LONG-TERM LUNAR STATIONS: N93-13995

SOME ECOLOGICAL CONSIDERATIONS

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A major factor for long-term success of a lunar station is the ability to keep an agroecosystem functioning at a desirable, stable steady state with ecological stability and reliability. Design for a long-lived extraterrestrial manned station must take into account interactions among its subsystems to insure that overall functionality is enhanced (or at least not compromised). Physical isolation of food production, human living areas, recycling, and other systems may be straightforward; however, microbiological isolation will be very difficult. While it is possible to eliminate plant-associated microbiological communities by growing the plants aseptically, it is not practical to keep plants germ-free on a large scale if humans are working with them. Ecological theory strongly suggests that some kinds of communities or organisms effectively increase the stability of ecosystems and will protect the plants from potential pathogens. A carefully designed and maintained (lunar-derived) soil can provide a variety of habitats for effective microbial buffers while adding structure to the agroecosystem. A soil can also increase ecosystem reliability through buffering otherwise large element and compound fluctuations (of nutrients, wastes, etc.) as well as buffering temperature level and atmosphere composition. We are doing experiments in ecological dynamics and attempting to extend the relevant theories.

INTRODUCTION

The primary consideration of this paper is to outline some of the ecological design and management problems and possibilities of isolated human-containing, human-supporting agroecosystems, frequently referred to as Closed Ecological Life Support Systems (CELSS). Of the many possible topics within this category, those discussed here will be (1) problems related to the need of plant pathogen avoidance along with the necessary association between food-supporting plants and their unavoidably "dirty" human gardeners; (2) some possible stability problems stemming from possible internal dynamics of the human-plant agroecosystem; (3) a simple model of human nutritional requirements (except for substances, such as vitamin B_{12}, which are not produced by green plants) with appropriate portions of foods from the "recommended" CELSS food plant list, along with estimates of some of the requirements of the plants necessary for the production of the required amounts of the needed foods (including recommendation of animal use for food and companionship); and (4) a suggestion that somewhat more attention be given to the interacting needs and requirements of the components of (long-term) extraterrestrial station (ETS) support systems. In addition, brief descriptions of our preliminary and our current closed systems will be given.

PLANT-MICROBIAL INTERACTIONS

A major, but completely unavoidable problem is that human beings carry many species of microorganisms with them. Only about 10% of the approximately 10^{14} cells that we each carry around are really ours. The other 90% of these cells are of our "associates"; most of them are bacteria (*Savage*, 1977). Some of these microorganisms provide us with needed materials. For example, vitamin K, important in blood clotting, is normally provided to humans by some of their intestinal "associates." In any event, we cannot, in any practical way, produce trained, germ-free adult humans. (Imagine raising germ-free people from birth and then giving them adequate astronaut training while keeping them *completely* isolated microbiologically from the rest of the nonsterilized world.) In addition, extended experience on Earth shows that it will be extremely difficult to prevent those human-supporting agricultural plants in isolated agroecosystems from being exposed to people-carried microorganisms. We do not know what effects such microorganisms might have on otherwise germ-free plants.

The general method that no doubt will be used to avoid the introduction of plant pathogens into the system will be to eliminate plant-accompanying viruses and microorganisms (and other pests) from the agricultural plants by growing their progenitors axenically (in the absence of other species, including microorganisms). Elimination of pathogenic viruses will be more complicated than suggested by this simple prescription, but it can be done by use of already known techniques.

It is well known that plants naturally produce rich organic substrates around their roots and on the surfaces of their leaves and stems. For example, up to about 25% of the total carbon that the plant makes available to its roots may be lost from the plant by a combined excretion of low molecular weight organic molecules and loss of dead cells from the roots (*Barber and Martin*, 1976; *Newman*, 1978; *Burr and Ceasar*, 1984). Accumulations of rich and abundant microbial food material produced by the growth of the initially germ-free agricultural plants in an ETS would be rapidly invaded by microorganisms within the station. To the degree that the assortment of microorganisms within this system is restricted to species that are carried there by even well-washed humans, the invaders of the plant root zones (rhizospheres) and leaf surfaces (phylloplanes) will be of species that normally are associates of humans. Experience suggests that exclusion of all other (nonhuman associating) microbial species may also be difficult.

We do not have information concerning what effect these "unnatural" rhizosphere and phylloplane microorganisms might have on the plants. At best they will cause no difficulties. At worst, some of them will invade some of the plants and cause damage that will result in a reduction (perhaps catastrophic) of food yield. That some human-carried microorganisms will fall into the destructive category is strongly suggested by *Lasko and Starr* (1970), *Cooper-Smith and von Graeveniz* (1978), and *Starr* (1979). (See *Maguire*, 1980, for a more detailed discussion). *Lasko and Starr* (1970) inoculated plants with 45 different strains of the enterobacterium *Erwinia*, which had been isolated from various animals. Upon testing these by exposing plants to them, it appeared that 16 were harmless, 13 produced slight deleterious effects, and 16 damaged the plants as much as did strains of *Erwinia* that are considered to be serious plant pathogens. *Cooper-Smith and von Graeveniz* (1978) were concerned with cases of humans who were infected by a bacterium that later turned out to be *Erwinia herbicola*, previously recognized to be a plant pathogen. *Starr* (1979) summed up the situation by pointing out that some 200 species of bacteria and fungi were then known that attack and harm both plants and animals.

To counter this potential problem, as well as to help in the solution to several other problems that will be discussed below, we recommend that carefully selected and "purified" soil microbial communities be used. They should be inoculated onto the previously germ-free plants supplied to the ETS for food production. These "domesticated" microbial communities will use (and destroy) the organic materials released and cast off by the plants. They will be important in preventing other possibly damaging microorganisms from joining the plant-microbial community. These microbes also could be very beneficial in recycling sewage (kept free of heavy metals and toxic chemicals) that might be used to enrich the agricultural soils.

The theoretical reason that this strategy is expected to work is that plant pathogens have evolved a series of specializations that enable them to achieve contact with a host, penetrate that host in spite of the host's defences, and then to utilize the host as a resource on which to grow and reproduce. Because no organism can evolve to be maximally efficient in carrying out a large number of different kinds of tasks, what this means to plant pathogens is that they are generally inferior to many of the normal rhizosphere microorganisms in utilizing the exudates of roots and the roots' dead cells (such as hair cells and root cap cells) for their growth and reproduction. The normal rhizosphere organisms therefore tend to form a barrier, an important part of which is a zone of severe nutrient depletion around the plant roots that the pathogens must reach to be successful. The pathogens find this zone difficult to penetrate. However, with an absence (or low population level) of these normal rhizosphere microorganisms, which are specialized to use these organic root products, pathogens have much less difficulty in invading their host plants. In many instances, for reasons of these dynamics, the addition of populations of some kinds of soil or rhizosphere bacteria decreases the number of plants that are attacked by a pathogen (and also may decrease the severity of the symptoms in those plants that do come down with the disease). (See *Maguire*, 1980, for many references pertinent to the above.)

Considerable experimentation has been carried out in which various nonpathogenic microorganisms have been added to the rhizospheres of plants in frequently successful attempts to reinforce the normal rhizosphere microbiological community and better protect the plants from some of their important pathogens.

Results have been sufficiently successful that some commercial application of such biological control of pathogens has developed. A number of biocontrol agents have been used. Among them are the bacteria *Pseudomonas sp*, *Pseudomonas fluorescens*, *Agrobacterium rhizogenes* (strain 84), *Bacillus mycoides*, *Bacillus pumilus*, and *Enterobacter cloacae*, as well as the fungi *Coniothyrium minitans*, *Gliocladium roseum*, and a number of species of *Trichoderma*. *Lynch* (1987) provides an excellent (although brief) review of the current state of this art.

The naturally developing rhizosphere communities of different plant species (and even of different varieties of one species) are different. As one of many possible examples, the peanut (*Arachis hypogaea* L.) varieties "Virginia" and "Spanish" and even different subvarieties within these varieties had different numbers of total bacteria and of *Azotobacter*, in their rhizosphere microbial communities (*Joshi et al.*, 1987). Total number of bacteria in the rhizosphere also was positively correlated with the yield of the individual plants.

Various individual species of rhizosphere microbial communities also appear to stimulate the growth of the plants. Also, in addition to the direct plant-protective function of some microbial species, the presence of some other species results in an elevation of the numbers of living bacteria in the rhizospheres of plants. In another example, *Secilia and Bagyaraj* (1987) added cultures of species within four genera of vesicular-arbuscular (VA) mycorrhizae to pot cultures of Guinea Grass (*Panicum maximum*). They recorded considerable increase in the number of bacteria and nitrogen fixers in the rhizosphere communities to which one of these three species was added. Presence of the fourth VA species did not correlate with change in the number of bacteria or nitrogen fixers, but it did correlate significantly with the number of Actinomycetes present (one, but not the other two VA species also produced this pattern of increase of Actinomycetes). Finally, in their very brief comment on the nonantipathogen effects of rhizosphere microbial communities on plant growth, *Vancura and Jandera* (1986) report on the production of plant growth hormones (kinetins, gibberillins, indole-3-acetic acid, and so on) by some rhizosphere microbial species. These growth hormones may have considerable effect on plant growth and yield. It is clear that there is much work yet to be done on the systems briefly illustrated by these examples. Development of research in these directions might make important differences in the kinds of agroecosystems that will be most useful on the Moon and other ETSs, as efficiencies and rates of food production may be greatly elevated by proper choices.

Some of the products of the rhizosphere community include volatile chemicals (such as ethylene, which may act as a plant hormone under some conditions) that will need to be removed from the atmosphere of ETSs. If human-carried microorganisms invade the rhizospheres of the plants, they also could result in problem volatiles. As one small example, *Belay et al.* (1988) have isolated methanogenic bacteria from human dental plaque. If these methanogens (or those that inhabit the intestinal tracts of about one-third of the adults of the U.S.) should be present and have the opportunity to be too active in ETSs, there might also be the problem of having to remove this gas from the atmosphere.

Finally, for this portion of the paper, there is the problem of the relatively ready movement of genetic elements among many of the microorganisms that share some environment. This kind of movement is known to provide bacterial species with abilities that they previously did not have. It is what has provided our hospitals, for example, with strains of infective bacteria that are

resistant to a number of different kinds of antibiotics (which is why infections that one acquires in a hospital may be especially nasty and difficult to cure). *Davey and Reanny* (1980) present a "genetic network" in which they illustrate the known paths of phage and plasmid transfer of genetic elements among 21 genera of bacteria. Represented in this web are 2 genera from the rhizoplane, 11 from the rhizosphere, 3 from bulk soil, 9 from "soil feces," and 1 from a human gastrointestinal tract (with 1 genus being found in—and counted in—2 of these habitats). *Reanny et al.* (1983) reinforce the suggestion that genetic elements pass rapidly among many genera of bacteria within natural systems, and discuss the evolutionary implications of the patterns observed. Transfer of genetic components (including nuclei) is also well known in the fungi.

What the above sections tell us is that microbial communities cannot be avoided if agroecosystems are to be used. If we wish to establish ETSs on the Moon or elsewhere we need a great increase in our understanding of these communities if we are to avoid complex and potentially serious ecological problems. Some of these have been briefly considered above. It seems clear that we need to learn how to design microbial communities, and need to know which, out of a very large number of possible designs, will be most effective in helping us to avoid really serious agroecological problems (at the same time it would be nice to have microbial systems that would enhance agricultural yield and perhaps do other useful things for the ETS). We must do a great amount of ecological research if we wish to use agroecosystems for feeding the people in ETSs; the alternative is to continue to bring sandwiches from Earth, which is not a viable economic proposition in the long run, especially as the ETSs get to be farther and farther from Earth. We have a long way to go, and need to get started in a number of research directions.

Plants of ETSs on the Moon and other planetary bodies may well be grown in carefully designed and cared-for soils because (1) soil cation exchange capacities provide effective buffers for many plant nutrients (an alternative to fail-safe hydroponic control systems); (2) soils provide temperature buffers for the plant roots; (3) soils provide a variety of habitats for beneficial soil micro-organisms that could help to recycle sewage, and to destroy some kinds of toxic chemicals, in addition to protecting "their" plants; (4) soil provides support for plant roots and the plants themselves; (5) soil physical and chemical heterogeneity results in substantial increase in the range of chemical conditions that occurs among microhabitats reachable by plant roots and thereby possibly increasing the availability of nutrients needed by the plants (*Brady*, 1984); and (6) soils (of appropriate structure) on bodies that have planetary mass (such as the Moon) will be under the influence of gravity, which will cause them to drain under proper conditions. (Capillary forces within a soil, in the presence of no more than microgravity in nonspinning orbiting space stations, would prevent drainage of that soil. This is a very important factor in the consideration of use of soils in such non-spinning, orbiting stations.) Where there is adequate gravity, the use of soils requires less in the way of mechanics and control than do hydroponics, may provide other important benefits, and also may permit the people to spend less time in taking care of the plants.

STABILITY

There is controversy concerning ecological stability theory. We believe that *appropriate* communities of organisms (including communities made up of agricultural plants and "their" micro-

organisms) may be of considerable effect in increasing the stability of agroecosystems. Such systems may change less as a result of a given shock or perturbation than do simpler systems. In addition, these communities can return to approximately the preperturbation state more rapidly than others. (These properties are called, respectively, resistance and resilience by ecologists.) We are currently doing experiments to learn more about these ecological dynamics and to extend ecological theory concerning them.

The often-observed decrease in the number of kinds of micro-organisms carried by people isolated from others for long periods of time is an indication of the lack of internal stability in the human-carried microbial community. This pattern has been observed in long expeditions to the Arctic and Antarctic, for example, where the incidence of communicable disease (flu and colds, for example) drops markedly after a while because there are no more susceptible people for the disease to be passed to and the disease dies out. This also happens with respect to microorganisms that appear not to induce immune reactions in the host. For example, *Taylor* (1974) points out that on longer space missions there is a reduction of the "normal" human-carried microbiota. It is as a result of this reduction, Taylor reasonably hypothesizes, that the potentially pathogenic yeast *Candida albicans* becomes more common as a result of the absence of some of its normal competitors. What these observations tell us is that the "normal" human microbial communities are not stable in and of themselves, and that in these isolated groups of humans, extinction of some microbial species is common. On Earth, the number of species in human-held microbial communities results in part from a continued reinvasion of each community by microorganisms from other humans (and from the environment in general). A number of these invaders are, at the time of invasion, new to the community. This also suggests that premission microbiological isolation, to reduce the amount of reinvasion (and the number of species carried), might be an important part of the preflight preparation of those leaving Earth to occupy ETSs, including those on the Moon.

Complex, steady-state communities of microorganisms are probably not to be expected (they are probably uncommon in natural ecosystems on Earth). Interaction among the processes of population growth, competition, and predation in complex biological systems probably at least sometimes makes for a system in which the dynamics, at least in detail, are fundamentally unpredictable (chaotic, in the formal, mathematical sense; see *Thompson and Stewart*, 1986, for a good, general introduction to chaotic dynamics). We predict that some of the dynamics, in both human-carried and plant-associated microbial communities, will turn out to have chaotic elements (see *Maguire*, 1978, for a model of a simple ecological community that becomes chaotic in both time and space). There are trends and patterns that are quite predictable even within the chaos of these ecological systems; the observed reduction of numbers of species in the microbial communities of small groups of isolated individuals is an example. Much more work on this and other aspects of the dynamics of these systems is needed.

HUMAN-SUPPORTING AGRICULTURE

One of us (*Maguire*, 1984) has published a very simple minimal model of a human-supporting agroecosystem. In this, some necessary quantitative and qualitative characteristics of the food produced by the crop plants suggested for CELSSs were examined. A mix of food from these plants was chosen such that the known

nutritional requirements of humans (except for vitamin B_{12} which is not produced by plants) would be met, while at the same time the amount of space required to grow the food would be kept low. Dietary needs considered by the computer nutritional program were calories, proteins (including amino acid content), fats, carbohydrates, vitamins, minerals, and trace minerals. From this analysis, a list was produced giving one possible set of average per-person daily amounts and kinds of food required. Only species on the current list of CELSS-acceptable crop plants were used (see Table 1). Also, as can be seen from this analysis, about 70 m^2 (and at least near optimum culture conditions) are needed to grow adequate amounts of each plant in this selection to provide a nearly adequate diet (it contains everything needed except for the vitamin B_{12}, also known as cobalamine) for one person. Transpiration of the agricultural plants required to produce this per capita kind and amount of food will be about 190 l of water per day. The per capita flows of some of the major components of

this human-supporting agroecosystem are illustrated in Fig. 1; these give an approximation of the magnitude of some of the required dynamics (and resultant machinery/management requirements).

As can be seen in Table 1, there are secondary and tertiary lists that were added to the list of primary CELSS-recommended plant list. Rabbits were included as they (1) do not eat the same food as humans and would use parts of the agricultural plants that are indigestible to humans (and therefore do not directly add to the cost as far as ecological or food chain energetics are concerned), (2) provide a good source of the otherwise problem vitamin, B_{12}, (3) provide for an important increase in the tastiness and satiation value of food produced by the agroecosystem, and (4) make satisfactory pets (provide companionship, can be "litter box" trained, like to be petted, etc.). Living in an ETS for extended periods is going to be difficult, and some small but important "luxury" items such as rabbits and roses appear to us to be well worth their costs. Onions, strawberries, and roses also are added to the proposed plant list, as tomatoes recently have been, because they (or other plant species to serve the same functions) can add considerable to life quality while adding little in the way of costs. Those who have been on long, isolated expeditions (as one of us has) well know the very great value of small amenities such as those we suggest.

CURRENT WORK

One of us (Maguire) developed, constructed, and successfully tested a preliminary closed system with which to ask some of the ecological questions posed above. Figure 2 is a photograph of part of this pilot system showing a variety of plants that (except for the red beets) were healthy and growing 20 days after the closure of the two experimental systems. Figure 3 gives the fluctuation of carbon dioxide over two 24-hour periods. It shows that there was substantial uptake of carbon dioxide during the daylight
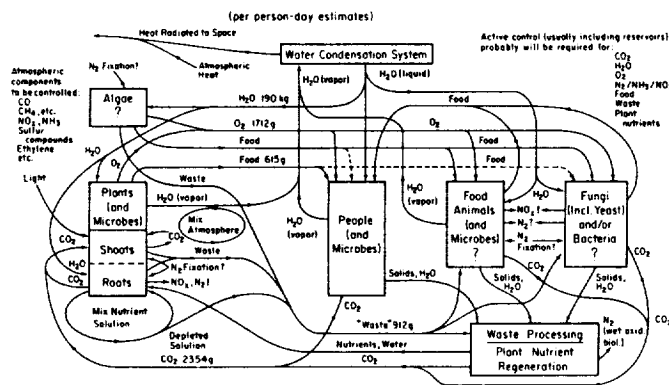

Fig. 1. Major material flows for extraterrestrial stations based on per-person daily requirements.

TABLE 1. Per-person daily food values, production, and requirements (cooked where appropriate) for extraterrestrial stations (see text).

Food	Amount	g(dry)	kcal	g carb.	g protein	g fat	g/day/m^2 (edible)	agri: m^2/ crew	agri:kg H$_2$O/ day/crew	nonedible (g/day)
Rice (brn)	1 cup	58	232	50.0	4.9	1.2	8.4	6.9	18.9	87
Wheat (brn, fl)	1 cup	106	400	85.0	16.0	2.4	16.4	6.5	17.8	159
Potato (white)	1 large	50	145	32.8	4.0	0.2	19.0	2.6	6.5*	13
Potato (sweet)	1	53	161	37.0	2.4	0.6	30.0	1.8	4.9*	14
Soybean	1 cup	52	234	19.4	19.8	10.3	6.8	7.6	26.6*	121
Peanut	1 cup	142	842	27.2	37.4	71.6	8.9	16.0	48.0*	331
Sugar (beet)	0.5 cup	108	410	106.0	0.0	0.0	32.4	3.3	6.6*	132
Broccoli	3 spears	8	24	4.2	2.7	0.3	1.3	6.1	16.7	19
Peas (green)	0.5 cup	14	54	9.4	4.1	0.2	1.7	8.3	22.7	33
Lettuce	1 cup	3	7	1.6	0.5	0.1	13.6	0.2	0.2	1
Strawberry	1 cup	15	55	13.0	1.0	0.7	2.1	7.1	15.8	—
Onion	0.5 cup	6	22	5.0	0.9	0.1	3.1	1.9	5.2	2
Total		615	2586	390.6	93.7	87.7	X = 12.0	68.3	189.9	912
Tomato	1 medium	9	27	5.8	1.4	0.2	11.3	0.8	—	—
Yeast (baker's)	1 package	5	15	2.0	2.2	0.1	—	—	—	—
Alfalfa	per m^2	16	—	—	2.6	—	16.0†	—	—	—
Milk (goat)	1 cup	32	168	11.0	8.7	10.1	—	—	—	—
Lamb	3 oz	32	158	—	24.4	6.0	—	—	—	—
Rabbit	3 oz	34	184	—	24.7	8.5	—	—	—	—

* Those species for which water use data are given by *Tibbits and Alford* (1982), and which were used to produce the average of 2.74 kg/water/m^2/day used to estimate water use of the other species (see text).
† Edible for goats, rabbits, termites, etc., not humans (although human-digestible leaf protein of good quality and quantity can be extracted from alfalfa).

hours, and substantial return of carbon dioxide to the atmosphere during the night by the respiration of the plants and microorganisms within these closed systems.

Figures 4 and 5 illustrate the current version that we have designed and assembled and which is in preliminary stages of experimentation. As can be seen in the photographs, the wheat is healthy in this completely closed system. The slightly spindly nature of the plants is the result of somewhat low light levels along with lack of thigmomorphogenesis (a thickening of plant

Fig. 4. Closed chambers of the current model, some containing 15-day-old wheat. Gas and water handling barrels, pumps, carboys, and tubing are at the upper left.
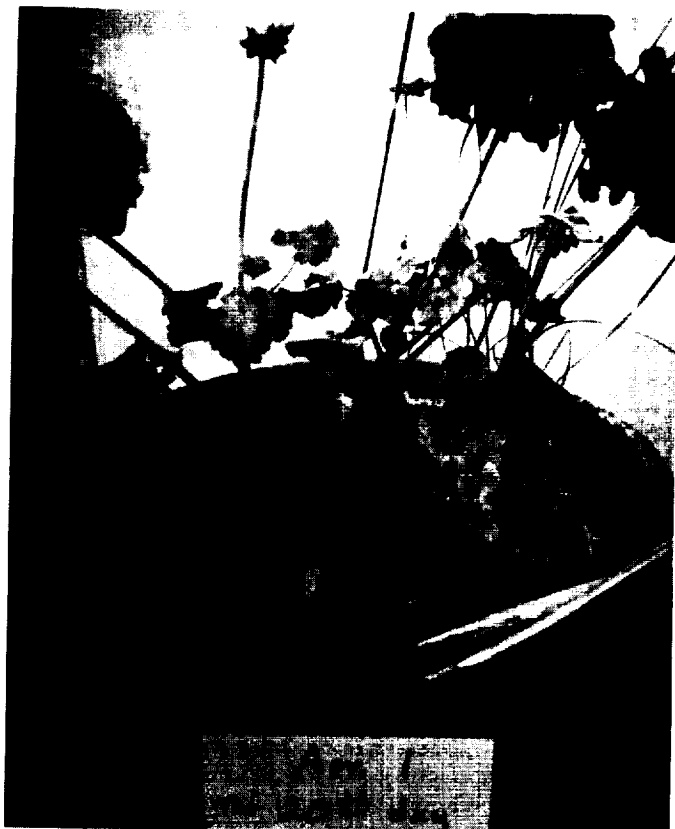
Fig. 2. Plants in a Closed Ecological Life Support System chamber 20 days after closure. Geranium, chrysanthemum, rye, turnip, and clover were healthy and growing, but the red beets were doing poorly.
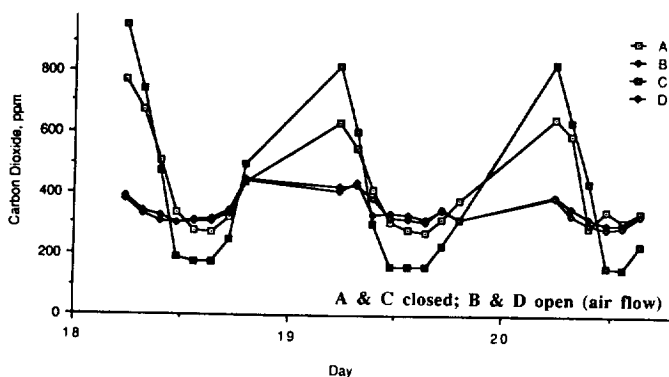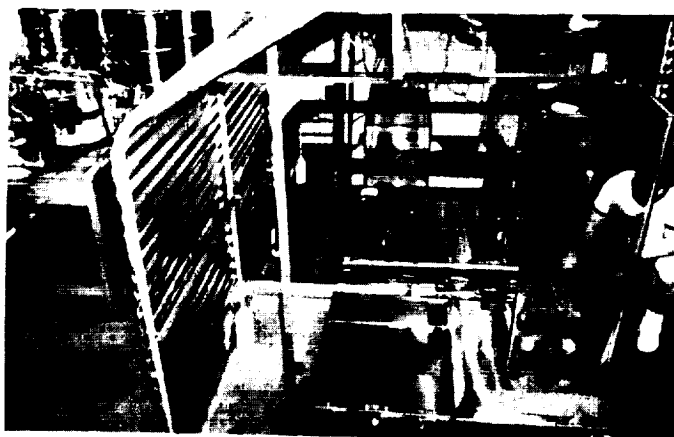

Fig. 3. The fluctuation of carbon dioxide in the chamber shown in Fig. 2 for the days 18 through 20 after closure.

Fig. 5. Current model closed chamber containing 15-day-old wheat. See text.

stems induced by their bending, and normally caused by winds blowing on the plant out in the wider world). After a little more testing of these closed systems, we will use them to examine the resistance and resilience of wheat growth rate to calibrated perturbations in the absence of and in the presence of microbial communities of various kinds and complexities.

As a final note, we suggest that careful consideration be given to placing ETSs on the Moon at or near to the poles so it will be possible to continuously take advantage of sunlight by use of solar cells and power lines. Even better, if possible, would be to use light-weight mirrors, perhaps in association with other technologies, to direct light to the agricultural plants continuously (rather than just during the lunar day). Because the Moon lacks an atmosphere, this could be done reliably and easily over considerable distances. Efficiencies of use of light reflected by mirrors would be considerably larger than efficiencies of converting sunlight to electricity and then back to light again. In addition, the appropriate use of "cold" mirrors (mirrors that reflect visible wavelengths of light, but do not reflect the infrared of the solar spectrum) could considerably reduce the amount of heat that the station would have to dissipate to space. Of course, some infrared (far red) is necessary to adequately stimulate some physiological processes, including flowering, in various of the agricultural plants.

A FINAL SUGGESTION

As our last suggestion, we hope to see the establishment of a truly wide-ranging committee (including both NASA and non-NASA dependent people) that would estimate as well as possible the various interactions that could occur between the major ETS subsystems. With this information, then it should be possible to modify features of some subsystems without seriously affecting their performance, but such that important negative effects on other subsystems are reduced (and possible positive effects are increased).

It is also important that options not be closed too soon. Considerable development is still occurring in our understanding of many of the subsystems that will be important to "self supporting" lunar (or other) bases. It may well turn out that total system optimization will require that some of the subsystems be considerably different from (and more difficult to build and manage than) those currently under consideration.

SUMMARY AND CONCLUSIONS

It is our conclusion that there are considerable problems to be solved with respect to use of agricultural plants for human support (food and oxygen) in ETSs, such as bases on the Moon. Nevertheless, use of standard agricultural plants to provide food for the people seems to be the most reasonable (these plants have been chosen and bred for their usefulness and efficiency of food production over large stretches of space and time). Plant pathogen exclusion from ETSs appears to be best achieved by rendering the agricultural plants germ-free and then supplying them with carefully developed microbial communities that will protect them from the microorganisms unavoidably carried to the ETSs by their human occupants. Questions of stability of the human-supporting agroecosystem also need to be examined, and ways found to reduce the possibility of serious deleterious changes in the internal ecological dynamics within this ecosystem. Much research in these directions remains to be done (it has not

even been really started). It is time for effective, long-term support of research to this end to be started, as it will take considerable time and effort to obtain the badly needed answers.

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