A METHOD FOR SCREENING OF PLANT SPECIES FOR SPACE USE

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INTRODUCTION

Higher plants are capable of performing important functions of environmental regeneration and food production in future space or planetary habitats. Also, tending of some plants within a spacecraft or habitat may have psychological and morale benefits to space crews. Success will depend upon the plant's responses to special environmental factors including reduced gravity. For example, plants may be cultured on a large scale in radically altered gaseous environments (e.g., very low pressures) which would offer considerable savings in structural and energy expenditures. However, if plants and other living organisms are rejected as part of future space habitats, it may result not from a lack of biotic capabilities, but from a failure on the part of biologists to demonstrate and express these capabilities in a rigorous, quantitative manner acceptable to space-planning engineers.

Technology transfer between the physical sciences and engineering is accelerated by the common disciplines and languages of thermodynamics, material properties, mathematics, and computer analyses. Conversely, the interface between basic biology and agriculture is highly subjective and slowed by specialized jargon, especially in the new areas of biotechnology. Historically, the quest for more detailed biological information has obscured the need for unifying concepts and theory. Thus while "genetic engineers" are rapidly learning how to modify individual genes, there is difficulty identifying the processes which need to be altered and quantitatively predicting improved performance under specified environmental conditions.

During the past 10 years, progress has been made in the development of "crop models" designed to predict the performance of certain species and cultivars in Earth environments. In the early days, these were primarily "black box" models based on easily measured overt behavior (e.g., growth, flowering, photosynthesis, etc.) fitted to environmental inputs by regression to linear or polynomial equations. These models were highly site specific and thus not easily transferred from one Earth environment to another, much less to space habitats.

Recent modelling efforts, including ours (i.e., Biosystems Research Group) have been more mechanistic, incorpora-

ting quantitative concepts of physics, thermodynamics, bio-chemical rate kinetics, feedback control, physiology, and anatomy. However, as models become more complex the likeli-hood of different models predicting similar overall behavior increases. Thus comparison, validation, and/or further de-velopment of such models requires experimental techniques capable of simultaneously measuring numerous interactive processes within the modelled organism.

Need for Innovative Experimental Methods. Developing and using plant models to select appropriate organisms for space environments, suggesting genetic improvements (by ei-ther conventional or molecular methods), and optimizing plant growth systems will require certain experimental information not fully available at present: (1) We must understand how plants will operate under altered atmospheric pressure; (2) We must optimize in terms of (a) selecting growth conditions within the chosen envelope of possible conditions dictated by structural engineering and operating parameters; and (b) selecting or breeding plant species and varieties most suit-able for use under the chosen conditions to meet human nutritional requirements.

The traditional approach to answering such questions is to conduct field or growth chamber tests in which potentially useful varieties are screened against the range of possible conditions and performance is judged, at some end-point, in

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terms of health, total growth, fruit yield, etc. Such an
approach is time-, labor-, and space-consuming but for stan-
dard terrestrial conditions is nonetheless relatively man-
ageable. However, if we consider the necessity of conduc-
ting such tests under altered atmosphere and pressure and
ultimately perhaps under altered gravity environment, the
logistical problems and associated costs become intolerable,
if not insurmountable. A means of greatly simplifying and
reducing costs of selective research is needed if we are to
accomplish the testing necessary to define the plant species
and varieties and the conditions under which these plants
will be grown.

A second major problem in evaluating plant responses to
environmental factors (e.g., temperature, gas composition,
nutrient supply, etc.) is in separating the primary effects
from secondary feedback and/or adaptive responses. This is
critical in the development of predictive, mechanistic mo-
dels. For example, the concentration of CO_2 directly and
immediately affects photosynthesis, plant responses to ethy-
lene, and perhaps a few other metabolic processes. After
prolonged exposure, however, other responses appear such as
altered carbohydrate allocation, premature senescence of some
leaves, and early flowering in some species. The problem
then is to determine which of these processes were affected
directly by CO_2, and which were induced indirectly by the
primary mechanisms.

The best means of discerning primary and secondary me-
chanisms is continuous, simultaneous measurement of key
processes in individual live plants responding to step chan-
ges in given environmental factors. Unfortunately, conven-
tional methods for measuring many internal processes require
destructive sampling of plant populations at various inter-
vals during an experiment. Such experiments are time con-
suming and expensive, and provide little assurance that the
important dynamic transients and interactions will be ob-
served.

In this paper, we will describe a cost-effective metho-
dology which approaches the rigorous demands of theory by
simultaneously monitoring numerous dynamic aspects of carbon
assimilation and allocation kinetics in live, intact plants.
Analogous methods can apply to nitrogen uptake and alloca-
tion.

This methodology capitalizes on the special properties
of the short-lived, positron-gamma emitting isotope ^{11}C,
especially when applied as $^{11}CO_2$ in a special "extended
square wave" (ESW) pattern. As indicated previously (Fares
et al., 1978; Magnuson et al., 1982; Goeschl et al, 1984),
the 20.4 minute half-life allows for repeated or continuous
experiments on the same plant over periods of minutes, hours,
days, or weeks. The steady-state isotope equilibrium ap-
proached during the ESW experiments, and the parameters which

can be analyzed by this technique are also direct results of
that short half-life. Additionally, the paired .511 MeV
gamma rays penetrate any amount of tissue and their 180°
opposite orientation provides good collimation and allows
coincidence counting which nearly eliminates background.

TECHNIQUES

The ^{11}C System. Schematically, the present ^{11}C label-
ling system operates as depicted in Figure 1. It has three
basic components:

1. An accelerator such as a cyclotron or a Van deGraaf
    accelerator in close proximity to, and capable of
    providing a continuous stream of radioactive CO_2 to:

2. A precisely controlled plant growth chamber; and

3. A set of radiation detectors and other measuring
    devices connected to a system of counters and other
    output devices which are on-line to a computer.

CO_2 is continuously fed into the accelerator target chamber
where it is activated to a mixture of gases including ^{11}CO.
The radioactive mixture is piped through a chemical conver-
sion system to remove unwanted gases and to convert the ^{11}CO
to $^{11}CO_2$. The labelled CO_2 is then mixed in the proper
proportions with a standard atmosphere and piped into a
clear, plastic photosynthesis chamber attached to the test
plant leaf where both $^{12}CO_2$ and $^{11}CO_2$ are taken up by the
plant and converted by photosynthesis into carbohydrates.
Movement of the ^{11}C-labelled photosynthates through the test
plant is monitored by a series of detectors attached at

chosen points on the plant. Various chemical and physical sensors measure system conditions, gas exchange, and other plant responses. That data is collected, stored, and displayed in a computer system to provide real-time experiment monitoring.
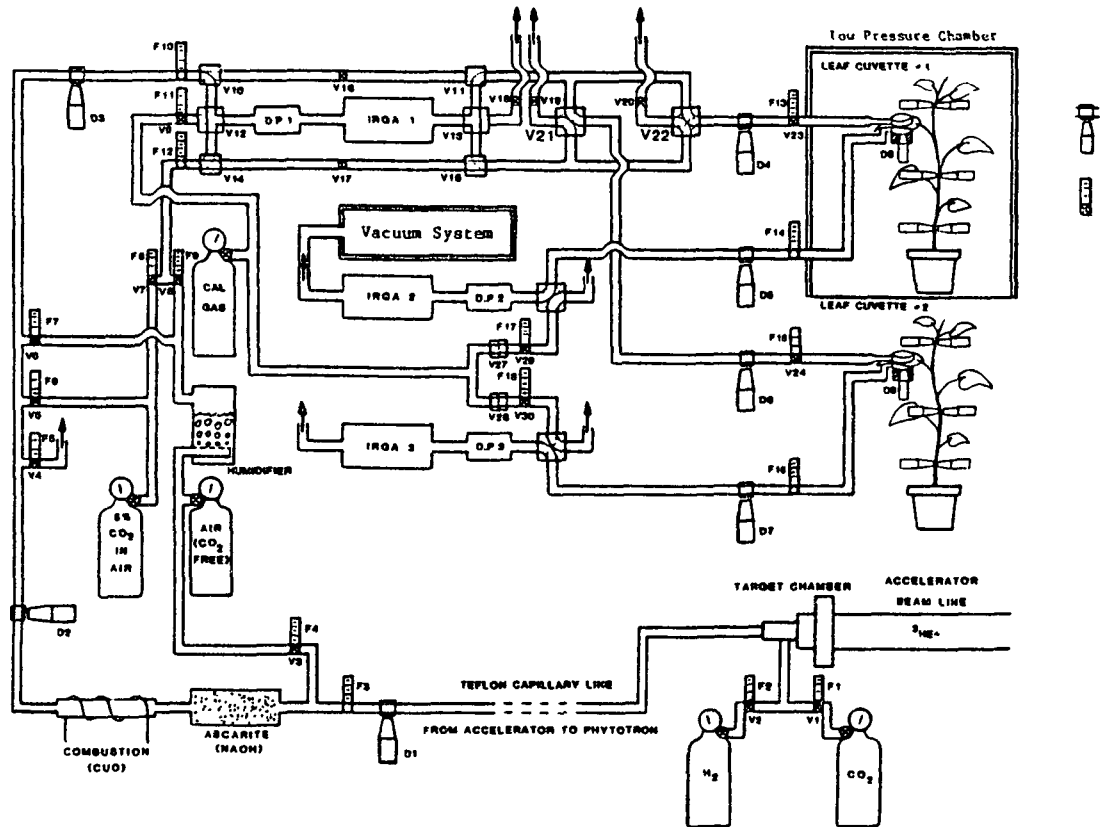

Figure 1. Schematic of the existing ^{11}C System. Lower right - beam line and target chamber; Lower left - chemical processing system; Upper left - gas mixing and measurement; Upper right - plants with leaf chambers and detectors. (Electronics and computer are not shown); Center - output gas measurement and calibration system. The components blocked with heavy (double) lines are the points at which low pressure modification would be made.

The Extended Square Wave. Figure 2 presents typical results of a ^{11}C study of carbon assimilation and allocation in a plant. These data are analyzed by computer along with the gas exchange data. Based on these calculations, the following parameters can be estimated:

1. Transpiration
2. Leaf Temperature } stomate conductance
3. Net Photosynthesis Rate (CER)
4. Rate of photosynthate storage in the leaf
5. Rate of photosynthate export
6. Export pool "size" or concentration
7. Export pool turnover time
8. Speed of translocation
9. Concentration of translocates
10. Unloading rates at various sinks

EXAMPLES OF RESULTS

Evaluation of Genotypes. Application of the technique to the problem of comparing genotype productivity potentials results in a genetic performance histogram like Figure 3. The histogram pinpoints the genetically controlled limiting property in a particular genotype. In the example shown, each bar represents the ratio of an activity or property of a Mississippi genotype of the C_4 grass Echinochloa crus-galli divided by the same activity or property of a Quebec genotype. The Quebec biotype is adapted to short growing seasons, and thus has a very rapid rate of dry weight accumulation for the first four weeks of growth compared to the
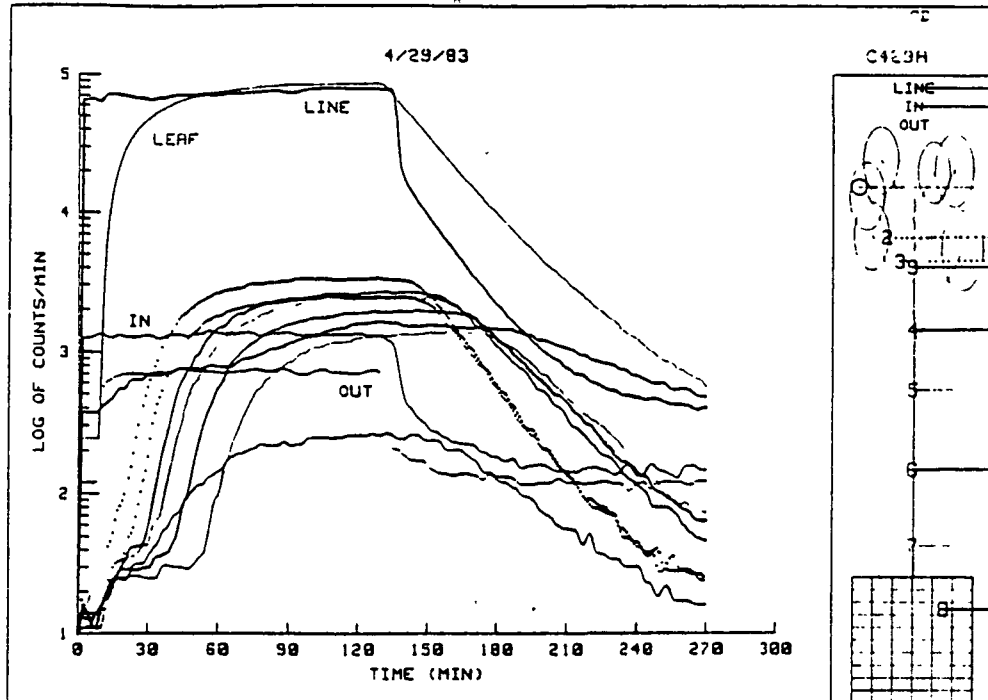
Figure 2. Raw data taken in real-time of a typical ^{11}C metabolism experiment. These traces are stored digitally and recalled for analysis as needed. Each trace represents the calculated ^{11}C concentration at a different position on the plant shown schematically to the right of the trace.

Mississippi biotype (Potvin et al., 1984). The upper five open bars relate growth and net productivity of the genotypes. The remaining open bars represent processes involved in the rate of dry weight gain. The solid bars represent some of the plant's genetically controlled properties which control those processes.
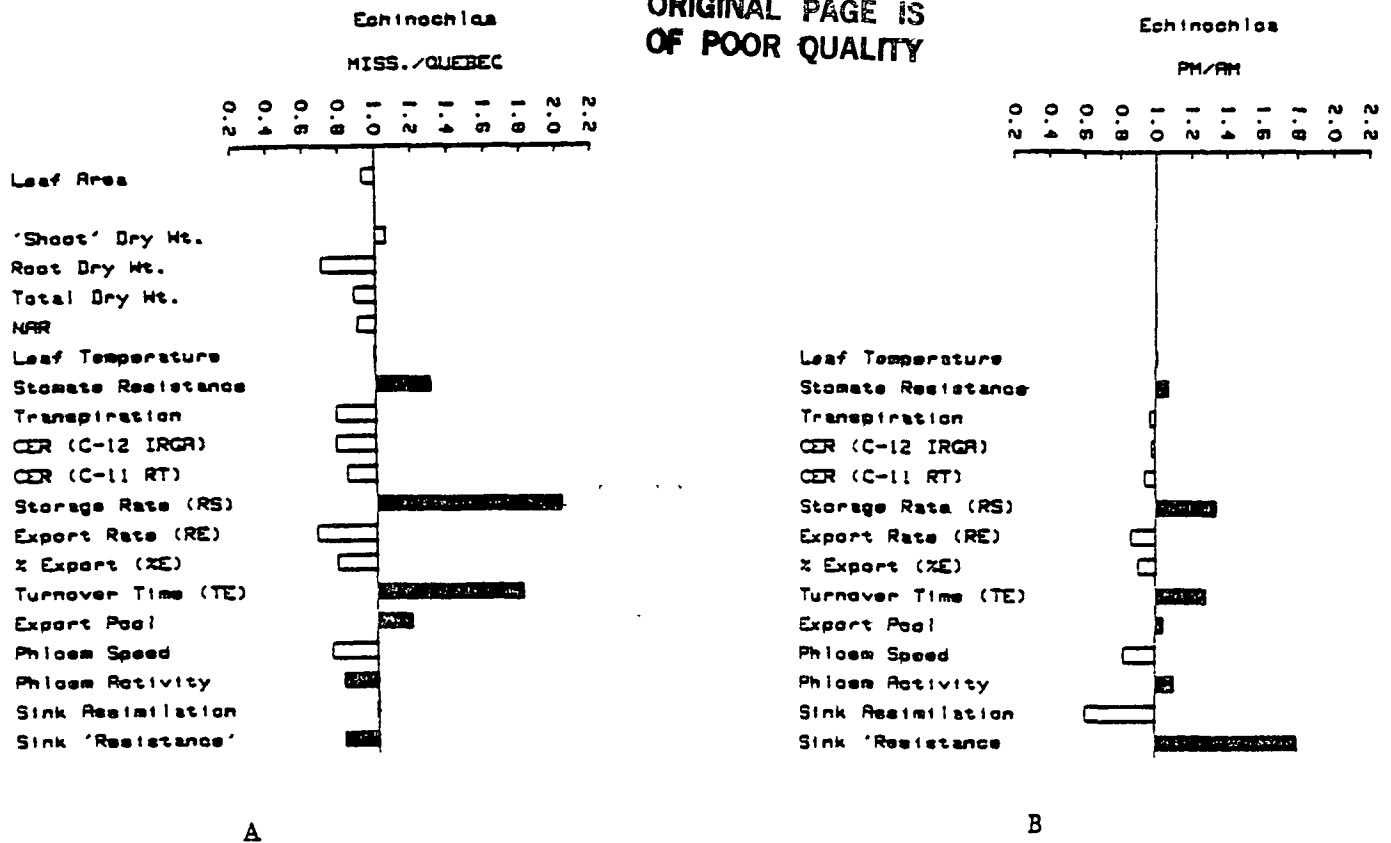
Figure 3. Genetic Performance Histogram (GPH) of cool climate and warm climate biotypes of barnyard grass Echinochloa crus-galli. A - Comparison of performance of the two biotypes; B - Morning versus afternoon performance of the biotypes. Each bar represents an activity or property of the Mississippi biotype divided by the same activity or property of Quebec.

To find the genetically controlled parameters responsible for the lower productivity of the Mississippi genotype, begin at the bottom of the histogram and move upward to the first solid bar to the right of the axis. In this case, that bar represents the concentration of exportable products in the leaf. This suggests that loading of these products into the phloem transport system is a major limitation, as further

reflected in the slow turnover time of these products and the increased rate of storage. If photosynthesis *per se* were limiting in the Mississippi genotype, one would expect storage and export pools to be lower, i.e., these solid bars would be to the left of the axis.

Sources of variability. Differences in the rates of various processes and the properties which control those processes are often as great between morning and afternoon as they are between genotypes. In Figure 3-b, data are based on the afternoon/morning (PM/AM) behavior (in controlled environment conditions) of the same plants shown in the Mississippi/Quebec comparison (Figure 3-a). Note, particularly, that if only the normal parameters of net photosynthesis (CER), transpiration, and leaf temperature were measured, little difference would be observed.

Other similar sources of variation include recovery of plants from experimental "set-up" procedures, effects of day/night temperatures, or pot size. The important point is that many of the physiological responses would not be detected were it not for the real-time, non destructive nature of the short-lived isotope methods.

CONCLUSIONS

These techniques have been used to observe other phenomena, some of which had not been previously observed, but were predicted from biophysical-thermodynamic theory of

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carbon transport in plants (DeMichele et al., 1978; Goeschl et al., 1976; Magnuson et al., 1979). One of these phenomena, the spontaneous blocking and unblocking of phloem transport (Goeschl et al., 1984), was particularly striking when observed with our unique ^{11}C steady-state labelling system. Also, we have recently reported experimental evidence supporting an hypothesized role of the phloem transport system in the response of plants to drought stress (Goeschl et al., 1984; Fares et al., 1984).

The above observations illustrate the potential capabilities of the short-lived isotope labelling techniques as means to (1) evaluate the performance of plants in specialized environments; (2) distinguish and quantify primary environmental effects and secondary physiological adaptations; and (3) provide validation and further development of predictive, mechanistic plant models for use in the engineering design and management of space habitability systems.

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