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-- SECTION II: --

CONTROLLED ECOLOGICAL LIFE SUPPORT SYSTEMS:
DEVELOPMENT OF A PLANT GROWTH MODULE

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INTRODUCTION

This section summarizes the results of a workshop held at NASA-Ames Research Center in September 1984. The purpose of the workshop was to begin definition of the scientific and technical requirements for the design and construction of a ground-based plant growth facility. The energy, mass, volume and cost considerations of the Plant Growth Module (PGM) are not included in this report, but are left for consideration by design engineers. Building on the previous work of the CELSS program, the attendees consolidated their thoughts on science design criteria for the PGM, and this section reports those considerations.

The PGM workshop served as the preliminary step in the design and construction of a functional plant growth module. The topics of discussion in the workshop covered the major design elements of the PGM. Individuals with expertise in each particular sub-area were invited to discuss and propose what they thought the requirements of those design elements should be. Decisions of each group were recorded and reported by the chairman. These reports were extensively reviewed by the members of the group and by CELSS program scientists. The results of the many meetings, discussions and reviews were then incorporated into this section, in the format that each of the chairmen considered most appropriate.

IRRADIATION

John Sager, Chair

I. Definition of parameters affecting plant growth.

A. Irradiance

Adjustable levels from 0 (visually dark) to 1000 micromole $s^{-1} m^{-2}$ with an operational range of 400-700 micromole $s^{-1} m^{-2}$ (90-160 $W m^{-2}$) measured at the top of the plant canopy. Levels greater than 1000 micromole $s^{-1} m^{-2}$ may be required for high CO_2 experiments. Adjustment of irradiance must be adapted to the specific lamp types used in the canopy. Options include the dimming systems available for HID and fluorescent lamps (for HID, Widelite Inc., San Marcos, TX; for fluorescent, CESI, Rockville, MD). Other less expensive options for irradiance control include the use of an absorbing screen to reduce radiation transmitted to the plants or symmetric reduction of lamp number -- applicable chiefly to fluorescent systems on the scale contemplated.

B. Spectral Distribution

Maximize photosynthetically-active radiation (PAR) based on the relative quantum efficiency of photosynthesis (McCree, K. J. 1972, Agric. Meteorol. 9:191-216) and the light energy utilization efficiency of photosynthesis from the various sources (Sager, J. C., J. L. Edwards, and W. H. Klein 1982, Transactions of the ASAE 25(6):1737-1746). In addition, the light provided must include far-red and UV portions of the spectrum and the spectrum must be balanced to achieve the desired physiological and morphological development of the particular species. Examples of such control include the germination of some seeds, such as Grand Rapids lettuce, which require a red irradiation. In this case far-red, and in some instances blue radiation, provides maximum inhibition of germination. Nonetheless, far-red promotes flowering in conjunction with photoperiod, and therefore must be provided. These effects can be attributed to phytochrome photo-equilibrium (the P_{fr}/P_{tot} ratio) during the photoperiod (Vince-Prue, D. 1975, Photoperiodism in Plants, McGraw-Hill). With these effects in mind, the spectral characteristics of the light source must be known from 250 nm through the thermal range (about 50 micrometers), with particular care given to limiting the radiant loading on the plants.

C. Spatial Distribution

Horizontal variation should be $\pm 10\%$ of irradiance over the plant canopy area. Vertical variation (lighting at the

IRRADIATION (cont.)

top of the growing canopy) should be $\pm 10\%$ for the life of a crop or during the course of an experiment. Vertical variation might be minimized by using reflective sidewalls and a lamp arrangement designed to promote uniform distribution (area sources such as fluorescent lamps). Consideration should be given to inclusion of side and base sources to optimize irradiance within the unit.

D. Barrier

Light barriers should have high transmission with consideration given to using filters for undesirable wavelengths, such as ultraviolet or infrared. The transmission of barriers should be characterized from 250 nm to 50 micrometers.

E. Photoperiod and Photocycle Regulation

Photoperiod should be variable to allow any day/night length. To study rapid light/dark variations as a means of reducing the overall power requirements of an eventual CELSS, the ability to strobe light sources would also be desirable.

II. Equipment must meet the plant growth parameters as well as the following.

A. Sources

1. The lamp canopy configuration should be optimized for use of either fluorescent, HID or other light sources. Consideration should be given to supplying light via a "lightpipe", lens combination, such as the Japanese "Himawari".
2. The lamp canopy should allow for temperature control to maximize efficiency of different light sources.
3. The volume required for installation and the energy required for control should be minimized.
4. The light canopy and the plant chamber should have separate environmental controls to isolate their energy requirements and to optimize both environments.
5. There should be modular light canopies so that various light sources can be used interchangeably.
6. Maximize photosynthetic efficacy of source.
7. The total system, sources as well as barriers and light piping/lens materials, should be selected to filter the radiation and thereby optimize plant growth

IRRADIATION (cont.)

and productivity.

B. Barrier

1. Barriers must be compatible with the air distribution system.
2. Barrier design should allow quantification of the aging effects of the transmitted radiation, and eventual replacement of the barrier if needed.

C. Measurement Systems

Spectroradiometric measurements should be possible from 250 to 2000 nm with a bandwidth \leq 10 nm. At greater than 2000 nm the radiant energy should be measured at the minimum bandwidth permitted by the available instrumentation.

AIR FLOW

Larry Anderson, Chair

1. The air flow path over the plants should be kept as short as possible.
2. The air flow path should be vertical, from bottom to top.
3. The air velocity should be variable from 0.2 to 0.9 m/s. The plenum should be designed to be adjustable with a slotted base or with holes so that air flow variation is minimized across the chamber.
4. The air flow must be great enough to remove the heat load within the chamber.
5. Provision must be made for external or internal scrubbing, and for adding, diluting, mixing and completely purging the gases.
6. The design should permit a minimum of 2 to 3 air exchanges per minute within the plant canopy.
7. Air flow sensing can be accomplished by the use of portable instruments, except for safety controls to detect fan failure.

In an attempt to determine the air flow requirements within a plant growth module, the committee asked itself some basic questions:

1. What does the moving air accomplish? Is the moving air anything more than a transport mechanism?
2. What must the moving air do to optimize plant production?
3. What is happening incidentally as the primary function is being achieved?
4. Are there detrimental effects of moving air?
5. Could some other physical phenomena be used to achieve the same desired results?

Discussion of the first question used all the available time. The committee acknowledged that while the moving air stream is acting as a transport system, it is a special one where some of its components are being consumed or augmented. It was suggested that the requirement to move heat was very critical, and perhaps the most demanding. [Considering this, perhaps question 5 should be given further consideration. ~~Editors~~] One critical factor in a closed-loop control design such as the thermostatic control of a heated vessel is keeping the "transport lag" short. This means that the fluid (air) stream should be short, so that the controlled space is closely coupled to the controlling device (the

AIR FLOW (cont.)

heating and cooling elements). Similarly, to minimize temperature gradients the path from the inlet to the outlet (supply to exhaust) should be as short as possible. These criteria suggest a chamber design with air flow across one of the shorter dimensions with the fan/coil unit and ducts positioned to give the minimum length air path.

Since the chamber will be used to test hypotheses about the optimal conditions for plant growth, an effort must be made to subject all plants to identical growing conditions. This would suggest that if there is any temperature or humidity gradient inherent in the operation of the air conditioning system, that gradient should be along the axis of the plant stems, rather than from plant to plant. This consideration strongly favors a vertical air flow.

While the experience of a majority of the committee suggests an upward air flow through the plant canopy is better, there were counter arguments for downward flow. While it was suggested that upward flow would do a better job of permeating the canopy (because of the architecture of the leaf and the formation of a natural plenum by the canopy) there are benefits to using the cooler incoming air to scrub heat from the barrier to control long wave radiation. This need should be taken into account in the overall chamber design. [The evaluation of supply grills or ports designed to aspirate room air and provide some pre-mixing and reduce gradients will be useful. Editors]

To satisfy the requirements for air mixing, heat removal and control loop design, the air velocity should probably be as high as possible. However, excessive wind speed damages or disturbs the plants so that the optimum would be a velocity such that "the leaves just flutter slightly". While this may seem imprecise, it would indicate that the boundary layer of heat, moisture and "used" air is being scrubbed at the leaf surface, thereby providing the plant with the desired conditions.

AIR FLOW (cont.)

The total volume of air moving through the chamber per unit time, as distinct from the air velocity, must be such as to transport the heat and moisture being supplied or removed, and to refresh the supply of carbon dioxide and oxygen and to remove other gaseous materials released by the plant, or by components of the growth module itself. Practical experience suggests that 2 to 3 air exchanges per minute are required. Under some experimental conditions an exchange of chamber air for outside air might be required as well. If the system is to be run in a closed-cycle mode, provision must be made for internal or external scrubbing, adding, diluting, mixing and purging of the air and its components.

Except for safety controls to detect fan failures, the use of portable instruments to measure velocity will probably suffice. This would allow for measurements to be made at or near the active leaf regions as well as near grills.

PLANTING AND HARVESTING

Bruce Bugbee, Chair

I. Planting

A. Direct Seeding

1. Advantages

- a. Considerably less labor involved than transplanting
- b. More amenable to automation

2. Disadvantages

- a. Somewhat reduced uniformity
- b. Less efficient interception of radiation, unless variable spacing is used
- c. Increases the importance of careful seed selection prior to planting, though even seed selection could be mechanized

B. Transplanting

1. Advantages

- a. Plants can be selected for uniformity
- b. Doesn't require chamber space for germination in those experiments where that stage is not important to the investigation
- c. More efficient interception of radiation without variable spacing, because young plants can be started close together

2. Disadvantages

- a. Very labor intensive
- b. More potential for damage during the transplant operation, with a resultant increase in mortality or spurious results
- c. Would necessitate careful transplant selection, which would be difficult to automate

C.

PLANTING AND HARVESTING (cont.)

Automatic Seeding Devices

1. Most important with closely spaced plants such as wheat
2. Numerous commercial types are available
 - a. vacuum
 - b. pre-seeded cassette
 - c. seed tape
 - d. pneumatic

D. Automatic Transplanting Devices

Transplanting is labor intensive and is difficult to automate. Any available devices need to be investigated.

E. Planting Media

1. Must provide support and facilitate handling of young plants.
2. Should be recyclable if the ultimate concern is using the facility as part of a CELSS prototype.

II. Continuous vs. Batch Culture

- A. Both should be available.
- B. Environmental conditions for continuous culture may need to change in an operating CELSS, since young plants benefit from conditions that are different from those of mature plants.
- C. Continuous culture may be better for production objectives because plants can be sequentially harvested.
- D. Batch culture operation would be better for plant research because environmental conditions can be altered and plant response can be monitored at distinct stages of development.

III. Plant Spacing

- A. Fixed spacing has the advantage of much less complex design and construction.
- B. Variable Spacing

PLANTING AND HARVESTING (cont.)

1. Advantages

- a. Much more energy, mass and volume efficient
- b. Very important with directly seeded crops and for crops with vertical leaves

2. Disadvantages

- a. May prevent the use of certain types of nutrient delivery systems
- b. Roots may intermesh and prevent variable spacing unless some type of spacer is used to keep roots separate.

IV. Harvest

- A. Easily mechanized, though new designs would be needed for crops which would be continuously harvested
- B. Many automated devices are available
- C. Harvest system would also need to remove nonedible stems, roots and leaves

V. Additional Comments and Summary

- A. Variable spacing of plants beneath lamps is highly desirable. For wheat the anticipated range of variability would be from 2 mm by 2 mm at seeding, up to 50 mm by 50 mm at maturity. Calculations indicate that variable spacing could increase yield per unit area by up to 60% without increasing energy input.
- B. A gantry, bridge crane, or remote manipulator arm(s) could be very useful for plant manipulations.
- C. Flexibility of use for different crops and different cultural systems is critical.

CARBON DIOXIDE

Steve Schwartzkopf, Chair

Carbon dioxide is an extremely important factor in plant growth studies. Because of the different metabolic roles played by atmospheric CO₂ around plant leaves and roots, a primary recommendation made by the group was that the top and root zones be isolated from one another by a CO₂-impermeable barrier. Such a barrier will require that separate mechanical systems be developed for the two zones, but it will enable the collection of much needed information on carbon partitioning and mass balance. For discussion and design purposes, the group divided the topic of CO₂ into two functional tasks: monitoring and control.

For atmospheric CO₂, it was suggested that the monitoring system be designed to include an intake manifold, pump, flow regulator, gas drier, and particulate filter, and to use an infra-red gas analyzer (IRGA) as the monitoring device. It was also suggested that this design would make it possible to monitor CO₂ concentration throughout the entire chamber with only one IRGA. The IRGA itself, should be capable of achieving measurement precision to ± 10 ppm. The number of manifold inlets was not specified, however they should be large enough to insure that CO₂ concentrations within the chamber can be controlled to within ± 25 ppm or $\pm 5\%$ of the setpoint, whichever is greater. It was also suggested that provision be made in the manifold for use of two IRGA's, for those cases where an experimenter might want to control CO₂ at two widely disparate concentrations. (For example, a 350 ppm daytime concentration, and 10,000 ppm nighttime concentration. If two IRGA's are used, one could be calibrated for the range 0 - 1,000 ppm for daytime control, and the second could be calibrated for 9,500 - 10,500 ppm for nighttime control. Thus, both would have the same precision about their respective ranges.) It was also suggested that the IRGA monitoring system include automatic calibration, and that at least one spare IRGA be maintained for each unit in the system, as a replacement in case of malfunction.

It was suggested that the control system include provisions for both adding and subtracting CO₂. Addition would be most easily accomplished through the use of an injection manifold which introduced either a known volume or a known flow rate of pure CO₂ into the chamber. The number of points at which CO₂ should be added to the chamber was not specified, but the number should be sufficient to maintain the precision specified above. It was suggested that the CO₂ removal system utilize a regenerable adsorbent buffer, such as molecular sieves, if possible. In those cases where mass balance is not required by the experimenter, LiOH or a similar absorbent could be used.

Monitoring and control of CO₂ in the root zone is a more complicated task. Regardless of the specific culture technique used, either hydroponic or aeroponic, the concentration of CO₂ in both the gas and the liquid phase will be of interest. It will

CARBON DIOXIDE (cont.)

probably be possible to monitor and control CO₂ in the root atmosphere with the same system as used for the top atmosphere. CO₂ dissolved in the nutrient solution will be somewhat more difficult to deal with. Monitoring might be accomplished by ion-specific electrode, automated wet chemistry, or by some form of liquid chromatography. Control, however, will be difficult because of the necessity for simultaneously controlling both the pH and CO₂ concentration of the nutrient solution. No specific recommendations were made on how this would be most easily accomplished. It was presumed that the root atmosphere in the aeroponic system would likely be sufficiently mixed by the spray action of the aeroponic nozzles so that little or no additional atmosphere movement would be required to produce a homogeneous atmosphere. In a hydroponic system, however, it was felt that some form of air movement would have to be supplied to insure mixing of the root atmosphere. The precision of control and uniformity through the chamber was specified as the same values for the top atmosphere (± 25 ppm or $\pm 5\%$ of setpoint, whichever is greater).

The group felt that the range of control for CO₂ should include both values of interest from an experimental viewpoint as well as values that might arise in spacecraft cabins. The group defined this control range to be from 25 ppm CO₂ to 1% CO₂ (10,000 ppm). 1.5% CO₂ has been found in spacecraft atmospheres and it may be useful to allow for control up to 2%.

TEMPERATURE AND RELATIVE HUMIDITY

Craig McFarlane, Chair

I. Temperature

A. Air

Air temperature should be controllable between 5 - 40°C in dark and light. There should be the ability to vary control and set values over any period, i.e., provide for changing temperatures throughout a day or over the growth cycle of a plant. This should be available through advance programming. It should be noted that the range of control needed for research is much wider than the range acceptable in a spacecraft system.

B. Root Environment

Should be controllable between 5 - 40°C. If an aeroponics system is used, 2 solution tanks maintained at different temperatures should be used to provide light and dark nutrient solution temperature cycling coincident with conditions in the aerial environment.

C. Control

Should eliminate large variations which result from heater/chiller cycling. We recommend a fully proportional control system. Variation should be at most $\pm 0.3^\circ\text{C}$ at the control point. Spatial variation should be at most $\pm 1^\circ\text{C}$ within 90% of the plant growing volume, regardless of plant density or age.

D. Measurement

Bulk air temperature should be measured and continuously recorded. Provision should be made for temperature safety override alarm and shut down if needed. Provision should be made for both continuous and periodic air temperature monitoring, with ports, plug-in sensors or IR reflectance canopy and leaf monitoring.

II. Humidity

A. Control Limits

The control limits should be between 35% and 90% RH. Generally, humidity will be within the range of 50-80% RH for optimum plant growth. Requirements for lower humidity may exist when plants are maturing, for example when wheat is drying. This demand will generally be associated with conditions of low water insertion rates and thus represent a special condition. It is recommended that the condenser not be below 0°C because of the difficulty encountered

TEMPERATURE AND RELATIVE HUMIDITY (cont.)

with icing. Nevertheless, it is recognized that low humidities (at or below 35%) at low temperatures, especially in the dark, are impossible without a dew point below 0°C. This is a special condition of little importance. Thus, the limit of 35% RH applies only to lighted conditions and temperatures greater than 25°C. Low humidity may also be desirable for increasing potable water yield. That would not require below freezing dew points.

There is a need for lower humidity to examine the effect of high CO₂ on wheat and other crops. High CO₂ causes stomata closure, which reduces transpiration and thus movement of nutrients to the leaves. Decreased humidity could increase transpiration and possibly accommodate this need. In this condition water insertion rate would be low, temperature high (30 - 35°C) and thus dehumidification easier. Under these conditions, provide 20% RH in air.

B. Variability

Control should be within ±5%, or state of the art.

C. Humidification

Humidification will not generally be necessary because of the enclosed situation. However when plants are small, or when low temperatures are demanded, some low level of water insertion may be necessary. The source water must be pollutant free. It is suggested that ultrasonic atomization and steam injection be considered. The humidification system should not result in any droplet formation on the plants or the chamber.

D. Measurement

Measurements of the bulk air should be made and continuously recorded. We recommend that both IRGA and wet/dry bulb systems be evaluated.

OXYGEN

Robert MacElroy, Chair

1. C3 plant efficiency of vegetative growth can be increased if O_2 is decreased.
2. C4 plants are unaffected by O_2 concentration.
3. O_2 is needed for root growth; roots use O_2 to produce energy needed for transport.
4. O_2/CO_2 ratios are important and possibly also the O_2/CO_2 /Ethylene ratio.
5. Range for O_2 between 5% and 20% is OK.
6. There is no information on the effect of O_2 above 20%.
7. Physiological responses to O_2 are seen in the short term. Long term effects are not clear.
8. Decreased O_2 has inhibitory effects for roots and reproductive growth does not occur for some species (e.g., soybeans and wheat) at low O_2 concentrations.
9. Half normal concentrations of O_2 in the root zone are considered to be anaerobic.
10. There are effects of O_2 on microbial growth.
11. The demand for O_2 by roots is stimulated by heavy metal stress.
12. There are plant species differences in root O_2 responses.

CONSTRUCTION MATERIALS AND ACCESS

Robert Langhans, Chair

Construction Materials

It is vitally important that the materials used to construct the plant growth module not be phytotoxic. The two major sections of concern are the root and aerial zones.

Root zone: There is concern regarding the leaching of materials into the nutrient solution. Therefore, any construction material that is in contact with the nutrient solution or condensate from the cooling coils should be carefully screened for leaching of heavy metals like Zn, Cd, Ni, Cr, Cu, Co, Ag, Pb, and Al. Tubing used to transport the nutrient solution is also a source of phytotoxic material. Rubber and tygon tubing have been found to have toxic effects. Tubing made for moving food products, such as teflon and some polyethylenes (especially very high molecular weight, high density, polyethylene such as that manufactured by the Philips Petroleum Co. under the trade name Drisco) have been used successfully.

Aerial zone: Aerial parts of the chamber can be built of PVC, stainless steel, aluminum and glass. We suggest that care be taken in working with the following materials:

- . paint
- . sealants
- . gaskets
- . preservatives
- . glues
- . mastics
- . rubber

Any of the above materials should be screened to check for phytotoxicity. Materials that should not be used include:

- . galvanized steel
- . copper
- . brass

Testing

It is suggested that a biological test be used to test each of the materials used in the plant growth module.

Access

It became apparent in the discussions that access to the chamber will be a big problem. One of the objectives of the ground based plant growth module is to demonstrate that it can be kept gas-tight for a period as long as one year. Yet during this time a number of operations involving the plants and equipment will have to be performed. The following is a list of functions which might require working access to the plants.

CONSTRUCTION MATERIALS AND ACCESS (cont.)

1. Plant growth --- seeding, germination, transplanting, spacing, supporting, pollinating, etc.
2. Harvest --- removal of mature plants, fruits or seeds
3. Tissue and solution sampling
4. Observation of aerial parts of the plants
5. Measurement during growth of plant height, leaf width, fruit size, etc.
6. Manipulation of special equipment --- porometer light measure, leaf index, temperature probes, etc.

Three means are suggested to allow access for manipulating the plants.

1. Glove and access ports
2. A walk-in entrance, with a walkway and airlock if necessary
3. Robotics/Automation: Automated devices or robotics with video or optical monitoring. Although this area has been recognized to be important to PGM development it requires further definition.

There are pros and cons for each option. It may be that all will be necessary in designing the first breadboard unit.

The following parts would be required for maintenance or replacement of the mechanical equipment.

1. Cooling coils
2. Heating units
3. Humidity nozzles
4. Air handling equipment, such as fans, louvers, etc.

This equipment should be situated as close as possible to the chamber. It should be redundant and easily accessible for replacement (modular) without stopping plant growth or the operation of the module and (to the maximum possible extent) without breaking the gas-tight seal.

A priority list for system shutdown may be required to minimize the impact to experiments of power or other system failures.

CONSTRUCTION MATERIALS AND ACCESS (cont.)

Vibration

Vibration should be kept to a minimum. Excessive vibration can cause plant growth problems. The magnitude and frequency of vibrations should be no greater than that found in commercial plant growth chambers.

VOLATILE COMPOUNDS

Ted Tibbitts, Chair

Several different volatile compounds are known to be released in controlled growing systems. Some of these can be toxic to plants if concentrations are permitted to exceed certain limits. Other volatiles are not recognized to be toxic to plants but may be toxic if concentrations reach abnormally high levels. Of particular concern in this system are volatiles that are not presently recognized to be phytotoxic, but may be phytotoxic when the system is kept closed for long periods of time.

Principal emphasis in the report has been placed upon volatiles that will be released in the plant-growing subsystem. In an operable CELSS, information would also be needed about volatile compounds released from the other subsystems, such as waste processing, human habitation and algae growing areas.

Volatile compounds will originate both from living organisms and from hardware in the regenerative system. Compounds known to be released from plants and microflora in the plant growing sub-system include the following.

- Ethylene* (5 ppb)
- Carbon monoxide
- Terpenes
- Aldehydes
- Methane
- Other hydrocarbons
- Ammonia* (65 ppm)
- Amine oxides
- Cyanide
- Nitrogen oxides including NO, N₂O, NO₂,
- Sulphur compounds including H₂S, CH₃SH

* The starred compounds are of particular concern because they can cause injury to growing plants at the indicated concentrations.

Compounds which may be released from the hardware in the system or during system set-up and which would be phytotoxic are:

- Plasticizers that release methyl chloride, or other chlorine or fluorine compounds
- Freon
- Ozone
- Mercury
- Selenium
- Heavy metal particulates
- Cleaning solvents

VOLATILE COMPOUNDS (cont.)

No welding or soldering should be performed in the plant growth module during plant growth experimentation.

Research is needed to determine rates of release of volatile contaminants from plants, materials and machines under the range of environmental growing conditions within the regenerative system. There is also a need to determine the chemical, photochemical and biological transformations that may occur within the system.

It would be desirable to have the capability to monitor potentially phytotoxic gases on a continuous basis, or at least hourly. Compounds with no significant phytotoxicity would require monitoring only on a weekly basis. Monitoring will likely require several different analytical procedures including gas chromatography, mass spectroscopy, ion chromatography and specific ion analyzers.

One method of reducing high levels of contaminants is by use of a catalytic converter or similar air-cleaning device, as is done on submarines and on the space shuttle.

BACTERIA, STERILIZATION, AND FILTRATION

Mel Averner, Chair

I. Reasons for Wanting to Remove Microbial Populations

- A. To control plant pathogens
- B. To control human pathogens, such as enterics
- C. To control system pathogens, such as denitrifiers
- D. To examine the effects of microbial populations on plant growth parameters.

II. Reasons for not wanting to remove microbes

- A. Maintain selected microbial populations on plants and in the rhizosphere to minimize the invasion of pathogens.
- B. Sterile media increases the potential for invasion of pathogens.
- C. Plants will release organics which serve as a microbial substrate.
- D. To minimize the need for extensive sterilization procedures.
- E. Experiments need to be performed to define these symbiotic microbial communities.

III. General Rule

Once an infection begins, it is difficult to stop without interfering with plant growth. Therefore prevention by appropriate startup protocols and management is critical.

- A. Use construction materials which do not leach organics into the nutrient solutions.
- B. Plants will release organics into nutrient solutions.
- C. Perform appropriate clean-up between experiments.

IV. Techniques for Sterilization

A. Air

- 1. Filters -- will remove dust; will not kill microorganisms

BACTERIA, STERILIZATION AND FILTRATION (cont.)

- a. Electrostatic filters -- require maintenance
 - b. High-efficiency particulate air filters (HEPA) -- require maintenance
 2. Ultra-violet light -- will kill microorganisms
 3. Fumigation -- can kill microorganisms
 - a. Formaldehyde -- possible carcinogen, can be vented
 - b. Gluteraldehyde -- possible carcinogen, can be vented
 - c. Chlorine released from sodium hypochlorite -- can be vented
 - d. Ethylene oxide -- carcinogen, can be vented
 - e. Wet heat (steam)
- B. Liquid
1. Filters -- clog, require maintenance and replacement
 2. Bacteriostatic columns (Iodine, Ag) -- may leach and may have flow rate problems
 3. UV light -- can destroy chelator and acquire salt deposits
 4. Chlorozone -- may cause accumulation of ozone
 5. Antibiotics -- may affect plants and be taken up into food produced by plants
 6. Organic ion exchangers to remove substrates -- can leach organics
 7. Wet heat -- steam
 8. Alpha-radiation
- C. Surfaces
1. Hypochlorite -- standard, removable
 2. Organic iodine (wecodyne) -- may not be removable
 3. Iodine vapor -- may not be removable

BACTERIA, STERILIZATION AND FILTRATION (cont.)

4. Detergent/sulfuric acid mix -- removable
5. Wet heat
6. UV light -- shadowing
7. Quaternary amines (quats) -- may affect plants

D. Monitoring

1. Direct sampling methods
 - a. Air -- membrane filters
 - b. Liquids -- membrane filters, conductance?
 - c. Surfaces -- swabs
 - d. Counts from plant materials
2. Both species and numbers should be monitored, as should community physiological indicators.
3. Symptoms of plant stress should be monitored.
 - a. Ethylene, ethane, ABA
 - b. Plant temperature
 - c. Laser or spectrographic reflectance
 - d. Evidence of microbial activity
 - a. pH
 - b. Fourier transform IR for microbial "signature" molecules
 - c. Plant genetic markers

V. Miscellaneous Considerations

- A. Automation vs. human tending. The chamber should be designed with ease of microbial investigation in mind. If human entry is allowed, sterile suits may be required (will not be necessary if normal microbial populations are allowed).
- B. Disinfestation of propagules, seeds or tissues

NUTRIENT APPLICATION SYSTEMS

Cary Mitchell, Chair

Types of Culture

Since the PGM will be a research facility, the design should be sufficiently flexible to accommodate investigations employing any of the major types of soilless culture methods including the following.

- Batch (tank) hydroponics
- Aeroponics
- Solid matrix flush culture
- Nutrient film technique (NFT)
- Capillary mat bottom irrigation

No single nutrient application system was favored exclusively, since the choice may depend upon plant-species requirements. However, sentiment was expressed in favor of exploring nutrient systems not absolutely dependent upon gravity, since these might be more easily extrapolated from a ground based PGM to a space-deployed CELSS with a minimum of additional research and development.

Limitations

Several discussion groups favored separate compartments for shoot and root atmospheres in the ground-based PGM. One of the concerns that arose early and often in the Nutrient Applications group was the need for adequate aeration of nutrient solutions. Although the optimum oxygen concentration that must be maintained in solution is an R & D question for each combination of species and growing conditions, it should be as high as possible so as not to limit plant growth. For example, air-saturated H₂O contains about 9 ppm O₂ at 25°C. With root/shoot compartmentalized NFT, high flow rates of nutrient solution are anticipated in each culture trough to avoid O₂ and nutrient depletion along the trough, as well as a gradient of plant growth from inlet to outlet end of the trough. Use of air jets, manifolds, cascades, and turbulent circulation within nutrient reservoirs were suggested as ways to avoid such deficiencies.

Transverse rather than longitudinal flow of liquid through troughs within the proposed PGM was proposed to minimize the number of plants along a given NF trough, thereby minimizing the chances of O₂ and nutrient gradients. It was further suggested that as little as 3 ppm O₂ might be tolerated in a nutrient solution if solution flow rate across the roots is great enough. However, the O₂ concentration differential between the solution and root surface was stated as being more important to root growth than was flow rate per se. It was further suggested that turnover rate of nutrient solution be defined in terms of the amount of biomass being supported by a given volume of nutrient solution.

NUTRIENT APPLICATION SYSTEMS (cont.)

This is a research question.

Needs Within the Delivery System

A need for remote sensors for O_2 , specific ions (such as NO_3^- , K^+ , NH_4^+ , Ca^{2+} , Cl^- , etc.), pH, and conductivity will have to be accommodated at various appropriate places within any nutrient delivery system. Alternatively, automated sampling and analysis of inorganic substances by high performance liquid chromatography or atomic absorption spectrometry could also be developed. Once again, the goal would be to achieve reasonable uniformity within the particular system and the pertinent issue seems to be adequate mixing of flowing solutions along their pathway. Anecdotal observations suggest that mechanical disturbance of roots, such as by vigorous mixing or flow of nutrient solution, may be less disruptive to plant growth than mechanical disturbance of shoot parts.

Opportunities

Sentiment was expressed in favor of adopting the use of benevolent plant/microbial interactions to NFT or aeroponics in order to enhance delivery of nutrients to the roots of plants growing in solutions containing treated recycled sewage. Rhizobia to encourage legume roots to fix N_2 and Mycorrhizae to encourage uptake of phosphates and other nutrients from dilute, recycled waste solutions would be compatible with overall CELSS objectives.

Other Needs

Monitoring and control of individual nutrients will have to be tested in the PGM, with appropriate numbers and placement of remote probes in solution and sufficient analytical facilities and laboratory personnel to support maintenance of the nutrient delivery system. Once again, if partially treated, recycled wastes are incorporated into the nutrient solution, steps will have to be taken to avoid problems resulting from biodegradation of wastes, such as microorganism buildup and micronutrient accumulation to toxic levels.

Suggestions for Nutrient Delivery Systems

Unless some sort of growth block or solid substrate is used in conjunction with nutrient delivery systems, there might be a rhizosphere headspace above the nutrient solution. Concern was expressed regarding the air pruning, browning or desiccation of roots that often occurs above the liquid phase. Since it may be desirable to recover roots from the system without adhering substrate, a need to develop systems that overcome this problem

NUTRIENT APPLICATION SYSTEMS (cont.)

was expressed.

Concern also was expressed regarding the potential use of construction materials that potentially release toxic substances into nutrient solutions. Examples given included black polyethylene, which releases copper and zinc. Rigid PVC may adsorb organic contaminants with the danger that they might be released at a later time into nutrient solutions or onto root surfaces. Materials containing plasticizers such as phthalates that can support microbial growth should be avoided or treated. Teflon-coated surfaces or ultra-high molecular weight and high density linear polyethylene were identified as construction materials that might be used because they are particularly inert and non-reactive. Use of Porylene, an inert coating material, also was recommended.

The need to recover and recycle growth substrate, such as capillary matting material, following a production cycle also was stated. A substance which is inert, porous and resistant to the combustion or chemical treatments used to remove roots is needed.

Finally, development of a nutrient delivery system compatible with microgravity and with 1 g conditions was identified as a key issue. One hypothetical system proposed involved pumping nutrient solution from one collapsible bag to another, alternately filling and draining roots of plants contained in one of the bags. Aeration would occur during the drain cycle. Details of this system were not worked out, but it was stated that it would be analogous to the pumping of an artificial heart. This example is by no means the best or only system that could be developed for the PGM.

NUTRIENT MONITORING

Wade Berry, Chair

In order to evaluate reasonable sample sizes for nutrient monitoring some assumptions must be made about nutrient volume per chamber. For the first approximation we have assumed that each NASA plant growth chamber nutrient delivery system will be subdivided into four compartments; one or two to be used for control or reference groups and the others for treatment groups. We have assumed that each compartment in the hydroponic mode will contain between 400 and 4000 liters of nutrient solution. The upper limit represents a scale-up of the Salisbury-Bugbee growth chamber for wheat at Utah State University, while the lower limit reflects a concern that the volume of the nutrient solution be sufficient to resist a sudden change in composition, and to permit adequate sampling.

The minimum capability for the frequency of nutrient solution sampling should be 4 samples per day for an entire growth period of about 120 days. The sample size should be 10 ml per sample to provide solution for both routine analysis and archiving. The total volume of the monitoring sample over the entire crop period would therefore be $(0.010 \times 4 \times 120)/400 = 1.2\%$ of the suggested 400 L minimum capacity of each nutrient delivery system.

The preferred method of analysis for mineral nutrients would be inductively coupled plasma-emission spectroscopy (ICPES) analysis for the cationic and trace elements. For the anionic elements the preferred method of analysis would be HPLC ion chromatography. Each of these analysis methods would require less than 1 ml of sample. Approximately 8 ml of sample would remain to archive for future analysis. For example, archived samples would provide a means to evaluate contamination that had resulted in delayed toxicity.

Tissue samples for mineral analysis should be taken at least once a week to verify mineral nutrient availability to the plants. The tissue sample should be at least 100 mg of recently matured leaf tissues and young root tips. (Such tissue may not be available during the first few weeks of growth.) The tissue samples will need to be prepared and put into solution before analysis. The tissue samples should be analyzed for the same elements as the nutrient solution and by the same methods. Any extra tissue should be archived for later re-evaluation if that becomes necessary.

The nutrient analyses should be as nearly real-time as possible. It would be highly desirable if the analysis of the solution were automated and on-line, thereby providing for real-time control of the nutrient solution. This, however, would not alleviate the need for routine sampling and archiving of solution samples for future reference.

NUTRIENT MONITORING (cont.)

At least the following elements would need to be monitored.

1. Essential macronutrients
N, P, S, K, Na, Ca, Mg, Cl, Fe
2. Essential micronutrients
B, Mn, Zn, Cu, Mo, Si
3. Potentially toxic elements
Cr, Ni, Co, Ag, V, Pb, Cd, Se, Fl, Br

NUTRIENT pH AND CONDUCTIVITY

David Raper, Chair

Rationale

The discussion group viewed this topic as an exercise in both monitoring and control. The technology for monitoring is available in the form of pH electrodes and conductivity meters. Both these devices can operate in real time and continuously, but a question was raised as to whether the simultaneous use of pH electrodes and conductivity meters might result in mutual interference. Although both devices are available, their reliability and durability could potentially be improved. Control of conductivity can be achieved through microprocessor activation of injectors for replacement of nutrient ions in response to signals from a conductivity meter. Control of pH can be achieved through a selection of options in response to signals from pH electrodes. Monitoring and control of pH and conductivity should be easily accomplished in liquid culture systems. Monitoring and control would be more difficult to accomplish in solid media, especially in the rhizosphere.

Conductivity Control

Conductivity monitoring and control must be considered because it involves controlling concentrations of nutrient ions in solution. The real-time, continuous nature of conductivity measurements would complement nutrient monitoring and control which will probably be done at discrete intervals by adding specific ions to re-adjust concentrations to desired levels. Conductivity monitoring and control offers an interim system for avoiding nutrient depletion in excess of the desired range of control. The range and precision of conductivity control that will be necessary must depend on the nutrient application and monitoring systems. Furthermore, nutrient requirements can be expected to vary with the age and species of plant being grown. Finally, it should be recognized that organic acids entering the nutrient system from plant roots will alter the conductivity of solution. This means that conductivity measurements must be calibrated against the total of all the ions in solution measured by nutrient monitoring. For this reason, monitoring organic carbon in the nutrient solution may be a valuable supplement to conductivity measurements.

pH Control

Control of pH should be available over the biological range of 4.0 to 8.0. It is expected, however, that most control will be to a fixed point within the range of 5.5 to 6.5 with a precision of control to within 0.1 pH unit. There are several options available for pH control.

NUTRIENT pH AND CONDUCTIVITY (cont.)

1. Additions of acid, such as H_2SO_4 , or a weak base, such as $Ca(OH)_2$ to control pH to a chosen range.
2. Additions of phosphate salts can be used to buffer the pH.
3. Additions of nitrate and ammonium can be used to adjust pH by the uptake and subsequent release of counter ions by plant roots.
4. Exchange resins and other means can be used to remove specific ions or all of them, followed by reconstitution of the nutrient solution. (Exchange resins can also be considered as an option for a buffered solid medium.)
5. [Editors note: Electrochemical pH control is also a viable option.]

All these options have an impact on the nutrient supply and control systems and thus must be selected with consideration of this interface. It seems advisable to implement several or all of these options so that selection of a specific option at any time can be made in reference to maintenance of nutrient control. It should be noted that pH control eliminates utilization of pH monitoring as a diagnostic tool for stress or failure of the plant system; however, the frequency of correction through the controller microprocessor can serve as an indicator of problems in the plant system.