walker 1992). In this experiment, the fact that only one graph was chosen to represent the corn plant data may have been one of the potential causes of error. Had corn graphs been averaged, the averages may have shown corn as being less responsive to elevated CO_2 levels. It is also possible that the soybean plants used in this experiment may have had lower response rates due to the fact that they were used in experiments prior to this one. Also, only three corn and three soybean plants were used. Further experimentation would need to be conducted with a greater number of plants and a greater number of replications that could be averaged together with less error (a larger sample size reduces error). This might then show different results that may, in fact, support the hypothesis that soybean plants would be more responsive to elevated CO_2 concentration.

In this experiment, there was much room for error. Many variables were not controlled that, if accounted for, could have had significant effects on the data obtained. The sequence of the various treatments may have had an impact on the data obtained (i.e. if a plant was first treated with a 1050 ppm CO_2 concentration, then a 350 ppm CO_2 concentration, and finally a 700 ppm CO_2 concentration). The proper calibration of machinery is imperative in further experimentation. In this experiment some machinery was old and faulty. One gas pump was pumping at a much lower flow rate than others, and one infrared gas analyzer was reading CO_2 concentration much lower than the actual value. One way to reduce error in further experimentation would be to create all of the custom CO_2 mixtures using one machine. Then the CO_2 concentrations of all of the gas bags would err by a constant amount based on only one machine, as opposed to the many that were used in this experiment. Because of machinery errors, it was difficult to average data to obtain more of a group result. As one can see from Fig. 3, average data proved to be difficult to draw conclusions from, as error bars were large. Because of such large error bars, it could almost be concluded from this graph that transpiration rate increased. Thus, the reader is strongly cautioned against taking this information at face value until further research is conducted.

From this experiment and others, it can be seen that the transpiration and photosynthesis rates of C_3 and C_4 plants benefit from increased CO_2 concentration. In both types of plants photosynthesis rates increase and water loss decreases with increased CO_2 concentration. These factors may lead to faster growth rates and more abundant harvests in years to come. Further experimentation needs to be conducted to determine the effect of temperature on crops that are experiencing higher levels of atmospheric CO_2 concentration. Plants grown outdoors are not experiencing a constant room temperature as they are in the lab. The amount of light that the plant receives must also be taken into account, as plants outdoors will not experience a constant light flux of 1000 $\mu E/m^2*s$ as they did in the lab. A more long-term experiment monitoring plant growth of C_3 versus C_4 plants in enriched and regular CO_2 concentrations would also be advisable.

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