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PLANT HEALTH SENSING

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SUMMARY

When considering projects for the 1987/88 Advanced Space Design class, plant health and disease sensing stood out as an important problem area that needs to be extensively researched. Designing this type of project has not been exhaustively investigated, so NASA and the CELSS program will benefit from the work that could be done in this area. If plants are to be used as a food source for long term space missions, they must be grown in a stable environment where the health of the crops is continuously monitored. The sensor(s) to be used should detect any diseases or health problems before irreversible damage The method of analysis must be nondestructive and occurs. provide instantaneous information on the condition of the crop. In addition, the sensor(s) must be able to function in microgravity. This first semester, the plant health and disease sensing group concentrated on researching and consulting experts in many fields in attempts to find reliable plant health indicators. Once several indicators were found, technologies that could detect them were investigated. Eventually the three methods chosen to be implemented next semester were stimulus response monitoring, video image processing and chlorophyll level detection. Most of the other technologies investigated this semester are discussed in this report. They were rejected for various reasons but are included in the report because NASA may wish to consider pursuing them in the future.

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INTRODUCTION

Problem Description

In the near future, when long term space travel becomes a reality, the need for food sources will become one of the many crucial factors controlling the duration of a space flight. The ability to grow crops in space can provide a virtually unlimited source of food and oxygen, and when astronauts depend on that food source, their survival is dependent upon the health of those crops. The need for a way to determine the health of those crops will play a very important role. There must be some type of system that can monitor the food crops, determine if the plants are healthy or not, be able to diagnose the problem if the plants are not healthy and finally give an appropriate course of action or cure which can be followed by the crew to insure the survival of the crop.

This system would be subdivided into two parts. The first part should encompass a way of continuously monitoring the plants and detecting any possible problems. The second part must then be able to interpret the problem and determine an appropriate course of action. An expert computer system combined with remote sensors could accomplish this task. The remote sensors would provide a way of continuously monitoring the plants, and the expert system would act as a plant pathologist being able to interpret this sensory data and determine if a problem exists, and then reference its extensive knowledge and data bases to come up with a diagnosis and cure.

The success of this system depends upon the dependability of the remote sensor. There needs to be an effective way of monitoring the health of plants grown in space on a continual basis. Having a automated system would free the astronauts from the time-consuming task of visually inspecting the thousands of plants on a daily bases as well as the requirement of at least one of the crew members being an expert on plant diseases. The remote sensor is important because there are many plant diseases which can harm a plant before there are any visual signs of damage. For this reason, it would seem that the development of a reliable sensing technology would be very important since one would not be able to insure the survivability of the food crops based on visual observations from the astronauts alone.

Advances in the area of remote sensing of plants health has been very slow. There are a few techniques being used presently here on earth, however, there is still a reliance upon the visual observations of agronomists and pathologists to discover diseases among their crops. On earth, the losses of crops due to missed or late observations would not have the same impact as on a space mission with a limited number crops and crew members whose lives depend on those crops.

Project Description

The Sensor Project Group has selected to research and design a possible remote sensor or sensing technology which could be used to monitor the health of crops grown in space. This sensor will be required before the entire process of automated plant health monitoring can be implemented. There are many sources of knowledge in the field of computer expert systems but very little in the area of remote sensing for plant health, therefore, the development of such a sensor would be useful for both the NASA CELSS Project as well as for the rest of the agricultural community on Earth.

<u>Design Criteria</u>

In designing a remote sensor to monitor the health status of plants to be grown in space, several factors must be taken into consideration. One must not only consider the wide variety of plant types and plant sicknesses, but also the special requirements for such a system to work unattended in micro gravity. Several goals were set to guide the research into those sensing technologies which showed the highest probability of success.

The design criteria that a remote sensor must meet are:

- 1. It must act as an early indicator of health plant, that is, it must able to indicate a plant sickness (disease or deficiency) before irreversible damage has occurred.
- It must respond to a wide range of plant sicknesses which have various types of affects upon those plants. The sicknesses might include all types of pathogen invasion, nutrient deficiencies, environmental and water stresses, and physical trauma.
- 3. It must respond to a wide diversity of crops (or at least to the types which are being considered for space travel).
- 4. It must not be destructive to the plant. If a technology requires the sampling of a plant, it must not jeopardize its growth or survival.
- 5. It must sense an indicator of plant health which is accurate and dependable for all plants. There should be no false alarms nor late warnings.
- 6. It should give a real time estimate of the health status of a plant, instead of an analysis dependent on measurements over time. In this way, the condition of many plants could be monitored rapidly.
- 7. It should not contaminate the growth chamber. The generation of toxic waste products or particulates would have harmful effects.
- 8. It must fit within the growth chamber and be easily moved from plant to plant within a crop type.
- 9. It must operate in microgravity and must function properly independent of orientation.
- 10. The design and construction of such a sensor must fall within the resources of the Design class.

Background Information

The design, construction, and testing of a remote sensor will fall within a two-semester time frame. This entire process was divided into two phases, research and implementation, with each phase lasting one semester. During the first phase, research, information pertaining to plant diseases, nutritional deficiencies and various stresses were accumulated from numerous plant pathologists, agronomists, agricultural engineers, biologists and other scientists. The goal in this research was to find a common indicator of health for the crops being considered for space travel (potatoes, wheat, lettuce, and soybeans). Once a potential indicator(s) had been selected, our efforts were directed towards finding a technology that could detect that indicator.

The next part of our research called for finding any and all possible technologies that could possibly be applied towards the sensing of plants. This area of research was broken into three categories:

- 1. Investigating already existing technologies in the field of remote sensing of plants and assessing their suitability or adaptability for the established criteria.
- 2. Finding any technologies being used for remote sensing in other areas such as the medical and engineering fields, and estimating if they could be adapted to plant health.
- 3. The development of a new sensing technology based upon scientific and/or engineering principles that could be tested.

After finding the possible technologies that could be used to monitor or sense the indicator of health on a plant, we then narrowed down the various technologies to those which could work with all the food crops. We then eliminated potential sensing technologies due to their cost or because there already had been extensive work done in that area. The next phase would be the investigation of some potential technologies through design and testing of the sensor.

The second phase, implementation, begins next semester. Having narrowed down the potential sensing technologies, we plan to test each of our theories on live plants after developing the physical sensor itself.

CONCEPTS AND FINDINGS

List of Sensing Technologies Researched

Several technologies applied to remote sensing were researched during the semester and are listed below. Advantages and disadvantages of each were studied before narrowing down this list to three potential sensing technologies, which will be implemented next semester.

Many of these technologies that were researched and listed below have great potential for working as a successful remote sensor but most were not included in our implementation phase for next semester due to one or more of the following reasons: excessive equipment purchases, extensive research already done in this area, and excessive time requirements.

Technologies Considered.

- 1. Gas level/exchange monitoring
- 2. Infrared (IR) temperature monitoring
- 3. IR video imaging
- 4. Spectral reflectance using color IR film
- 5. Odor sensing
- 6. Ion detection/monitoring
- 7. Nuclear magnetic resonance
- 8. Electrical properties
- 9. Resonance frequency
- 10. Stimulus response monitoring
- 11. B/W video image processing
- 12. Chlorophyll level and light absorption

Description of Technologies Researched

<u>Gas Level/Exchange Monitoring</u>. Carbon dioxide and oxygen gas levels are good indicators of the health status of plants. During photosynthesis, sunlight is captured by the photosynthesis unit of a plant cell in a leaf, consuming carbon dioxide and producing oxygen and carbohydrate. The overall process can be summarized in the following equation:

 $SUN + 6 H_2O + 6 CO_2 = C_6H_{12}O_6 + 6 O_2$

A healthy plant at a certain age or size will take in a specific amount of carbon dioxide and expel a specific amount of These levels can be accurately monitored in a closed oxygen. environment and can be recorded as the proper levels of gases exchanging in that closed environment. These levels will relate to a normal photosynthetic rate of a healthy plant. Because CO₂ intake is a direct indicator of photosynthesis rate, it is used extensively in plant physiology as an indicator of the well-being of the plant. As a plant becomes diseased, the photosynthetic rate of the plant can increase or decrease (which is normally the case). By monitoring the levels of carbon dioxide in the growth chamber, one would notice any sudden changes in the photosynthetic activity of the plants. One advantage of this type of monitoring is that it can show changes in the plant health before signs of any physiological effects can be seen on the plants. One drawback of this technique is that it might not work for every disease, and studies have indicated that chlorophyll levels can drop by 40% before the photosynthesis rate drops noticeably. The second drawback is that the monitoring device must come into contact with the plant leaf to make an accurate measurement, and these measurements of the CO2 uptake rate are difficult to correlate to plant health when they are made in two different leaves or two spots on the same leaf. For these reasons, and since extensive research has already been done in this area, this technique will not be implemented [1].

Infrared Sensing Technologies. The use of IR imaging is a promising and extremely useful technology which can be used in the remote sensing of plants, however, it is also extremely expensive.

IR Video Image Processing: This technology has been used in various areas in engineering and medical research and uses an IR video camera system which is combined with a computer. The computer is used to process and analyze the video signals received from the camera system. The images produced are color pictures in the shape of the object being imaged, having different temperature regions mapped out in different colors.

Infrared imaging allows continuous monitoring of plants and the development of color images relating to the temperature patterns on the surface of the plant. These temperatures can correspond to the transpiration process (loss of water through evaporation) which is occurring from the surface of the leaves and which is a function of the ambient vapor pressure and temperature in the surrounding atmosphere and the amount of water within the plant. These color patterns can also indicate the presence of dead or damaged leaves since these areas will not be transpiring and will show up as areas of different temperature.

This technique appears to be promising for remote sensing because a plant's temperature and its transpiration rate are all good indicators of plant health. Color patterns for a normal healthy plant can be stored by a computer and used as a reference. The image processing software will then analyze the new image and determine deviations from the reference patterns which would indicate potential problems. By creating a library of color patterns of plants with various diseases as a set of references, the image processor would be able to identify a specific disease.

An IR camera can be easily manipulated throughout a growth chamber and between individual crops. It is nondestructive and could work with a wide range of plants. The sensor project

group, however, will not be able to test this type of system due to the enormous costs involved in obtaining the state-of-the art IR image processing equipment needed to implement this technology.

IR Temperature Monitoring: A simpler and cheaper form of infrared sensing used to monitor plants is the use of IR "guns" which simply measure the average temperature of an object, instead of the elaborate temperature differential given by an IR imager, thereby eliminating the need for expensive image processing equipment. This temperature reading of an object is easily obtained by turning on the handheld IR gun, and aiming/pointing the gun at an object. This device is extremely accurate and gives a precise measurement of temperature.

The main application of this technology is detection of water stress among crop plants. Many plant diseases can be directly related to water stress or can place a plant in a state of water stress. In either case, there will be some effect upon the transpiration rate of the leaves involved. Changes in the transpiration rate will cause changes in the temperatures on the surface of the leaves which can be detected by the IR "gun". By knowing the normal temperature ranges for various crops, one can detect changes in the transpiration rate due to some abnormality by using this IR "gun". The main disadvantage to this technique is that it cannot distinguish between the possible causes of an abnormality. The sensor group decided not to implement this technology during the second semester due to its limited diagnostic capabilities and the extensive work that has already been done using field crops [2].

Spectral Reflectance Using Color IR Film: One of the first technologies that was considered during this semester was nearinfrared analysis of plant health using color IR film. This process measures the reflected energy coming from the plant, as opposed to the heat given off. The film is sensitive to visible

and near-IR radiation, which lies between 0.58 and 0.9 microns of Pictures of healthy plant leaves as well as those of wavelength. plants infected with known diseases would be taken. These photos would be studied and compared until spectral reflectance signatures for each plant type and disease could be identified and documented. This analysis involves observing color and reflectance intensity variations on the film and deciding what conditions represented normal energy levels on the leaf, as well as those that are abnormal. On an IR photograph, this diseased area of the leaf would appear darker than the surrounding healthy Eventually, the actual health sensing procedure would region. involve taking pictures of the plants every day, developing them, comparing them to the standard signatures that have already been obtained and looking for changes in reflectance that may indicate that the plant has developed a problem.

Unfortunately, the technology required to fabricate this project was too expensive for the class. For use on a space craft, an IR video image processor would be needed to quantitatively compare the test photographs to the standard reflectance signatures. The skill and time required to perform this type of comparison, in addition to establishing the signatures themselves, would be too great for the sensing group to do manually. In addition, one of the design criteria established was that the sensor must work in real time, so pictures would have to been taken, developed and processed every day. Although this type of plant health sensing is not a technology that this class chose to pursue, NASA may wish to investigate it further, because spectral reflectance using color infrared film is one of the few nondestructive methods for identifying disease on the plants at an early stage.

Odor Sensing. A new indicator that was considered for detecting disease in plants is odor sensing. Originally, this idea specifically dealt with potatoes. In potato tubers infected by diseases such as Brown rot or Late blight, decomposition and fermentation occur and a foul odor is produced. When celery is attacked by disease it also releases an sweet odor. Since humans can smell the odor, it is feasible that a gas sensor placed above the crops in a closed environment might detect a problem before any major harm could occur.

After some research and consulting with experts, this sensing technology was rejected. In space the gas released from the infected root area may not rise directly above the plants and more likely will dissipate outward. Experimentation in microgravity may prove that a sufficient amount of gas may not be detected by the sensor placed directly above the crops. It was also found that potatoes, in general, must be severely infected by a disease before the roots release enough gas to detect. Lesions and discolorations on the leaves usually occur before decomposition of the roots. Remote sensing of the leaf surface would be a more effective early warning sensor than odor detection. Finally, this technology did not meet the predetermined criteria because its use is limited to potatoes and celery.

Ion Detection/Monitoring. Growing higher plants in a space environment involves a complex scheme of interrelated systems. Along with the concerns about containment, plant support, planting, and harvesting is the area of plant nutrition. The productivity of the plants to be used as food must be maximized due to space and resource limitations. Since the nutrition of the plants grown will probably be delivered by hydroponic systems, control of the composition of the nutrient solution must be constant and precise. However, analysis of the solution for the widely varied concentrations of numerous individual nutrients would require a method or methods within the range of skills of the operators, either human or mechanical. Also, on deep space missions, equipment must be sufficiently durable to last the mission, or it must be repairable or replaceable by the crew or the mechanical devices aboard. If they are to be replaceable or

manufactured, they must require a small amount of material and the manufacturing process must not present a hazard to the crew.

Current methods for analysis of plant nutrient solutions involve the use of atomic absorption or inductively-coupled plasma spectroscopy, which analyze for discrete elements. These methods require detection devices specific for each element, and the use of high-pressure gases to deliver the solution to be analyzed to either a flame or an induction furnace. The routine use of such systems requires a human operator with considerable skill in both the use and maintenance of the equipment. These analyses also take a substantial amount of time, with occasional repairs that shut the system down completely. These are fairly complicated methods which require a high degree of user attention, and which also are potentially dangerous in space.

Another method that is being developed for routine analysis of ions in solution is ion chromatography. This method has the advantage of analyzing for a number of ions within the same solution. The machinery involved requires a fairly high level of skill to operate and maintain, and current models appear to be too delicate and temperamental for use in space. However, this does not prevent their use in the future if certain problems can be worked out in their design.

An alternative method is the use of ion-selective electrodes. These function on the principle that a specific ion will interact with a specific membrane to generate an electrical potential that is proportional to the concentration of the ion in solution. These are very sensitive to their respective ions, but other ions in the solution can interfere with the measurement of the desired ion. They are also specific only for the particular ion, they require periodic refurbishment, and manufactured electrodes are fairly large and expensive.

However, an alternative design for ion-selective electrodes has been developed [3]. These electrodes consist of a film of a suitable polymer containing the desired ion coated on a conducting metal, and have response characteristics equal to or

They are small and better than the manufactured electrodes. inexpensive to make, can be produced on site, and can be custom designed for a wide range of ions. The manufacture of the electrodes would use either platinum or copper for the wire, and either polyvinylchloride (PVC), poly(methyl methacrylate), or epoxy resin as the coating substance. The appropriate salts of plant nutrients (which could include calcium, magnesium, potassium, ammonium, nitrate, iron, zinc, manganese, sulfate, or phosphate) would be incorporated into the polymer, and the wires would be coated by repeated dipping and drying. Construction of microelectrodes on a microchip is a present technology [4], but the number of elements tested may be limited. This adds a level of miniaturization, such that the astronauts could take a supply of these chips along without much weight.

While this method has potential for the monitoring and maintenance of the nutrient solutions used for plant growth, this technology lies outside the range of plant monitoring for this study.

<u>Magnetic Resonance</u>. This technique operates on the principle that transitions between magnetic spin energy levels of certain atomic nuclei can be induced in a magnetic field. The energy required to cause these transitions in the radio frequency range. The use of an interaction between an applied magnetic field, radio waves and atomic nuclei provides a direct noninvasive monitor of biochemical events in selected regions of living cells, tissues or organs of live animals or humans [5].

While there has been great emphasis on applications to the investigation of cell membrane function, biochemical reactions, and NMR imaging of human subjects, little or no information has been generated on these characteristics in plants. Also, the equipment used is massive, requiring a large electromagnet, a radio wave source, and sophisticated support computers. At its current stage of development, the weight and size of the apparatus would make it unusable in space craft. The pursuit of

investigation of this technology would be inappropriate for our class project. However, under circumstances which would allow the use of this technology, much information about the physical and biochemical nature of plants could be generated.

Electrical Properties. Plant tissues are conductors because of the content of ions in the cell sap, and any stress or physiological imbalance may affect the ionic composition. Because of this, measurement of different types of electrical properties of plant tissue may give information on the physiological health of the plants being monitored. Many of the electrical properties of plant tissue involve dielectric constants or permittivity. A number of these types of measurements are included under this heading.

Electrical Properties: The interaction of electric and magnetic fields with organic matter have been considered as biological effects of nonionizing radiation, even though these fields do not involve any radiation. The wavelengths of these fields are so large that they do not produce any effects of radiation, such as ionization, and these fields have components that store energy without contributing to radiation. However, electrical fields of sufficient magnitude can orient dipoles, or move ions or polarizable neutral particles. Microwaves (in the radio frequency range) have also come into use for their heating effects, but all biological effects of radiated radio frequency power do not necessarily arise from temperature changes. The magnitude of the external or applied electrical field is always larger by several orders of magnitude than the resultant internal electrical field.

Most electrical processes known to occur naturally in biological systems (action potentials) are limited to direct current and extremely low frequencies. Some physiological effects may occur even if the magnitude of these fields is not large enough to produce thermal effects [6].

Electric fields can affect root growth by affecting the transmembrane potential [7], while magnetic fields can affect the orientation of shoot growth [8]. Electrical fields have been used to form channels between contacting cells, resulting in a fusion of plant cells, with the resulting mixing of genetic material resulting in new varieties of plants [9].

While there are distinct effects of electrical fields on plant tissues, there is little or no information on how these effects could be used as a means of monitoring plant health. These stimuli might be used in later research, but the present stage of development does not permit their use in a project on the scale of the Design class.

Electromagnetic Testing Methods: Eddy current testing involves the use of alternating magnetic fields and can be applied to any conductor. When an alternating current is used to excite a coil, an alternating magnetic field is produced and magnetic lines of flux are concentrated in the center of the coil. As this coil is brought near an electrically conductive material, the alternating magnetic field penetrates the material and generates continuous, circular eddy currents. Larger eddy currents are produced near the test surface; as the penetration of the induced field increases, the eddy currents become weaker. The induced eddy currents produce an opposing (secondary) magnetic field in the opposite direction to the generated (primary) magnetic field. This opposing magnetic field, coming from the material, has a weakening effect on the primary magnetic field and this change can be sensed by the test coil. In effect, the impedance of the coil is reduced proportionately as eddy currents are increased in the test material [4].

Any parameter that can affect the electrical conductivity of the test area can be detected with eddy currents. Since plant cells are conductors, a baseline conductivity for selected plant organs could be determined under optimum conditions and used as comparison during growth cycles. When used with plants, the

sensor would be a coil positioned above the surface of the plant part, probably the leaf. However, when attempting to measure changes in conductivity, changes in the distance to the material being measured are not desireable, so the measuring probe would need to accurately positioned at the leaf. A variation of this configuration is a probe in which the detector coil is positioned directly opposite the excitation coil may not be feasible, since the results are affected by the thickness of the material being tested, and plant leaves can vary greatly in thickness.

There is little information about application of this technique for plant materials, and the precision with which the probe must be positioned at the leaf surface is beyond the capabilites of the class.

Resonance Frequency. One original method for detecting plant health, which involves measuring the resonant frequency of a leaf, was researched by the group this semester. The idea involves oscillating a leaf at a known frequency, then using an image processor or perhaps a simple laser "gate" to measure the resonant frequency that the leaf reflects back upon the device holding the leaf. The frequency of the leaf would be proportional to the rigidity or stiffness of the leaf, and this in turn could determine the water pressure within the leaf. Through research and by consulting experts, measuring the level of water stress is a good way to assess plant health. A fully turgid plant has as much water as it can hold, and its elasticity is at a maximum. If root rot, stem rot, or wilt attack the plant, the vascular pressure and water content is reduced. These diseases stop the roots from moving water up through the plant, thus reducing water pressure. It takes time for the water pressure of the plant to decrease to a critical level, so if a decrease in resonant frequency was detected early, the disease could be treated by adding more water or changing the humidity and temperature in the environment, and the plant could be saved.

The technologies that are used today to measure this type of water stress are destructive. They involve either taking a small piece of a leaf, or taking a sap sample from the vascular system and measuring the water content using expensive equipment. Finding a leaf's resonant frequency looked very good as long as this measurement related to the water stress in the plant. Unfortunately, the stiffness of a plant depends on more than just the water pressure. For instance, if the leaves and stem have accumulated a high level of starch, the leaves may be leathery and inflexible, even though the plant may be fully turgid. Plants grown in lower light and humidity may be more flexible. Because a resonant frequency measurement may result in misleading information about the water stress in the plant and a visual measurement of frequency would probably be inaccurate, this technology will not be further researched in the second semester.

Technologies To Be Implemented

The following are the three methods chosen for further investigation in the next semester. These will be discussed in more detail than the previous technologies, along with plans for the construction and testing of the methods chosen.

Stimulus Response Monitoring. Since a plant is a living organism, a plant might react to an externally applied stimulus. By subjecting a plant leaf to an electrical, heat, intense light, chemical or physical source as a stimulus, the leaf might "react" and plant responses could be detected. Responses to stimuli (Figure 1) could show noticeable differences in the reactions of healthy and sick plants. Reaction times to the stimuli could be different as well as the changes themselves, and these differences could be catalogued.

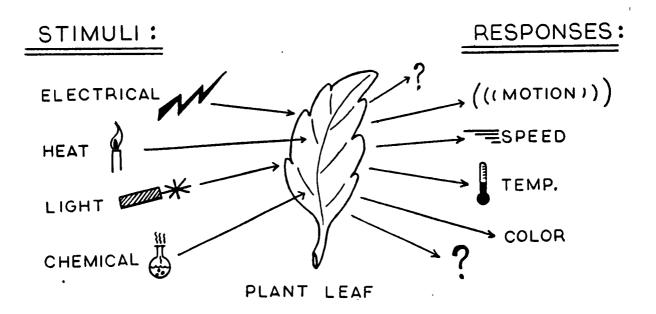


Figure 1. Plant Response to Stimuli

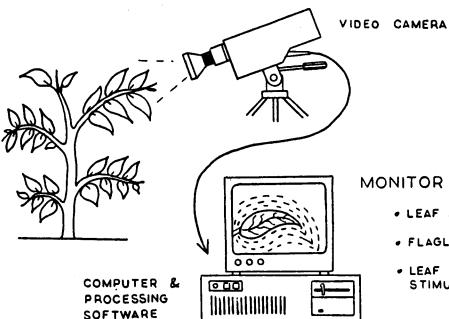
A number of methods can be used for this depending on the nature of the stimulus and the response being measured.

Visual Inspection: A long-distance microscope would enable the user to obtain microscopic examination of objects (in excess of 100x magnification) at relatively long distances. This would be an non-invasive technique and would allow for close inspection of the plants in the growth chamber without opening the chamber. It can also be used with photographic and video equipment. As with other video applications, the images could be enhanced and digitized for comparison to a library of information for the particular plant being studied [4].

Acoustic Emissions: Mechanical or thermal stress of materials, if continued to deformation or fracture, can generate an acoustic signal that warns of the impending failure. However, some deformations and fractures are so minute that extremely sensitive listening devices must be used to hear them. Many of these acoustic emissions are beyond the range of normal hearing and well into the ultrasonic range. Therefore, acoustic emission signals are both difficult to measure and difficult to simulate.

The combination of a transducer, preamplifier, amplifier, and oscilloscope form the basic acoustic emission monitoring system. The transducers used to acquire these signals are piezoelectric sensors, which can be used in the sonic and ultrasonic ranges. However, these techniques require actual contact with the surface being tested, or, as in the case of some ultrasonic applications, the immersion of the sensor and the material of interest in water [4].

Further information will be generated about the applicability of this technique in the next semester.



MONITOR SMALL CHANGES IN:

- LEAF AREA CHANGES
- . FLAGLEAF GROWTH
- LEAF MOVEMENT DUE TO STIMULUS RESPONSE

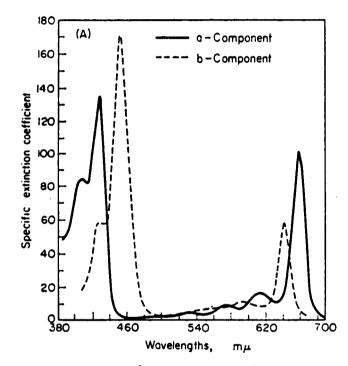
Figure 2. Black and White Video Image Processing

Black and White Video Image Processing. The video processing system, which includes a black and white video camera, personal computer and image processing software, can be used to accurately monitor very small changes in size, area or motion of an object. This system could be used to monitor the growth rate of a plant by looking at various growth aspects of a plant. It could monitor the overall canopy growth rate, the change in area of a single leaf, or search and find the flagleaf to measure its change in size. The flagleaf is the newest (and usually the smallest) growing leaf on a cereal type plant, and is a good indicator of the production of new plant tissue. The normal growth rates of a healthy plant would have to first be established, then this data could be stored and compared to new images of a plant that is currently being scanned (or monitored) to determine if it is growing at the normal rate of a healthy plant (Figure 2).

An image processing system for the analysis of plant health can be broken down into two subsystems: the image acquisition system and the image analysis system. The image acquisition subsystem could be either a image digitizer that scans the image field and produces a binary file or a standard television camera combined with a computer system that converts the image into a binary file.

The data file can be in one of three forms for black and white images. The first is the standard, unprocessed form, which consists of a field of pixels, each with its own intensity ranging from black to white (there are usually 256 possible intensity values). The second type is called a halftone image (similar to a picture in a newspaper), and consists of black dots of varying sizes. The unprocessed image is divided into fields of 64 pixels each (8 x 8), and each field is assigned a black dot whose size is proportional to the average intensity of the pixels in its field. Thus the original image data is "compressed" to about 1/8 of its original size, while still retaining good resolution. Some types of image processing will work better with

halftones, since the data to be manipulated is much smaller in size. The last type is the threshold image. To produce this type, the scanned pixels are assigned a black or white value (0 or 1) according to intensity relative to a predetermined value. This method works best with images on high contrast; most of the information is lost when this is used on a object with extensive shading or opaquity. None of these systems present any technological problems, for the technology involved has existed for dozens of years.



Absorption spectra of chlorophyll a (solid line) and chlorophyll b (broken line).

Figure 3. Chlorophyll Absorption Spectra

Chlorophyll Level and Fluorescence. The choice of chlorophyll activity as a plant health indicator was derived from the study of micronutrient levels in the plant as health indicators. It was thought that these levels could be monitored nondestructively, that is, while the micronutrients were still in the plant leaf, and the simplest way to do this seemed to be by spectroscopic measurement. Spectroscopy is based on the fact that atoms and molecules can absorb and emit light of specific wavelengths corresponding to the energy levels of their valence

electrons. Thus each molecule has its own characteristic spectrum composed of sharp peaks of either light absorption or light emission (Figure 3). Spectroscopic measurements are made by exposing the sample (in this case the plant leaf) to light of a certain wavelength and measuring the transmitted light level. The problem with using this method to measure micronutrient levels in plants is that there are about 15 micronutrients that need to be monitored, and such a system becomes complicated both in the monitoring of the micronutrient levels and the interpretation of the data. It is much simpler to measure the uptake level of micronutrients by monitoring the growth solution with ion-selective electrodes than to analyze the plant leaf.

Although it is not feasible to measure the micronutrient levels of plants spectroscopically, there are other concentration levels that can be measured using this method. The most important of these was found to be chlorophyll activity, which will give information on the health of the photosynthetic system of the individual plant [10].

Chlorophyll activity has the advantage over other plant health indicators in that it looks at the source of plant growth, rather than measuring the growth itself or a consequence of this growth (or lack of it). One of the most sensitive plant health indicators, carbon dioxide intake, is an indirect measurement of photosynthesis. The problem with this type of indirect measurement is that it suffers from a lag time between the beginning of harm to the plant and the drop of carbon dioxide intake. In other such secondary indicators this lag is even more profound; it takes time for growth rates to change, for transpiration to rise, and for other properties to react. This is a primary advantage of chlorophyll level measurement, for it is measuring the definition of plant health, and not the lack of health.

There are other methods for measurement of the chlorophyll activity in plant leaves, but spectroscopy is the simplest and most accurate method available [11]. Several methods of chromatography are currently used to measure concentrations of chlorophyll, but these methods are destructive and require the removal, transportation, and preparation of leaf samples, thus adding the complexity of mass transport and contamination of the growth chamber. There are also the traditional chemical laboratory techniques, but these share the same disadvantages as chromatography. Thus the spectroscopic plant health sensor (hereafter referred to as SPHS) system has an inherent advantage over destructive testing because the sample in kept in the leaf and there is no problem of loose samples floating around in microgravity.

A spectroscopic system for measurement of plant health would be very simple in design. It would consist of a light source and light intensity meter with the optics necessary for handling the light, a robotic device to bring the source and meter to the plant, and a computer to interpret the data. The light source could be either a laser tuned to the required wavelength of light or a high power lamp source with a monochromator to obtain the needed wavelength [12, 13, 14]. Fiber optics could possibly be used so that both the light source and intensity meter could be remotely located, thus reducing the size of the system/plant The light intensity meter would consist of a interface. sensitive photodiode, with the electrical output going to the computer. The interpretation of the single reading (multiple readings could be averaged to obtain a mean) would be simple and could be done on the average personal computer. The interpretation program will contain data about the average absorption reading and acceptable range for each type of crop in their various stages of development. The program will compare the input from the photodiodes to the stored data and determine whether the health of the plant is satisfactory.

The intentionally vague term "photosynthetic activity" was used to describe the parameter to be sensed by the SPHS because there are two types of spectroscopic measurement that give information on the health of the plant; light absorption and light fluorescence by the chlorophyll. The two types differ in the phenomenon that they are analyzing, and thus will provide different types of information as to the plant health. These systems also vary slightly in the design of the spectroscopic system but are similar enough to be incorporated in the same design.

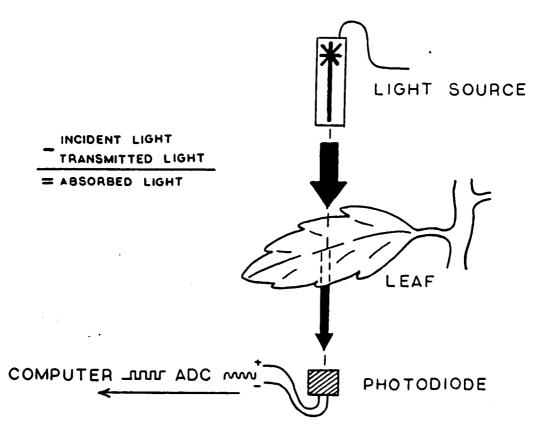


Figure 4. Absorption Spectroscopy

The first, absorption spectroscopy, is done by comparing the intensity of a beam of monochromatic light at the wavelength of an absorption peak that impinges on a leaf with the intensity of the transmitted light. Chlorophyll A (the more prevalent type) has two absorption peaks; one at 422 nm and one at 640 nm [15]. One can calculate the concentration of the chlorophyll that is absorbing that specific wavelength of light. It is not important, for the purposes of this system, to calculate the

actual concentration of the chlorophyll [16]. The plant health analysis program would contain data on the acceptable absorption reading for the plant leaf and take into account the crop type, age of the plant, and any other factors that may affect the absorption (Figure 4).

The use of light absorption as an indicator stems from the easily observable phenomenon of chlorosis. Chlorosis is the loss of the green color in the plant leaf due to the decomposition of the chlorophyll in the plant cells as a result of stress. This is the cause of the yellow or dark green color in sick house plants, and on initial observation of a stressed plant, chlorosis is usually the first visible warning of declining plant health [17]. Chlorosis is an early indicator of a wide variety of plant stresses, the most important being improper light level, microand macronutrient deficiencies, and pathogen invasion [18]. It is simple because the result of a measurement consists of a single value of the percent light absorbed and requires little computer processing, as opposed to image processing plant health systems that must process data in the megabyte range. In a image processing system, it is often difficult to reach a conclusion as to the meaning of the sensed data, whereas a spectroscopic system could provide a yes/no answer as to the question of plant health with a high degree of confidence. Also, because the system is simple, the dependability of the system should be good and the MTBF should be higher than a more complicated system. The main disadvantage of this system is the need to make the measurements at the same location repeatedly. Some leaves have higher chlorophyll levels and thus higher absorption levels, and the absorption on a specific leaf varies with age and location on the leaf [19]. This problem should be able to be overcome by insuring that readings are taken repeatedly in a predetermined spot on a specific leaf and by accounting for possible variations in the absorption reading in the plant health analysis software.

The second type of measurement that indicates plant health is the fluorescence of the photosystem. Fluorescence is a complicated phenomenon, and occurs when light of a certain wavelength strikes a chlorophyll molecule or light gathering dye and momentarily raises the energy of the molecule. The molecule stays at this higher energy state for a short period of time (in the picosecond range) and then emits this energy as light of a different wavelength (Figure 5) [20].

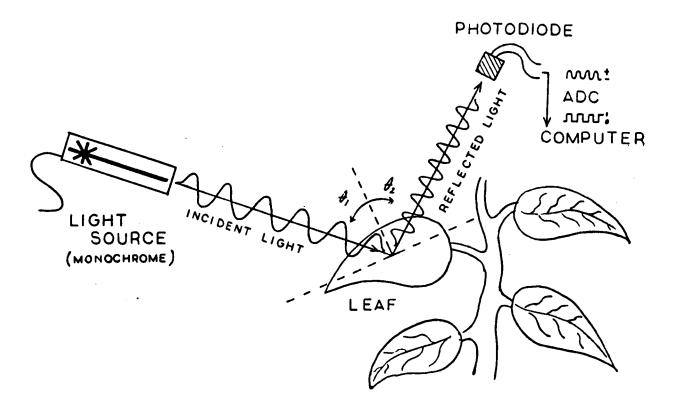


Figure 5. Fluorescence Spectroscopy

The interactions that occur in the molecule during fluorescence are complicated and are the subject of much current research [21]. When the leaf is exposed to a short burst of light, the time-varying fluorescence signal (usually with an intensity of about a thousandth of the incident signal) reaches a peak after a short time and approaches a minimum asymptotically (Figure 6).

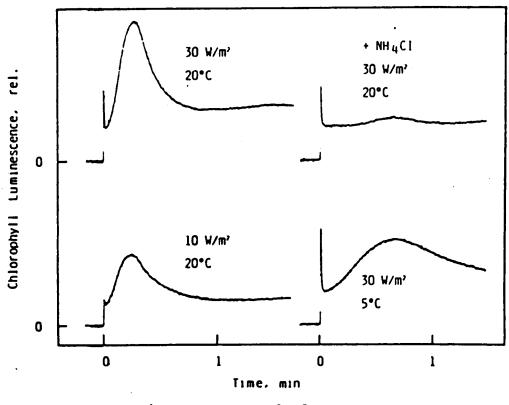


Figure 6. Leaf Fluorescence

This signal can be compared to others from similar plant types, or can be integrated to give a single value that can be processed in the same way as the absorption signal.

Spectroscopic measurement of fluorescence differs from absorption measurement in that it is looking at the "state" of the photosynthesis system rather than the concentration of chlorophyll in the leaf. Fluorescence characteristics of a plant leaf change when subjected to some types of stress such as temperature drop, CO_2 starvation, and water stress [20, 21]. It has the advantage of earlier indication, for the fluorescence of the chlorophyll is very sensitive to changes in the plant environment and will change as a result of stress long before the chlorophyll begins to decompose and the absorption characteristics change. Also, the exclusion of external light from the measurement area on the leaf is not as critical, for the signal from the photodiode can be compared to a base value that is caused by the ambient light that hits the leaf before the excitation pulse of light is shone on the leaf [24]. The main disadvantage of fluorescence measurement is that there are environmental factors that are unimportant to plant health but may cause variation in the readings while the health of the plant is unaffected. Such variations are plant age, measurement spot choice, concentration of specific molecules, and even pressure on the leaf surface from the measurement device, and these variations are much greater than those that would be measured with an absorbance method [19]. Thus there seems to be a tradeoff in these two systems between sensitivity (fluorescence) and correlation of data (absorption).

The SPHS seems to be the most promising system for the indication of plant sickness in a growth environment such as that planned for use by the CELSS project. Its primary advantage over CO_2 sensing in that the plant or leaf will not have to be enclosed to make a measurement. Other advantages are simplicity of design, thus leading to a lighter, smaller system with minimal maintenance. The greatest possibility of failure comes from the light source and the photodiode. Another advantage is the sensing of the widest range of plant sicknesses, since the photosynthesis system is usually the first thing plant system to be affected by stress (except for mechanical damage). Another advantage is the high degree of confidence in the technique, since very little information processing is required.

As a design project for EGM 4000, the SPHS seems to be one of the more promising. More research will have to be done in the area of spectroscopic design and in the effects of stresses on the photosynthetic system. Once a satisfactory system is built, a program will have to be written that will gather data from the spectroscopic sensor and store it in a file that can be accessed by the plant health analysis. The actual programming will be very simple, for it will only have to compare a single measurement to a database of stored reference measurement, and from this draw a true/false conclusion as to the health of the individual plant.

CONCLUSION

After careful consideration, the last three technologies discussed were chosen for design projects next semester. Hopefully, they will provide useful information about sensing plant health as well as detecting disease in plants at an early stage. By the time long-term space flight is possible a highly automated sensing system must be workable that can maintain the health of the crops without the need for human intervention.

The plans for next semester include planting four of the crops considered for growth in space in a growth chamber that has already been constructed. The crops are potatoes, dwarf wheat, soybeans and lettuce. We will use the black and white video image processor to measure the growth rate in the stages from seedlings to full grown plants. Also the chlorophyll level monitor will be constructed. Healthy and diseased plants will be tested using this device as well as performing stimulus response experiments. The results will be documented and analyzed with the help and advice from experts. The results from these experiments will be successful and progress will be made in the area of plant health and disease sensing.

REFERENCES

- Jones, Dr. Pierce. 1987. Personal communication. Dept. of Agricultural Engineering, University of Florida.
- 2. Allen, Dr. Hartwell. 1987. Personal communication. Dept. of Agronomy, University of Florida.
- 3. Cunningham, L., and H. Freiser. 1986. Coated-wire ionselective electrodes. In <u>Fundamentals_and_Applications</u> <u>of_Chemical_Sensors</u>. D. Schuetzle and R. Hammerle, eds. American Chemical Society, Washington, D.C.
- 4. Mix, Paul E. 1987. <u>Introduction to Nondestructive Testing</u>. John Wiley & Sons, New York, New York.
- 5. Chien, S., and Ho, C. (eds.). 1986. <u>NMR in Biology and</u> <u>Medicine</u>. Raven Press, New York, New York.
- 6. Polk, Charles. 1986. Introduction. In <u>CRC Handbook of</u> <u>Biological Effects of Electromagnetic Fields</u>. Polk, Charles, and Postow, Elliot, (eds.). CRC Press, Inc. Boca Raton, Florida.
- 7. Miller, Morton W. 1986. Extremely Low Frequency (ELF) Electrical Fields: Experimental Work on Biological Effects. In <u>CRC Handbook of Biological Effects of</u> <u>Electromagnetic Fields</u>. Polk, Charles, and Postow, Elliot, (eds.). CRC Press, Inc. Boca Raton, Florida.
- 8. Frankel, Richard B. 1986. Biological Effects of Static Magnetic Fields. In <u>CRC Handbook of Biological Effects</u>. <u>of Electromagnetic Fields</u>. Polk, Charles, and Postow, Elliot, (eds.). CRC Press, Inc. Boca Raton, Florida.

- 9. Barnes, Frank S. 1986. Interaction of DC Electric Fields with Living Matter. In <u>CRC Handbook of Biological</u> <u>Effects of Electromagnetic Fields</u>. Polk, Charles, and Postow, Elliot, (eds.). CRC Press, Inc. Boca Raton, Florida.
- 10. Banford, Dr. Amanda. 1987. Personal communication. Dept. of Botany, University of Florida.
- 11. Christian, Gary D., and Feldman, Fredric J. 1970. <u>Atomic</u> <u>Absorption Spectroscopy: Applications in Agriculture,</u> <u>Biology, and Medicine</u>. Wiley Interscience, New York, New York.
- 12. American Society for Testing Materials. 1959. <u>Symposium</u> <u>on Spectroscopy</u>. Third Pacific Area National Meeting; Oct. 12-15, 1959. ASTM Technical Publication.
- 13. James, J. F., and Sternberg, R. S. 1969. <u>The Design of</u> <u>Optical Spectrometers</u>. Chapman and Hall Ltd, London.
- Bally, E. C. C. 1927. <u>Spectroscopy</u>. Longmans, Green, and Co., New York, New York.
- 15. Street, H. E., and Cockburn, W. 1972. <u>Plant Metabolism</u>. Pergamon Press. Oxford, England.
- 16. Jursinic, Paul, and Dennenberg, Ronald. 1985. Reconciliation of the absorption change at 325 nm and other flash-yield determination of concentrations of active Photosystem II centers. <u>Archives of</u> <u>Biochemistry and Biophysics</u>. 241:540-549.
- 17. Comber, Norman M. 1968. <u>An Introduction to Agricultural</u> <u>Chemistry</u>. Edward Arnold and Co, London.

- 18. Kabata-Pendias, Alina, and Henryk Pendias. 1982. <u>Trace</u> <u>Elements in Soil and Plants</u>. CRC Press, Inc., Boca Raton, Florida.
- 19. Koch, Dr. Karen. 1987. Personal communication. Dept. of Fruit Crops, University of Florida.
- 20. Krause, G. Heinrich, and Weis, Englebert. 1984. Chlorophyll Fluorescence as a tool in Plant Physiology. <u>Photosynthesis Research</u>. 5:139-157.
- 21. Fraser, D., Colbow, K., Popovic, R., and Vidaver, W. 1987. Oxygen quenching of chlorophyll fluorescence in barley leaves at various irradiances. <u>Photosynthetica</u>. 21:76-81.
- 22. Prange, Robert K. 1986. Chlorophyll fluorescence in vivo as an indicator of water stress in potato leaves. <u>American Potato Journal</u>. 63:325-333.
- 23. Wong, Suan-Chin, and Woo, K. C. 1986. Simultaneous measurements of steady state Chlorophyll a fluorescence and CO2 assimilation in leaves. <u>Plant Physiology</u>. 80:877-884.
- 24. Schreiber, Urlich, and Schliwa, Urlich. 1987. A solid-state, portable instrument for measurement of chlorophyll luminescence induction in plants. <u>Photosynthesis Research</u>. 11:173-182.