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Paper Session I-A - Development of Technology and Experimental Designs for Plant Growth Studies in Space

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Development of Technology and Experimental Designs for Plant Growth Studies in Space

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Abstract

Plants will be a critical component of future Bioregenerative Life Support Systems that will be implemented on long duration space missions. We describe here a novel microgravity-rated plant growth apparatus that is targeted for use on the International Space Station (ISS) in the 2004-2005 timeframe. The system contains six modular units capable of utilizing either porous tube and/or substrate-based nutrient delivery approaches. Heat pulse moisture sensors are used to both monitor and control root zone wetness levels. In addition, a fixed-feed water delivery algorithm is available which meters out appropriate levels of water based upon plant life cycle stage. Fifty miniature color cameras will image the plant specimens throughout the experiment, permitting real-time assessments of plant performance over time. Alternative experimental strategies suitable for implementation on the ISS are discussed.

Experiment Justification

The Advanced Life Support Program seeks to utilize plants to recycle air, water, wastes, provide food and contribute to the psychological well being of the crew during extended space flight missions. It is believed that the provision of adequate levels of water and oxygen to the plant root zone are the most crucial components holding back major advancements in this area. The dominance of the surface tension of water under microgravity conditions has often been thought to create extremes of water delivery, e.g. either over- or under-watering of root zones. Differences in plant growth responses between space flight experiments and their ground controls can therefore be expected based merely upon differences in moisture distribution patterns between the two conditions. Until we have a better means of controlling these critical aspects of plant culture, all experimental results involving space-grown plants will be subject to question as to whether they are related to "direct" effects of microgravity or “indirect” effects attributed to a microgravity-altered culture regime. Such an altered regime could produce results less optimal than would be the case had the growing conditions been better tuned for space flight conditions.

The WONDER (Water Offset Nutrient Delivery ExpeRiment) project will address the question of comparability of environmental conditions between the space flight and ground control experiments by employing three different porous tube and substrate compartment wetness level treatments (Levine *et. al*., 1999). It is anticipated that different wetness level set-points than those used on Earth will be required to support optimal plant growth in space. Once this relationship is determined, the scientific community will be able to focus their efforts on a diverse array of research questions without concern for superimposed complications relating to unknown variations in water/nutrient delivery rates. In short, we wish to quantify the shift in the water delivery algorithm used to support plant growth in microgravity in order that we can optimize the root environment for growing plants in space.

Prototype Porous Tube Insert Module (PTIM) Hardware

A Porous Tube Insert Module (PTIM) prototype apparatus (Figure 1) that approximates the unit that will fly in space (Wells *et. al*., 2000; Burtness, *et. al*., 2002) has been fabricated and used for ground studies in which dry wheat seeds (*Triticum aestivum* cv Yecora rojo) have been automatically imbibed and germinated. As described below, this prototype unit can operate under either: (1) a moisture sensor feedback control mode, or (2) a programmable fixed feed mode. Typically, 20-24 seeds are glued to capillary mats that wrap around water input tubes within each experimental treatment. The tubes receive water from one end and are closed off at the opposite end. The three Substrate Nutrient Delivery System (SNDS) compartments (on the right in Figure 1) are typically filled with 1-2 mm Turface™ (a calcined montmorillonite clay) which contains 5 g/L of slow release Osmocote™ fertilizer pellets (NPK = 14-14-14). The experiments are conducted under anticipated flight conditions of 23º C, 95% RH for the first 48 h and thereafter 75% RH, 1,500 ppm CO_2, and total darkness for the first 24 h followed by constant light at 185 µmoles m^{-2} s^{-1} (as measured at the top of the PTIM root module tray). Based upon our experiences with this unit, a flight-rated hardware design has been baselined (see next section).

Figure 1. Porous Tube Insert Module (PTIM) Prototype Apparatus. The wheat plants depicted are the result of an 18 day ground experiment. The left half of the apparatus consists of six porous tubes nutrient delivery systems (PTNDS), and the right half consists of three substrate nutrient delivery systems (SNDS). Each PTNDS and SNDS unit is capable of being independently controlled with respect to water provision regime.

Flight-Rated Porous Tube Insert Module (PTIM) Hardware

An overview of the flight-rated PTIM hardware design (i.e., as it will fly in space) follows.

Close-up views of individual Substrate Nutrient Delivery System (SNDS) and Porous Tube Nutrient Delivery System (PTNDS) units are presented in Figures 2 and 3 respectively. Each SNDS compartment will have a cover to prevent particle escape, and there will be perforations on the top, bottom and sides to permit gas exchange through Teflon membranes. Two moisture sensor trunks (not visible in Figure 2) will lie across the top of the substrate compartments. These will have arms protruding downward into the substrate along which the moisture sensors will be situated. Within the PTNDS units, moisture sensors will be incorporated into the construction of the capillary seed mats (not shown in Figure 3). In both cases, the units will be removable via quick disconnects for crew-facilitated harvesting and replanting operations. Figure 4 presents a bottom view of the PTIM base when the three PTNDS and three SNDS units are attached to it.

In Figure 5, the PTIM base can be seen within the four side-walls (two of which are rendered transparent in this depiction). All six experimental treatments are visible. These will be as follows: (1) PTNDS wetness level treatment 1, (2) SNDS wetness level treatment 1, (3) PTNDS wetness level treatment 2, (4) SNDS wetness level treatment 2, (5) PTNDS wetness level treatment 3, (6) SNDS wetness level treatment 3. Thus, there will be three side-by-side pair-wise comparison treatments between the two types of Nutrient Delivery Systems (NDS'). One end-wall can be seen to be populated with 25 (side-imaging) cameras that will be used to document wheat shoot growth rates over the course of the experiment. The opposite end-wall (not visible) will have another 25 cameras. In each case, the arrangement consists of 5 columns of five cameras, with each column looking down the row <u>between</u> one of the PTNDS treatments and one of the SNDS treatments.

Figure 2. Individual SNDS compartment.

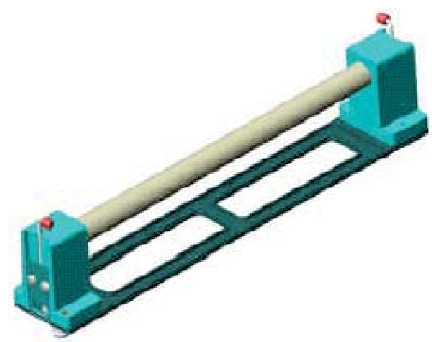


Figure 3. Individual PTNDS unit.

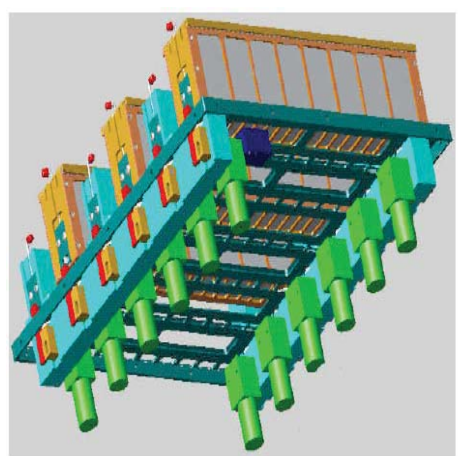


Figure 4. Bottom-up view of PTIM base populated with 3 PTNDS and 3 SNDS units.

Figure 6 depicts a bottom view of the PTIM base with the centrally located two liter reservoir and the four air blowers visible. A bottom-up air flow pattern is generally considered to be more advantageous for plant growth, and it will facilitate the extraction of accurate leaf length data by minimizing the splaying out of the leaves. It should also decrease the risk of poor seed germination results stemming from the drying out of seeds during the imbibition process.

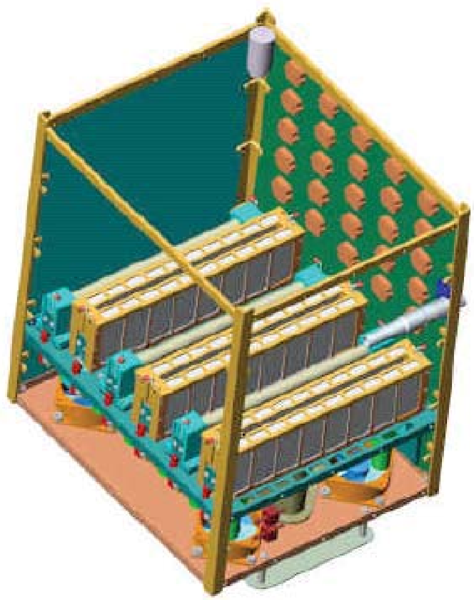


Figure 5. Top-down view of PTIM.

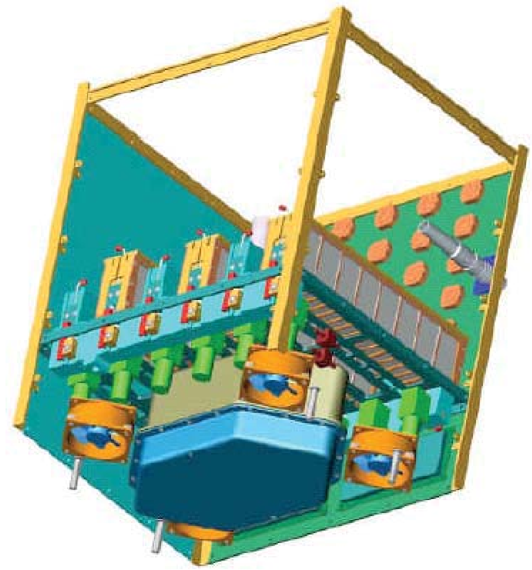


Figure 6. Bottom-up view of PTIM.

Wetness Level Control Strategies

The WONDER payload has been designed to both monitor and control root zone moisture level based upon output from heat pulse moisture sensors (Figure 7; Levine *et. al*., 2002). While this technology has been around since the 1930's, the most recent implementation has been developed by Orbital Technologies Corporation (Orbitech) under the NASA SBIR program with special reference to space flight applications. These sensors have the advantages of being small in size and exhibiting a better uniformity in response relative to earlier implementations of this technology.

In operation they are supplied with a fixed voltage for ca 10 seconds. An internal heating element (e.g. resistor) converts the voltage into heat and an adjacent temperature sensing device (e.g. RTD) monitors temperature changes. The change in temperature (delta T) between the initial (pre-heating) and final (after 10 seconds of heating) conditions is used in conjunction with moisture probe calibrations to determine the Relative Water Content (RWC) of the surrounding substrate. Conceptually, the wetter the substrate surrounding the sensor the faster the generated heat is dissipated away from the sensor and the lower the final (post-heating) temperature achieved. Therefore, higher delta T's are indicative of dryer conditions.

Figure 7. TMAS Heat Pulse Moisture Sensors. Grids = 1 cm.

Readings from six heat pulse sensors situated within the PTIM substrate compartments (at 2-6 cm depths) are averaged to provide the moisture values used for control purposes. Prior to use in the PTIM, each sensor is calibrated in the 1-2 mm Turface substrate at RWC levels of 0%, 100% and several points in-between. Once initiated, the PTIM command program injects 12 mL to fill the porous tubes followed by 340 mL to saturate the substrate contained within each of the three Substrate Compartments (SCs). Water is delivered sequentially at a rate of 100 $\mu L\ s^{-1}$. The program then reads the sensors at hourly intervals. If the moisture level is low, the program calculates the volume of water required to bring the moisture level up to set-point and feeds that volume in. If the moisture level is high, the program allows the substrate compartments to dry out through evaporation. For the example given in Figure 8, set-points for SC A, SC B and SC C were 65%, 75% and 85% (RWC) respectively.

Figure 8 presents the overall sensor-averaged values for the heat-pulse moisture sensors situated within each of the three SCs. For SC A (blue line), all sensors reflected an initial fully-saturated condition which slowly dried down to the experimental set-point (65%) by day 7. For SC B (red line), the pattern is similar, with the set-point value (75%) being achieved slightly earlier (ca day 6 plus 8 hours). For SC C (black line), the 85% set-point was achieved about day 4. It can be seen that the initial dry down pattern was similar between the three compartments and that control was well-maintained for this 11 day interval.

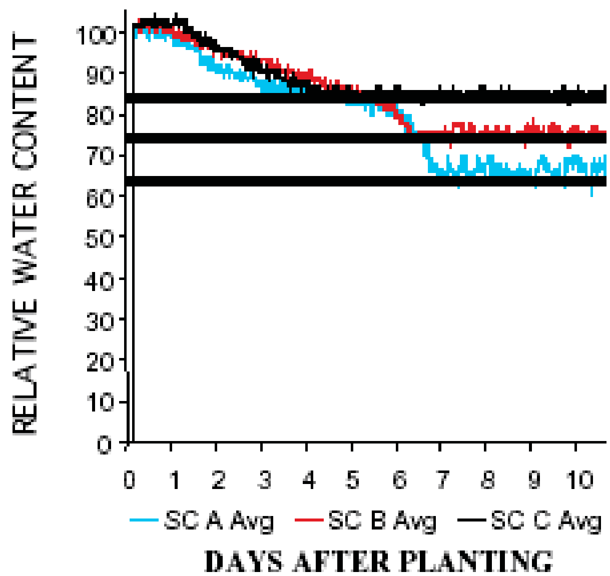


Figure 8. Set-point maintenance using moisture sensor feedback control.

We have also been developing a fixed feed water delivery scenario for use in WONDER. The fixed feed approach functions as a back-up water delivery system that can be implemented in the event that the primary approach (based upon the moisture sensor feedback control strategy described above) fails. Alternatively, if WONDER flies as an ISS payload, sequential experimental runs become possible, in which case the fixed feed mode may be implemented for assessment (as described below). We are developing different water delivery algorithms that predict how water usage rates change as the plants grow. As an example, we present the water usage algorithm depicted in Figure 9, which was empirically generated under conditions of 185 μmoles $m^{-2}\ s^{-1}$; 16:8 L/D; 23^{o} C; 75% RH; 1,500 ppm CO_2. The change in rate of water loss (in mL per hour) is fairly linear up through day 24, after which water usage rates decrease as vegetative growth ceases. At this time the plants are partitioning all of their energy reserves into seed development.

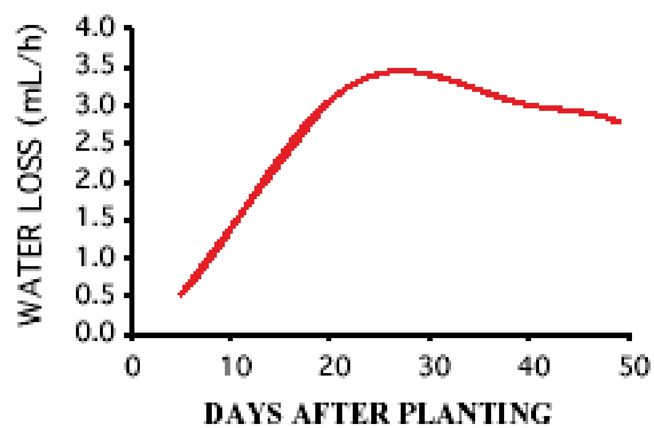


Figure 9. Substrate compartment water loss algorithm for a 50 day growth interval.

In addition to knowing how much water to inject on a daily basis, there's value in determining how often the water should be injected, i.e., what are the merits of alternative water delivery scheduling regimes. For instance, if a water delivery rate of 4 mL/h is called for (= 96 mL/day), all 96 mL could be delivered at one time, or 48 mL could be delivered at 12 h intervals, 24 mL at 6 h intervals, or 1 mL every 15 minutes. One of the key implications of these different scheduling options is the effect they each have on the substrate's dry-down profile. It may be that when there is a significant dry-down interval the ability of oxygen to permeate into the substrate is enhanced. This could be a key characteristic under microgravity conditions where the elevated moisture profile within the substrate (due to the absence of a gravitational force pulling it downward) may act to block oxygen penetration into the root zone.

Alternative Experimental Designs

We present below four alternative options as examples of the types of experimental strategies that become possible with an ISS payload.

***Option 1*: Three Sequential Short Duration (21-24 Day) Experiments:**

Each of the three experiments given below can be both initiated and harvested on-orbit by the crew. Harvest operations include some combination of freezing and/or chemical fixation activities. Alternatively, Ex. 3 could be harvested by the principal investigator's science team at landing.

Ex. 1: PTNDS = 3 Moisture Sensor controlled Wetness Levels (constant set-point maintenance)
SNDS = 3 Moisture Sensor controlled Wetness Levels (constant set-point maintenance)

Ex. 2: PTNDS = 3 Algorithm controlled Wetness Levels (constant set-point maintenance)
SNDS = 3 Algorithm controlled Wetness Levels (constant set-point maintenance)

Ex. 3: PTNDS = 3 *Refined* Algorithm controlled Wetness Levels (constant set-point maintenance)
SNDS = 3 *Refined* Algorithm controlled Wetness Levels (8/d set-point reestablishment*)
* This refers to an 8 times per day water delivery regime, i.e., every 3 hours.

***Option 2*: One Middle Duration (30-60 Day) Experiment:**

In this scenario, the experiment is initiated on-orbit by the crew 30-60 days prior to the expected time of landing. The science team harvests the tissues at landing (minimizing crew-time requirements). Flexibility exists in terms of crew initiation since study duration need not extend to the length of time required for seed production.

***Option 3*: One Short Duration (21-24 Day) Plus One Middle Duration (30-60 Day) Experiment:**

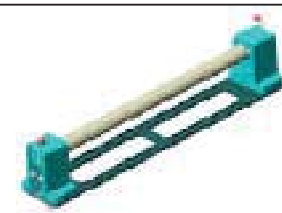
Ex. 1: The first experiment will be of a 21-24 day duration as described above in ***Option 1 Ex 1***.
Ex. 2. The second experiment will be of a 30-60 day duration as described above in ***Option 2***.

***Option 4*: One Long Duration (75-90 Day) Seed-To-Seed Experiment:**

In this scenario, the experiment is initiated on-orbit by the crew and allowed to proceed for 75-90 days (a full increment). The science team harvests the tissues at landing (minimizing crew-time requirements). Either the original set-point design (***Option 1 Ex. 1***) or one of the modified strategies as given in ***Option 1 Exs. 2 & 3*** can be used. This alternative may incorporate: (a) sampling of experimental plants over time, (b) a one time harvest on-orbit, or (c) a bring-them-back alive approach with harvest operations conducted by the science team at landing.

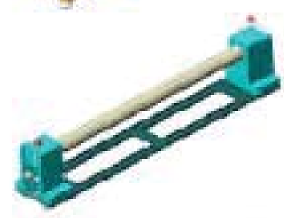
Figure 10 presents a diagrammatic representation of the various experimental design options as described above. For any one experimental run there can be three PTNDS plus three SNDS treatments. A final decision has yet to be made on exactly what the wetness level set-points will be, but they are operationally defined at this time as being either low, medium or high. Any of the six treatments can be based upon using the moisture sensor feedback control set-point maintenance strategy (Figure 10 Option 1), or the fixed-feed algorithm set-point maintenance strategy (Figure 10 Option 2). For the latter, either a constant set-point maintenance strategy or a periodic set-point reestablishment strategy (e.g. 8 times per day) can be implemented.

PTNDS Treatment #1: Low Wetness Level Set-Point
Option 1: Moisture Sensor Feedback Set-Point Maintenance
Option 2: Fixed Feed Algorithm Set-Point Maintenance
Constant Set-Point Maintenance Strategy



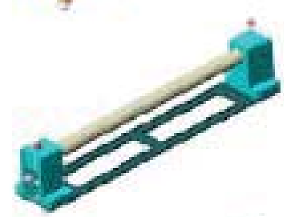
SNDS Treatment #1: Low Wetness Level Set-Point
Option 1: Moisture Sensor Feedback Set-Point Maintenance
Option 2: Fixed Feed Algorithm Set-Point Maintenance
Constant Set-Point Maintenance Strategy
8 Times Per Day Set-Point Reestablishment

PTNDS Treatment #2: Medium Wetness Level Set-Point
Option 1: Moisture Sensor Feedback Set-Point Maintenance
Option 2: Fixed Feed Algorithm Set-Point Maintenance
Constant Set-Point Maintenance Strategy



SNDS Treatment #2: Medium Wetness Level Set-Point
Option 1: Moisture Sensor Feedback Set-Point Maintenance
Option 2: Fixed Feed Algorithm Set-Point Maintenance
Constant Set-Point Maintenance Strategy
8 Times Per Day Set-Point Reestablishment

PTNDS Treatment #3: High Wetness Level Set-Point
Option 1: Moisture Sensor Feedback Set-Point Maintenance
Option 2: Fixed Feed Algorithm Set-Point Maintenance
Constant Set-Point Maintenance Strategy



SNDS Treatment #3: High Wetness Level Set-Point
Option 1: Moisture Sensor Feedback Set-Point Maintenance
Option 2: Fixed Feed Algorithm Set-Point Maintenance
Constant Set-Point Maintenance Strategy

Figure 10. Overview of Experimental Design Options.
PTNDS = Porous Tube Nutrient Delivery System. SNDS = Substrate Nutrient Delivery System.

Conclusions

1) The Porous Tube Insert Module (PTIM) is capable of scientifically assessing the effects of alternative wetness level set-points on plant growth utilizing both porous tube and substrate-based nutrient delivery approaches.
2) Fifty miniature color cameras image the plant specimens throughout the experiment, permitting real-time assessments of plant performance over time.
3) Heat pulse moisture sensors are used to both monitor and control root zone wetness levels.
4) A fixed-feed water delivery algorithm mode is available which meters out appropriate levels of water based upon plant life cycle stage.
5) Alternative water delivery scheduling regimes are also capable of being assessed.
6) Several experimental strategies suitable for implementation on the ISS are discussed.

Acknowledgements

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