

POLY: Resurrection in Space

An Active Biological Experiment to Measure Plant Revival Using Chlorophyll Fluorescence

(STS-85 SEM Experiment)

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Introduction

One difficult challenge facing small payload experimenters is to design an active biological experiment that works. The long and often unpredictable pre-flight storage period makes it difficult to find a suitably hardy living specimen that can survive a long period of complete dormancy and still produce useful data during the short time period of a shuttle flight. **BARI**, the CAN DO Project's first attempt at an active biological experiment to grow bacteria cultures in space was largely spoiled by an unexpectedly long flight delay and resulting storage period. The mechanism worked perfectly but the bacteria growth was severely inhibited by the dehydration of the culture media. This led to an effort to find a more stable specimen.

***Polypodium polypodioides* – The Resurrection Fern**

A middle school science class taught by Ms. Rie Cowan originally nominated the common Resurrection Fern as a possible experiment. Resurrection Fern (*Polypodium polypodioides*) is a common sight on rough tree bark and rocks throughout forests of South Carolina and the Southeastern United States. Students gathered the sample flown on STS-85 from a backyard on Seabrook Island, South Carolina.

A true epiphyte, the fern uses the trees only for physical support and gathers all nutrition from the air, not through the roots. When water is available, the fern fronds unroll, become green and produce spores. In times of dryness, they curl up and turn brown. They can remain in this dormant state for prolonged periods and still “green-up” in a matter of hours after a rain. This ability to “resurrect” from a state that appears dead and dried out to the casual observer gives the plant its name.

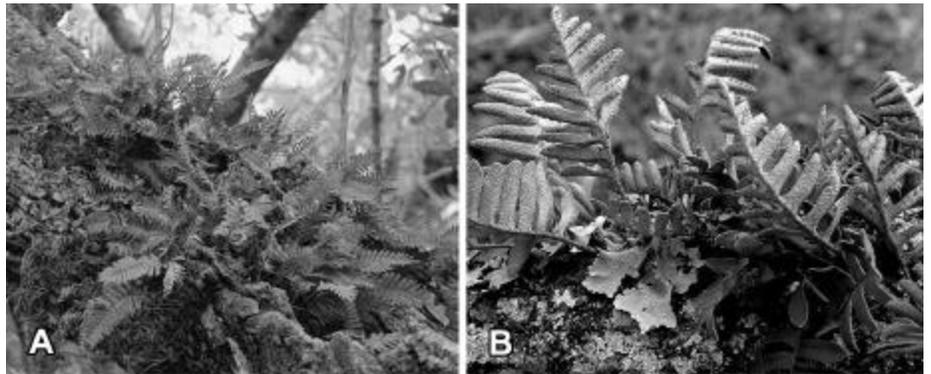


Figure 1. Resurrection Fern in the wild. (A) Growing on a tree (B) Close-up

Experimental Design

The design involves two separate steps: one to provide water, carbon dioxide and light to stimulate revival and two, to measure the return of biological activity.

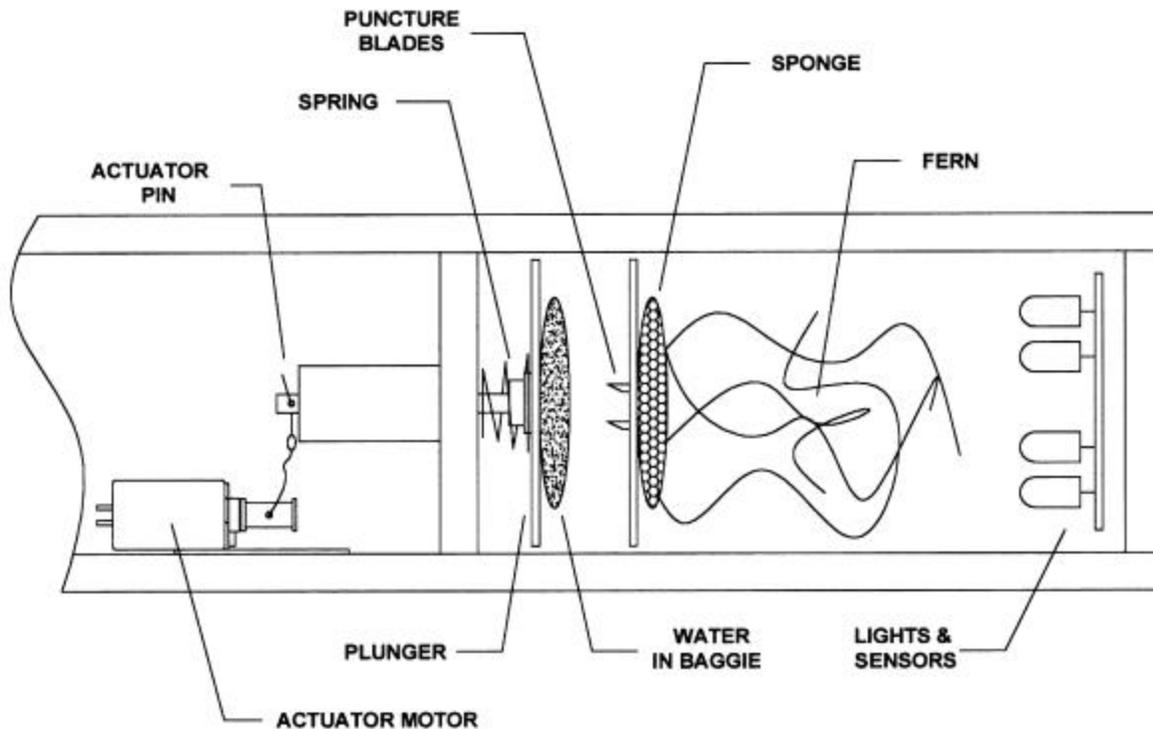


Figure 2. Poly housing and automatic mechanism

The SEM timeline software was used to control the mechanism and to gather measurements at the proper interval. A simple mechanism was designed to use a spring-loaded plunger to push a “seal-a-meal” plastic pouch of water onto sharp razor points. The water was then absorbed into a gauze pad to which the specimen was attached. A ground-up

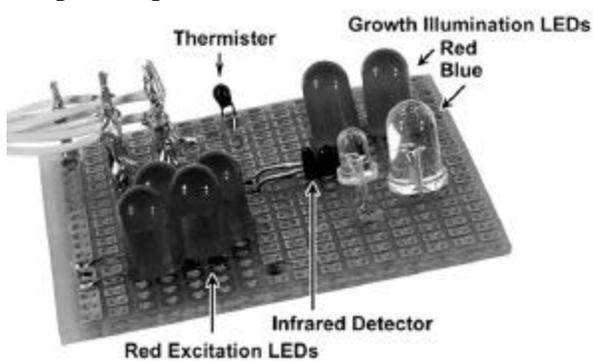


Figure 3. LED lamps and detectors

Alka-Seltzer tablet that was sprinkled onto the gauze pad provided carbon dioxide, automatically activating in the presence of water. The SEM time line software then energized a bank of high output blue LEDs to provide several hours light for a period of plant growth stimulation. Plant biological activity was monitored through the measurement of chlorophyll fluorescence. Light energy absorbed by plant chlorophyll is used first to produce chemical energy available to the plant. The capacity for photochemistry is limited however, and excess energy is often dissipated by re-emission, mostly as heat. A smaller but measurable amount is given off as red and near-infrared light. This fluorescence can be induced by a brief exposure to red light allowing a measurement of active chlorophyll levels. Red LEDs

were activated for a brief excitation period followed by an immediate measurement using a phototransistor to look for any induced fluorescence. Baseline fluorescence measurements were made before and after plant activation without red light excitation to check for any autofluorescence. A series of measurements with red excitation were made before and after the plant growth interval and then after a two day latent period (without growth light) to see if the chlorophyll levels would drop back towards baseline. This measurement was followed by another period with growth light provided to check for a recovery in chlorophyll activity.

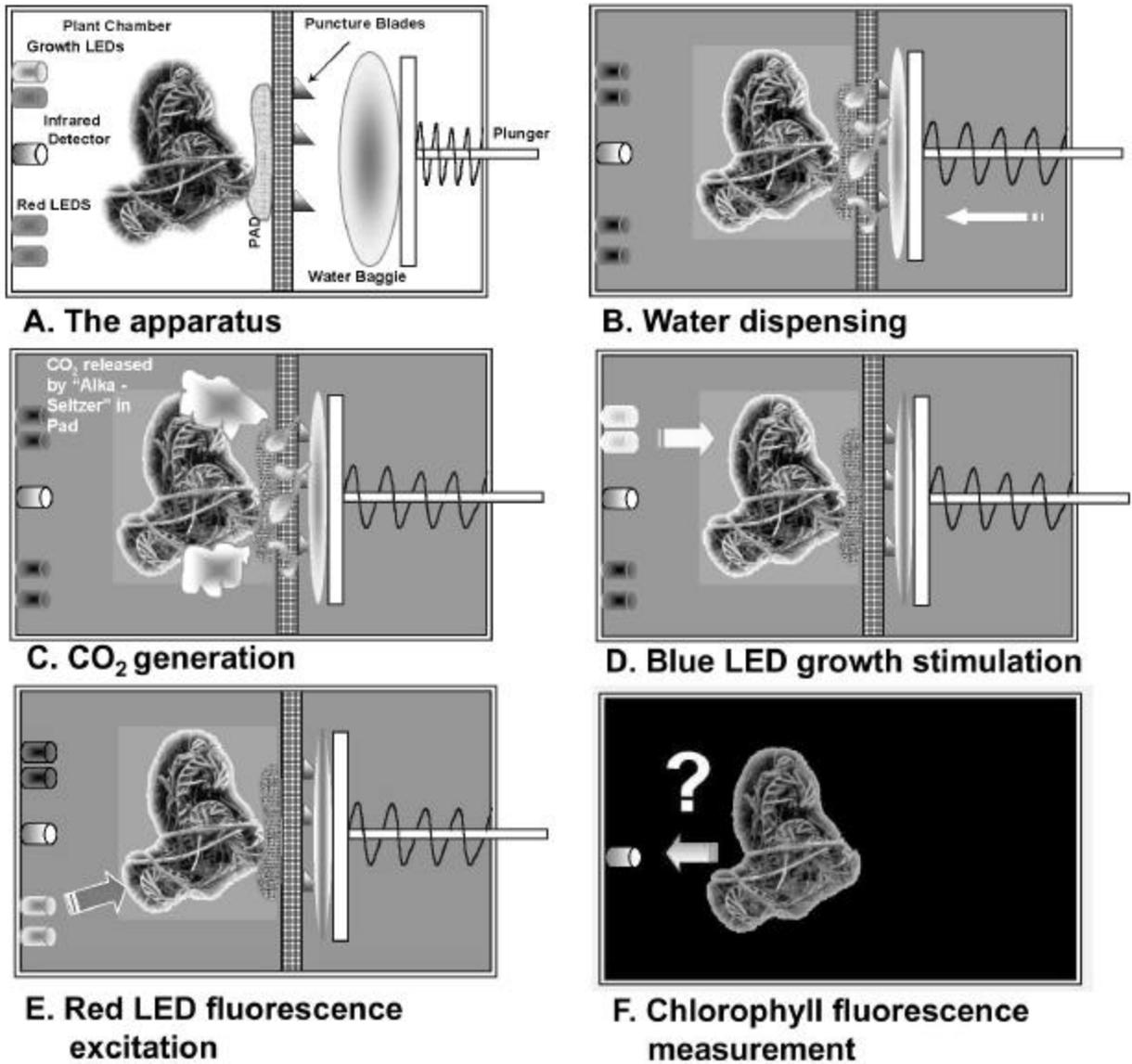


Figure 4. The individual experimental steps conducted under the control of the SEM timeline software program

MEASUREMENT OF BIOLOGICAL ACTIVITY

The apparatus to provide the necessary water, CO₂ and light to the plant is fairly simple in concept. The more difficult challenge was to design a simple and reliable method to measure the physiological activity level to verify successful revitalization. Various methods including photography and O₂ measurement were considered and rejected because of size, weight, complexity, and cost. Measuring chlorophyll production showed the most promise. Chlorophyll is produced in all green plants during biologically active periods, but it breaks down quickly in plants that are dead or are shut down into completely dormant states. A rise in chlorophyll levels is a sure indicator of active plant metabolism.

Chlorophyll actively absorbs light energy to drive the photochemical process in the plant cells. It converts some of the light energy into chemical energy that then becomes available to the plant. The capacity of this photochemistry to absorb energy is limited and excess energy has to be removed. Most excess energy is re-emitted as heat but a small amount is given off in the form of red light. This fluorescence can be measured to show chlorophyll activity.

RESULTS

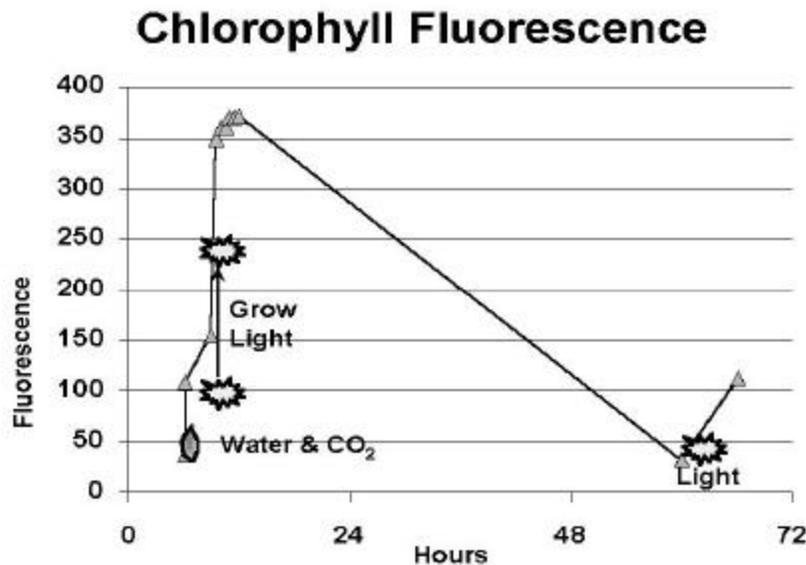
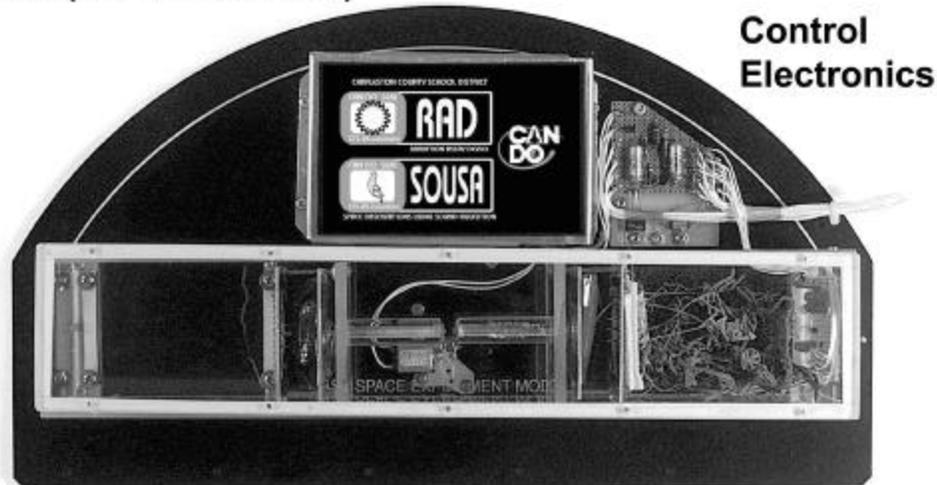


Figure 5. Fluorescence Vs Timeline showing the rise in chlorophyll signal after stimulation by light and water

As can be seen in the graph, chlorophyll fluorescence dramatically increased after each period of growth light activation and returned to baseline after a two-day dormant period. This result matches the expected biological behavior of the plant and demonstrates rapid revival of the Resurrection Fern after a long dormant interval. It also demonstrates the potential value of chlorophyll fluorescence as a method to monitor plant activity, which could be applied to other biological experiments. *POLY* proved to be a hardy space traveler and exhibited regeneration patterns unaltered by its orbital adventure.

**RAD (radiation experiment)
SOUSA (sound recorder)**



OPIE (paint drying experiment) POLY (Resurrection Fern experiment)

Figure 6. SEM Module with 3 active (POLY, OPIE, and SOUSA) and one passive (RAD) experiment. Note the clear acrylic chamber within which OPIE and POLY share a common activation motor in the center section. The motor pulls a pin releasing a spring activator for each experiment. In the righthand section of the chamber the Resurrection Fern sample can be seen.

REFERENCE

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