

## **Laboratory Packages for Experimental Biology Incorporating Computer-Monitored Sensors.**

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### **ABSTRACT**

Lack of suitable and affordable equipment is a major problem in developing research-based experimentation for undergraduate biology courses. Expensive research-level instrumentation is often more complex and sophisticated than is required for undergraduate projects, discouraging some students, or creating a "black box" attitude to data collection that fails to teach students the principles of the experimental method being used.

To overcome these problems, we have developed a series of laboratories that incorporate inexpensive sensors for monitoring physiological processes in plants and animals. Sensors are not built into instruments but are externalized so that students can better appreciate their form and function. Sensor outputs are recorded by data acquisition software via an A/D interface, and data is plotted to screen in real time. Sensors include a gas phase O<sub>2</sub> sensor for monitoring photosynthesis in plants and respiration in animals (including humans), a CO<sub>2</sub> sensor for studies of respiration, photosynthesis and photorespiration, and a H<sub>2</sub> sensor for investigating N<sub>2</sub> Fixation in legumes. We have documented numerous experiments that have been conducted with great success in the Introductory Biology (Bio 101), and Plant Physiology (Bio 301) courses at Queen's University. Upper-level students are encouraged to use the sensors in independent research projects.

The popularity of our laboratories with undergraduates, and the considerable interest in them shown by academic visitors to Queen's, has prompted the development of a company (Qubit Systems Inc.) for supplying the laboratories to other universities worldwide. Qubit has incorporated the sensors into a series of "Laboratory Packages" that include all the hardware, software and manuals required to conduct investigations into specific areas of plant and animal science. Packages are priced to accommodate undergraduate teaching budgets, the Photosynthesis Package and N<sub>2</sub> Fixation Packages each selling for less than \$1000 US. These packages are currently used by 200 universities in the USA and in universities in 13 other countries.

### **Introduction**

Effective teaching of biology requires an active laboratory or field component in which students investigate and measure the processes described in lectures. Preferably, these investigations should arise from hypotheses advanced by the students themselves, with the teacher acting as a guide to keep the investigations within achievable limits. When the investigations involve the use of laboratory instrumentation the following criteria should be met:

- (a) *Every student is actively involved in the experiment.* Ideally, students should work alone, or in groups of no more than 4 individuals, so that every person has both an intellectual and active input.
- (b) *Equipment must be affordable and robust.* Small lab groups require a greater amount of equipment per student, so expense is a critical concern. Many universities have opted to limit equipment expense by introducing simulation software into their biology labs. While such software may be useful to teach students the theoretical aspects of biology, as a complement to lectures, it cannot replace the experience gained in hypothesis advancement and problem-solving that is inherent in hands-on research labs. Equipment expense may also be reduced by cycling groups of students through different lab stations so that each group conducts a different experiment, using different equipment, during any one lab session. This, however, makes it very difficult to co-ordinate lecture material with laboratory studies. It is vital, therefore to provide teaching labs with accurate, inexpensive equipment that can withstand the rigors of regular student use.
- (c) *Equipment should be technologically advanced but simple to use.* When teaching a technologically advanced science it is inappropriate to use antiquated methods when more accurate modern methods are available. However, it is also important that students understand the function of the equipment that they use, rather than just the procedure for obtaining measurements. For this reason, research-based instruments are often inappropriate in the teaching laboratory because many such instruments provide data without requiring the user to understand how this data is obtained. Features of good teaching instruments include:
- (i) Externalization of components so that their operation can be seen and explained. 'Black Box' technology is to be avoided.
  - (ii) Reduction of extraneous features that distract the student from the main functions of the instrument.
  - (iii) Options for data output that involve the student calculating rates of processes rather than accepting rates displayed by the instrument.
  - (iv) A data acquisition system that is intuitive and flexible.
- (d) *Equipment should be usable in more than one course.* To minimize expense, equipment should be simple enough for use at freshman level, but have the sophistication required for use in more advanced research-based courses. In this way, the students can become familiar with the function of the instrumentation in their introductory biology courses, and gain the confidence to use the instrumentation creatively in more advanced courses.

Using the criteria listed above, a series of laboratory packages for teaching biology have been developed at Queen's University. These packages contain all the hardware, software and written protocols required to conduct numerous experiments in different fields of plant and animal biology. Each package contains several computer-monitored sensors that measure environmental variables such as CO<sub>2</sub> concentration, O<sub>2</sub> concentration, H<sub>2</sub> concentration, irradiance, temperature and humidity. As described below, these

sensors can be used to investigate numerous aspects of photosynthesis and  $N_2$  fixation in plants, as well as respiration in plants, animals, humans and most other organisms. The experiments described in each package include detailed protocols for both the lab instructor and student. In addition, the instructor's manual contains many suggestions for varying the protocols to suit students of different levels of ability and for independent research investigations by students.

### **The Photosynthesis Package**

A diagrammatic representation of the photosynthesis package is shown in Fig. 1. A leaf is enclosed in a transparent cuvette incorporating a sensor that measures photosynthetic  $O_2$  evolution. Calibration of the  $O_2$  sensor requires only that its output is adjusted to read atmospheric  $O_2$  level (20.7%). The student fills a gas bag with breath (approx. 17%  $O_2$  and 3%  $CO_2$ ) which is then flushed through the chamber. The chamber is sealed, the leaf illuminated with a halogen light source, and chamber  $O_2$  concentration increases as photosynthesis progresses. Light level may be varied using a dimmer control, and the level used in the experiment is monitored by a light sensor situated beneath the leaf chamber. Light sensor output is given in  $\mu E/m^2/s$  and no calibration is required. Analog outputs from both the  $O_2$  sensor and the light sensor are converted to digital signals by a serial box interface, and are recorded and displayed to screen using "Data Logger" data acquisition software. The software is also used for subsequent data analysis.

Data Logger software is extremely versatile, allowing students to display their data in a variety of ways, and providing numerous options for data analysis including regression analysis, curve fitting, integration, and derivatization. Students can display raw data to screen, or plot any two data sets against each other. For example, Fig.2A shows the raw data from an experiment in which the leaf chamber  $O_2$  concentration is monitored as irradiance is gradually reduced. In Fig. 2B the derivative of  $O_2$  concentration against time has been calculated to produce a data set representing photosynthetic rate (% $O_2$  increase/min.). This derivative has been plotted against irradiance to generate a photosynthetic light response curve.

Documented experiments with the photosynthesis package include:

*Measurement of the light dependence of photosynthesis including calculation of light compensation point, light saturation point and photochemical efficiency.*

*Wavelength dependence of photosynthesis and measurement of the action spectrum.*

*Temperature effects on photosynthesis*

*Comparison of Sun and Shade Plants*

*Comparison of C3 and C4 Species*

### **The Nitrogen Fixation Package**

Despite being a process of fundamental importance to life on earth,  $N_2$  fixation is rarely studied in undergraduate laboratories. This is because the standard method for measuring  $N_2$  fixation, the acetylene

reduction assay, involves the use an explosive mixture of 10% acetylene in air, as well as an expensive gas chromatograph. We have overcome these problems by developing a unique flow-through H<sub>2</sub> sensor that measures the production rate of H<sub>2</sub> from N<sub>2</sub>-fixing tissues as shown in the reaction below:



Measurement of H<sub>2</sub> allows continuous *in vivo* measurement of nitrogenase activity in real time, unlike the acetylene reduction assay that only allows intermittent measurements.

A diagrammatic representation of the N<sub>2</sub> fixation package is shown in Fig. 3. Nitrogenase activity is measured by monitoring H<sub>2</sub> evolution from N<sub>2</sub>-fixing material in an open-flow gas exchange system. Since H<sub>2</sub> is a natural product of the nitrogenase reaction, experiments are conducted under physiological conditions. Gas of a known composition (usually air) is pumped through a sealed pot containing a nodulated legume root, or a cuvette containing N<sub>2</sub>-fixing material (e.g. detached nodules). Flow rate of the gas is measured by a bubble flow meter. H<sub>2</sub> evolved from the nodules is monitored by a H<sub>2</sub> sensor, and other sensors are provided for measuring either temperature or O<sub>2</sub> concentration. All sensor outputs are displayed to the computer screen using Data Logger data acquisition software. The software is also used for later data analysis.

Unlike other methods of measuring nitrogenase activity, the H<sub>2</sub> evolution assay allows quantification of the different components of the activity. Total activity, N<sub>2</sub> fixation rate and activity related to H<sub>2</sub> evolution are all easily calculated, giving students a comprehensive view of the N<sub>2</sub> fixation reaction. As well as studying the physiology of N<sub>2</sub> fixation, the package can be used to investigate enzyme kinetics. For example, Fig. 4 shows data from an experiment in which students used H<sub>2</sub> and temperature sensors to investigate aspects of nitrogenase kinetics without the need for tissue extraction and wet-lab procedures. The data from this study allows students to calculate Q<sub>10</sub> values and make Arrhenius plots.

Documented experiments that may be conducted with the N<sub>2</sub> fixation package include:

*Temperature effects on nitrogenase activity.*

*Oxygen regulation of N<sub>2</sub> fixation.*

*Measurement of nitrogenase electron allocation coefficient.*

*N<sub>2</sub> Fixation and photosynthate supply.*

*Inhibition of N<sub>2</sub> fixation by fertilizers.*

### **A CO<sub>2</sub> Analysis Package for Plant Studies**

Measurement of CO<sub>2</sub> allows investigation of many aspects of photosynthesis, photorespiration and respiration that cannot be conducted with the O<sub>2</sub>-based photosynthesis package. However, most commercially available CO<sub>2</sub> analyzers designed for measurement of photosynthesis cost upwards of \$5000, and most designed for field use cost more than \$15 000. We therefore designed a low cost CO<sub>2</sub>

analyzer that is portable, rugged and very inexpensive (costing \$995), that has a resolution of 1 ppm CO<sub>2</sub>, a range of 0 - 2000 ppm CO<sub>2</sub>, and the accuracy required for use as both a teaching and research instrument.

The CO<sub>2</sub> analyzer has been packaged with numerous other sensors and devices required to measure photosynthesis and respiration in both the field and the laboratory. These are illustrated in Fig. 5 and include a flow-through leaf chamber, a variable light source (halogen or LED), a temperature sensor, a light sensor, a humidity sensor, AC and DC gas pumps and either a 2 channel or 4 channel data acquisition system. The CO<sub>2</sub> analyzer, DC pump, LED light source and humidity sensor may be powered simultaneously from a battery pack, allowing use of these components in the field. The A/D interface box may also be powered by the battery pack allowing data collection in the field using a lap-top computer.

Among the many experiments possible with different components of the CO<sub>2</sub> analysis package include:

*Investigation of the CO<sub>2</sub> dependence of photosynthesis including measurement of CO<sub>2</sub> compensation point, CO<sub>2</sub> saturation point and carboxylation efficiency.*

*Comparisons of methods for measurement of photorespiration.*

*Effects of stomatal conductance on photosynthesis and transpiration.*

*Investigation of factors promoting photoinhibition.*

### **CO<sub>2</sub> and O<sub>2</sub> Analysis Packages for Animal Respiratory Studies.**

While invasive techniques are an essential part of many physiological investigations, there is a growing movement to study animals without the need for surgery or euthanasia. We have designed several systems for monitoring gas exchanges from animals that do not require the animal to be harmed in any way. Respiratory metabolism may be measured inexpensively in the laboratory using extremely accurate sensors that monitor O<sub>2</sub> and CO<sub>2</sub> exchanges in real time. Insects, reptiles, small mammals and humans may all be studied using our gas exchange systems.

For real time measurements of CO<sub>2</sub> exchange in insects, and in animals with low metabolic rates, the CO<sub>2</sub> analyzer described above, with a range of 0 - 2000 ppm CO<sub>2</sub>, may be used. For measurements of CO<sub>2</sub> from larger, or more metabolically active animals, we have developed another CO<sub>2</sub> analyzer with a 0 - 10% CO<sub>2</sub> range, and a resolution of 100 ppm CO<sub>2</sub>. Both analyzers may be used in either open circuit (flow-through) or closed circuit gas exchange systems incorporating sample chambers housing the organism of interest. The CO<sub>2</sub> analysers may also be used in conjunction with the O<sub>2</sub> sensor that constitutes part of the photosynthesis package described above, since this is equally effective in measuring O<sub>2</sub> consumption and O<sub>2</sub> uptake. Together, CO<sub>2</sub> and O<sub>2</sub> exchange rates may be used to calculate respiratory quotient (RQ). RQ values provide important information about the organism's respiratory pathways, and changes in RQ can be related to changes in diet, exercise and O<sub>2</sub> availability. For example, an organism metabolising carbohydrates aerobically will have a RQ close to 1.0, while metabolism of fats

will reduce the RQ to about 0.7. Under O<sub>2</sub>-limited conditions, or during strenuous exercise, anaerobic respiratory pathways will cause the RQ to increase above 1.0.

The CO<sub>2</sub> analyzer and O<sub>2</sub> sensor can be used in a variety of ways to study respiratory metabolism in humans. Exhaled breath may be collected in a Douglas bag, (or equivalent) and analyzed by being pumped from the bag through an open gas system. Comparison of O<sub>2</sub> and CO<sub>2</sub> levels in the inhaled and exhaled air can be used to determine CO<sub>2</sub> and O<sub>2</sub> exchange, as well as respiratory quotient.

Experiments in animal physiology that may be conducted with the CO<sub>2</sub> and O<sub>2</sub> sensors include:

*Effects of temperature on the respiratory metabolism of ectotherms and endotherms.*

*Effect of diet on respiratory quotient in small mammals.*

*Control of respiration by CO<sub>2</sub> concentration.*

*Effects of exercise on respiration and respiratory quotient in humans of different fitness levels.*

## **Software**

All of the sensors we have developed produce an analog output from 0 - 5 V, and may be monitored by analog recorders, or by digital data acquisition systems after A/D conversion. We recommend the use of Data Logger software, developed by Vernier Software of Portland, Oregon, since this is very inexpensive (about \$50) and requires a very inexpensive interface box (about \$140). Also, the software will run on any PC (286 or better) or any Macintosh (MacPlus or better). Thus, computers that would otherwise be consigned to the scrap heap may find new life in the teaching lab. Vernier Software also produce a 4 channel software program called Logger Pro that requires a 4 channel interface box. Together, these sell for about \$400 and represent extremely good value for data acquisition. At present only a PC version of Logger Pro is available, and this requires a 486 computer, or better. A Macintosh version of the program will be available in April of 1998.

## **Equipment Availability and Cost**

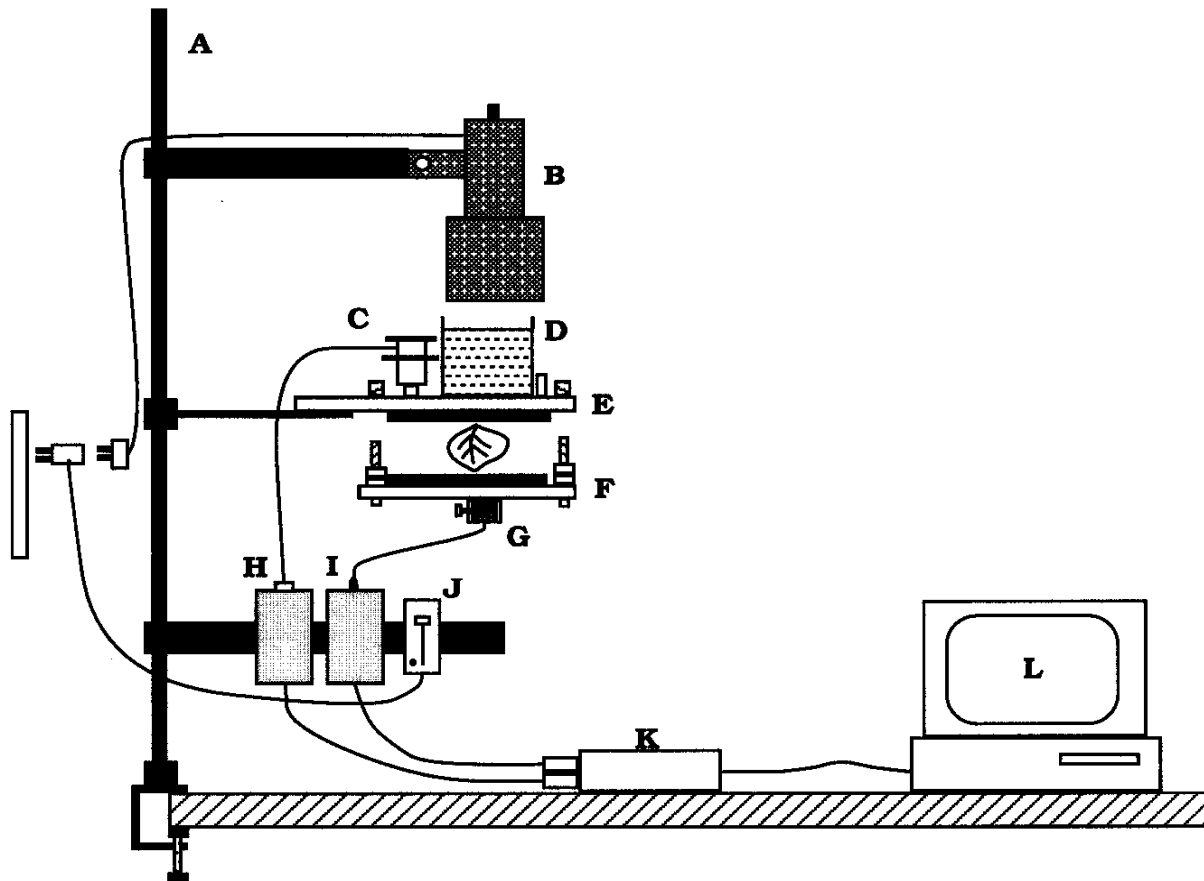
The packages described above have been tested in undergraduate courses at Queen's University and have been very successful with both students and instructors. Based on this success, a company called QUBIT SYSTEMS Inc. (Queen's University Biological Instrumentation and Technology) has been established at Queen's University to make the packages available to other universities. So far, the packages are in use in 200 institutions in 14 countries. Packages include all the hardware and software required to conduct investigations, as well as detailed manuals for both the students and the instructor. The photosynthesis package and N<sub>2</sub> fixation package sell for \$945, and different CO<sub>2</sub> analysis packages range from \$1700 to \$2500. Also, all components of the packages are available for individual sale. Because all packages use the same software, and share some other common components, users may purchase a one package, and then obtain the components of the other packages individually. This modular design helps to minimize costs and also provides a great degree of flexibility.

Qubit Systems Inc. is located at 134 Albert St., Kingston, Ontario, Canada, K7L 3N6; Phone (613)-549-3199; Fax (613)-549-3198. Qubit can also be contacted by email at [qubit@biology.queensu.ca](mailto:qubit@biology.queensu.ca). The company web site contains details of all the packages described above as well as prices for packages and components. The web address is [www.queensu.ca/parteq/qubit](http://www.queensu.ca/parteq/qubit).

## **Legends to Figures**

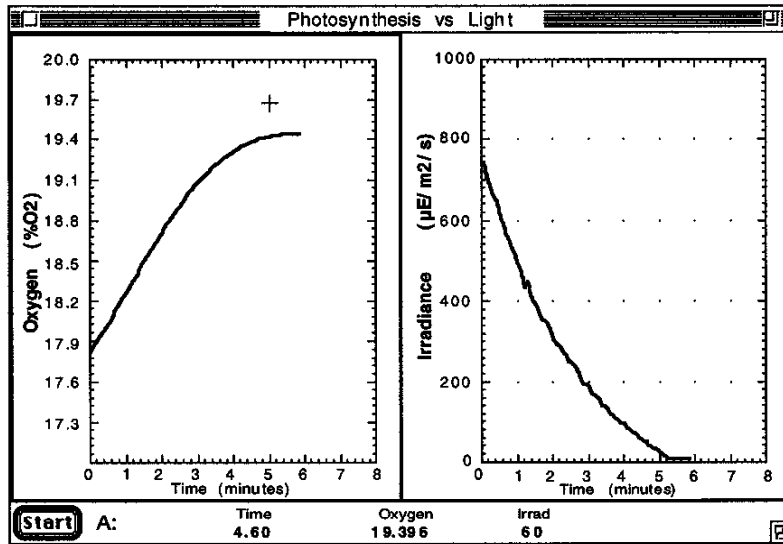
- Figure 1. A laboratory package for measuring photosynthetic O<sub>2</sub> evolution in leaves, and environmental factors that affect photosynthetic rate.
- Figure 2. (A) Raw data displayed in Data Logger software during an experiment using the photosynthesis package. The graphs show the change in O<sub>2</sub> concentration within a sealed leaf chamber as irradiance is decreased gradually.  
(B) Derivatization of the data in Fig. 2A using Data Logger software to obtain a light dependence curve for photosynthesis.
- Figure 3. A laboratory package for measuring N<sub>2</sub> fixation by monitoring H<sub>2</sub> evolution from nodulated roots of legumes.
- Figure 4. Data displayed in Data Logger software after an experiment in which nitrogenase activity was measured in the nodulated root of a soybean plant treated with a gradual increase in temperature.
- Figure 5. Components of a CO<sub>2</sub> analysis package for measuring photosynthesis, respiration and photorespiration in leaves. Components may be selected for use in the laboratory or the field.

**Figure 1**  
**Photosynthesis Laboratory Set Up**

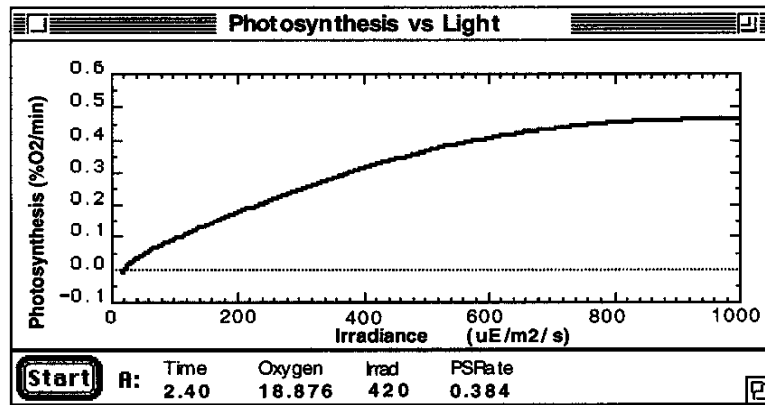


- A = Laboratory Stand with Mounting Brackets**
- B = Light Fitting with 50 W Halogen Source**
- C = Oxygen Sensor**
- D = Water Filter (200 mL Beaker)**
- E = Upper Surface of Leaf Chamber**
- F = Lower Surface of Leaf Chamber**
- G = Light Sensor in Mounting Cylinder**
- H = Amplifier for Oxygen Sensor**
- I = Amplifier for Light Sensor**
- J = Power Regulator Control**
- K = Serial Box Interface**
- L = Macintosh or PC Computer (not supplied).**

# Figure 2A

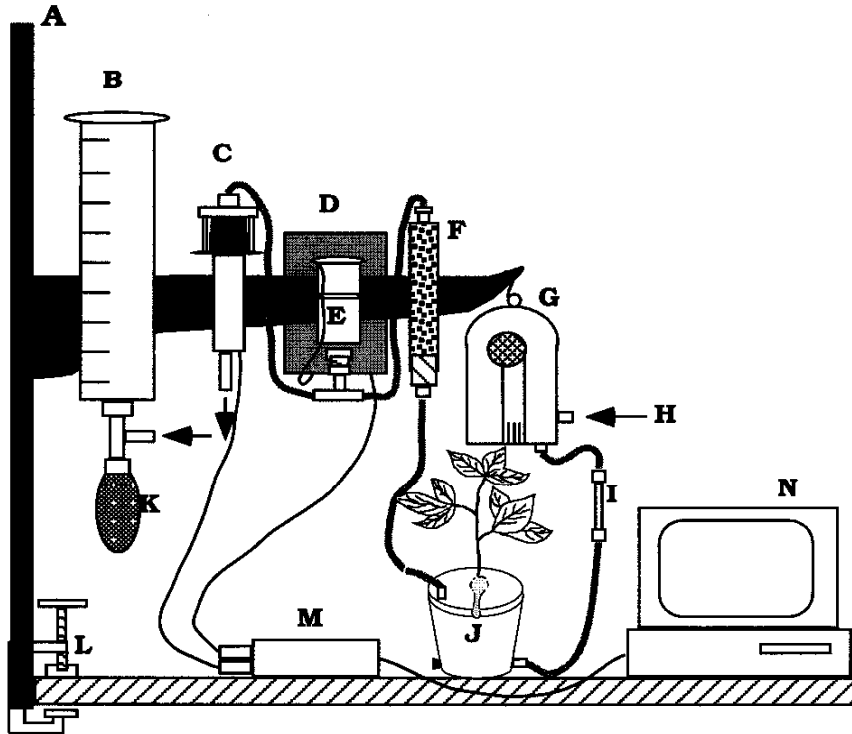


# Figure 2B



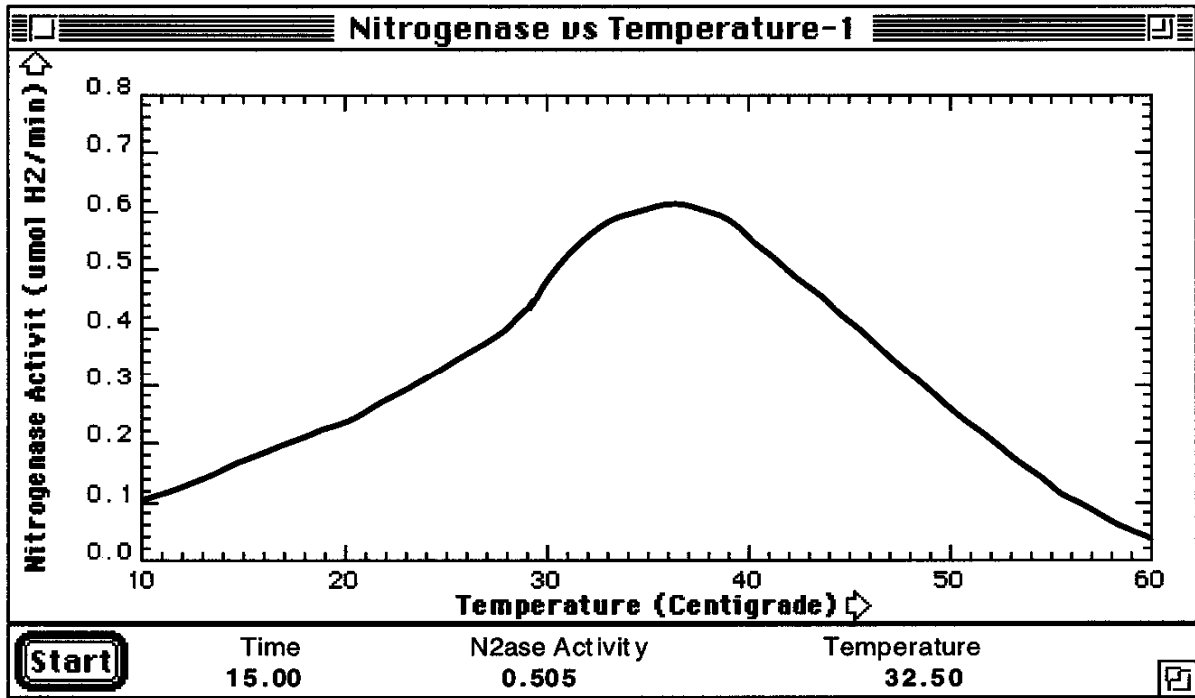
**Figure 3**

**N<sub>2</sub> Fixation Laboratory Set-Up**



- A = Laboratory Stand
- B = Bubble Flow Meter
- C = Hydrogen Sensor Inlet
- D = Oxygen Sensor Amplifier
- E = Oxygen Sensor
- F = Desiccant Column
- G = Air Pump
- H = Gas Inlet on Pump
- I = Flow Restrictor
- J = Legume in Sealed Growth Pot
- K = Rubber Bulb with Soap Solution
- L = Clamp to Mount Stand to Bench
- M = Serial Box Interface
- N = Macintosh or PC

# Figure 4



# Figure 5

## CO<sub>2</sub> Analysis Package Components

