The Influence of Microgravity on Plants

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Arabidopsis thaliana
Primary Plant Model Species
Eventually … ➔ Moon & Mars ➔ Planetary Surface Bases ➔ Become More Complex Over Time
Launching Food Makes Less Sense as Mission Duration

Providing Food, O₂ & H₂O in space = costly

Currently ➔ via stowage & resupply

As Mission Durations ➔ Costs ➔

Therefore:
Long-Term Missions ➔ Plants = Key ALS Components ➔
recycle wastes, remove CO₂, purify water & produce O₂ & Food

Understanding BOTH Science & Engineering Issues ➔ Critical for Success

Waste Integration

- food
- water
- oxygen
- CO₂
- urine
- feces
- Graywater
- Nutrients & CO₂
- Inedible material & O₂
Some findings from NASA Ground Testing

Recirculating Hydroponics

1) Conserve Water & Nutrients
2) Eliminate Water Stress
3) Optimize Mineral Nutrition
4) Facilitate Harvesting

High Light & CO₂ Produce High Yields

1) Wheat: 3-4x World Record
2) Potato: 2x World Record
3) Lettuce: Exceeded Commercial Yields
KSC Biomass Production Chamber (BPC)
(W. Knott, J. Sager, R. Wheeler, et. al)
NASA has funded university researchers to perform cultivar comparisons (wheat, potato, soybean, lettuce, sweetpotato, tomato) and develop strains appropriate for spaceflight applications.

**Utah State University**
- Super Dwarf Wheat
- Apogee Wheat
- Perigee Wheat
- Super Dwarf Rice

**Tuskegee University**
- ASP Sweetpotato
Substrate-Based & Porous Tube Nutrient Delivery Systems for Space

A. Cross section view of a porous tube (T. Dreschel).
B. Exploded View of porous tube components.

WCSAR Plant Tray with Wet (Left) and Dry (Right) Porous Tubes prior to Substrate addition.

A slice has been made along the root mass revealing the porous tube beneath (H. Levine).

Orbitec Plant Tray with Porous Tubes and Substrate containing slow release Nutrient Pellets.
Simulated Water Distribution in Svet Root Zone
(S. Jones & D. Or)

At 1G ➞ Gravity drains Water to Field Capacity

⇒ Vertical Moisture Gradation
⇒ Top = Dryer
⇒ Bottom = Wetter

At µG ➞ Water is held near the Water Delivery Tubes by Capillary Forces

⇒ Excessively Wet in Middle
Water Flow within Substrate Pores is Altered in Microgravity
(S. Jones and D. Or)
Psychological Value of “Salad Machines” (Vegetable Production Units)
# Targets for Plant-Related Life Support Applications

<table>
<thead>
<tr>
<th>Mission</th>
<th>Plant Contribution</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISS</td>
<td>- Dietary Supplement</td>
<td>Salad Machine Electric Lighting</td>
</tr>
<tr>
<td>Transit Vehicles</td>
<td>- Dietary Supplement</td>
<td>Salad Machine Electric or Direct Lighting?</td>
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<tr>
<td>Planetary Surface (Near-Term)</td>
<td>- ~5-10% Food Prod.</td>
<td>Large Garden System Electric Lighting or Small Greenhouses</td>
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<tr>
<td></td>
<td>- ~100% Water Processing</td>
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<tr>
<td>Planetary Surface (Mid-Term)</td>
<td>- ~50% Food Prod</td>
<td>Intermediate Greenhouse Suplmt. Electric Lighting</td>
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<tr>
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<td>- ~100% O₂ Production</td>
<td></td>
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<tr>
<td></td>
<td>- ~100% Water Processing</td>
<td></td>
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<tr>
<td>Planetary Surface (Far-term)</td>
<td>- 90% Food Prod.</td>
<td>Large Greenhouse Suplmt. Electric Lighting</td>
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<tr>
<td></td>
<td>- ~100% O₂ Production</td>
<td>Nuclear Power ?</td>
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<tr>
<td></td>
<td>- ~100% Water Processing</td>
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</table>
Ability to grow plants in space = improved

- but many fundamental processes of plant adaptations to spaceflight environments
  - just beginning to be understood

- These issues relate to both the **direct effects** of microgravity on plant development & physiology

- and **indirect effects** of space environments

- tightly closed atmospheres
  - accumulate VOCs

- poor water & air movement through rooting media

- elevated radiation levels

- spectral effects of electric lighting systems, etc.

"Direct" vs "Indirect" Effects of Spaceflight on Plants

Introduction of Ambient Ethylene to MIR.

Ethylene (and VOC) scrubbing ➔ critical for successful seed production (SVET Studies).
Key Questions

How does the gravity environment shape/alter the way plants grow and reproduce?

- **G** → directs Root & Stem Growth
- **G** → requires Gravity Perception
- **G** → Transfer of Info
- **G** → Sites of Reaction
- **G** → Reaction to Signal by Cells

NASA-sponsored studies

- Identifying G-Perceiving Cells
- Threshold Values
- How G-vector is Perceived

Growing plants within the Astroculture plant chamber on ISS.
Types of Plant Tropisms

**Gravitropism**
- Shoot Gravitropism
  (Negative = Away from Gravity)
- Root Gravitropism
  (Positive = Toward Gravity)

**Phototropism**
- Shoot Phototropism
  (Positive = Toward Light)

**Hydrotropism**
- Root Hydrotropism
  (Positive = Toward Water)

\(\mu G \Rightarrow\) used to investigate & clarify G-obfuscated phenomena (e.g. Phototropism)

The fundamental knowledge gained through these investigations aids in our ability to better control plant use on earth in agriculture (and other) applications.
Model of Plant Gravity Perception

Lost \rightarrow Wind & Rain \rightarrow Crops Fall Over \rightarrow Complicate Harvest
Plants Recovery = Gravity Response \rightarrow Reorient Shoot Growth \rightarrow Upright

Discovering Underlying Mechanisms \rightarrow allow develop of crop plants + stronger & faster G-responses

Root growth downward is key to their being able to locate and take up water.
Plants store food (starch) in Amyloplasts = involved G-Perception (left).

Amyloplasts = Denser than Cytoplasm ➞ Fall to Lower Cell Surfaces

Starchless Mutants ➞ respond sluggishly to G
   ➞ therefore Amyloplasts involved but not absolutely critical for G-Response
Effects of Microgravity on Plant Secondary Metabolism

Secondary Metabolism affected by altered G (both hypo- and hypergravity)

Brassica rapa → ISS Grown & Fixed (23)
- Nominal Growth & Leaf Chlorophyll & Starch & Soluble Carbohydrates
- [Glucosinolate] ↑75% greater in μG vs GC

μG Grown Seeds
- Altered Biochemical make-up vs GCs
  - [Chlorophyll] & [Starch] & [Soluble CH₂O]
  - [Protein]
  - Storage Reserve Deposition

μG Grown Seed Embryos
- Embryos at a range of developmental stages
- vs GC embryos = uniformly @ a single stage of development.

Therefore: Spaceflight environment influences metabolite production in ways that may affect flavor and nutritional quality of potential space produce.

Classes of Secondary Metabolites

<table>
<thead>
<tr>
<th>Class</th>
<th>Example Compounds</th>
<th>Example Sources</th>
<th>Some Effects and Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>NITROGEN-CONTAINING</td>
<td>Alkaloids</td>
<td>nicotine, cocaine, theobromine</td>
<td>interfere with neurotransmission, block enzyme action</td>
</tr>
<tr>
<td></td>
<td>Glucosinolates</td>
<td>sinigrin, cabbage, relatives</td>
<td></td>
</tr>
<tr>
<td>TERPENOIDS</td>
<td>Monoterpenes</td>
<td>menthol, linalool</td>
<td>mint and relatives, many plants, interfere with neurotransmission, block ion transport, anesthetic</td>
</tr>
<tr>
<td></td>
<td>Sesquiterpenes</td>
<td>pentalenic acid, Priterium and relatives</td>
<td>contact dermatitis</td>
</tr>
<tr>
<td></td>
<td>Diterpenes</td>
<td>gossypol</td>
<td>cotton, block phosphorylation, toxic</td>
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<tr>
<td></td>
<td>Triterpenes, cardiac glycosides</td>
<td>digitoxigenin, Digitalis (foxglove)</td>
<td>stimulate heart muscle, alter ion transport</td>
</tr>
<tr>
<td></td>
<td>Terpene polymers</td>
<td>carotene</td>
<td>many plants, antioxidant, orange coloring</td>
</tr>
<tr>
<td></td>
<td>Sterols</td>
<td>spinasterol, spinach</td>
<td>interfere with animal hormone action</td>
</tr>
<tr>
<td></td>
<td>PHENOLICS</td>
<td>Phenolic acids</td>
<td>coffee, oregano, all plants, cause oxidative damage, browning in fruits and wine</td>
</tr>
<tr>
<td></td>
<td>Coumarins</td>
<td>umbelliferone</td>
<td>carrots, parsnip, cross-link DNA, block cell division</td>
</tr>
<tr>
<td></td>
<td>Lignans</td>
<td>paeonol</td>
<td>mayapple poison ivy, cathartic, vomiting, allergic dermatitis</td>
</tr>
<tr>
<td></td>
<td>Flavonoids</td>
<td>anthocyanin, catechin</td>
<td>almost all plants, flower, leaf color, inhibit enzymes, anti- and pre-oxidants, estrogenic</td>
</tr>
<tr>
<td></td>
<td>Tannins</td>
<td>gallotannin, condensed tannin, oak, hemlock trees, birdfoot trefoil, legumes</td>
<td>bind to proteins, enzymes, block digestion, antioxidants</td>
</tr>
<tr>
<td></td>
<td>Lignin</td>
<td>lignin, all land plants</td>
<td>structure, toughness, fiber</td>
</tr>
</tbody>
</table>


Long-Term Space Exposure To Seeds

MIR μG Tomato Seeds (6 years) (3)

⇒ Significant Differences in Growth (Yield) and Development (Fertility plus Structure of Cell Walls, Chloroplasts and Mitochondria) relative to GCs.

⇒ DNA variations relative to the ground controls (4).

Short-Term Space Exposure To Seeds

Chinese Space Research (5-9)

⇒ Claimed Genetic Mutants from Plants/Seeds flown in Short Duration Space Experiments.

This conflicts with results obtained by American and Russian investigators (10-13).

Why?
The use of Spaceflight to study Endogenous Plant Movements

**Additional Plant Patterns (14-15):**
Circumnutation & Negative Thigmotropism

G ➔ Amplifies minute oscillatory movements
   ➔ Circumnutations (16)
   ➔ Movements still occur in µG

µG ➔ New Facets of Leaf Movements (17)
   ➔ Ultradian Patterns
   ➔ Effects of Transitions to Darkness or Light
   ➔ Several Heretofore Unknown Movements

TROPI Experiment (18)
   ➔ Gravitropism:Phototropism Interactions
   ➔ European Modular Cultivation System

This Research greatly benefits by the use of microgravity to eliminate one of the parameters (gravity) obfuscating results in earth-based experiments.
Pollen and Seed Development in Space (M. Musgrave et. al.)

Can Plants carry out normal reproductive processes in space?

Plants in $\mu G$ $\Rightarrow$ fertilization & early seed development require CO$_2$ enrichment & air-exchange (19, 20)

**HOWEVER:**
There are still differences attributable to the spaceflight environment (21, 22).
- $Brassica$ seeds and pollen produced in $\mu G$ $\Rightarrow$ physiologically younger than GCs
- Speculation: $\mu G$ limits mixing of the gaseous microenvironments inside the closed tissues and that the resulting gas composition surrounding the seeds and pollen retards their development.
Enhanced Root Production vs GCs (24-28)

Physiological Basis
Spaceflight-Associated Artifact
(more even distribution of moisture in root zone?)

Arabidopsis thaliana ➔ Germinated & Fixed in µG (30)
Root Cortical Cells Proliferated at a Higher Rate
Possibly the result of an Accelerated Cell Cycle

Medium Samples Extracted from the Root Zone (29)
2X Difference Between the Final [K] vs GC

Physiological Basis
Spaceflight-Associated Artifact
(↑ Root Production ➔ More K Uptake)
Ultrastructure Results

Do \( \mu G \)-Grown Plants exhibit alterations in ultrastructure?

Mitochondria in Root Statocytes of \( \mu G \) Soybean Seedlings (31)
  \( \Rightarrow \) Round / Oviform \( \bigoplus \) Low Electron Density of Matrix

Mitochondria in Root Statocytes of GC Soybean Seedlings
  \( \Rightarrow \) Polymorphic Shape \( \bigoplus \) Higher Matrix Electron Density

\( \mu G \) Grown Soybeans (32)
  \( \Rightarrow \) Changes in Vascular Structure
  \( \Rightarrow \) Speculation
  \( \Rightarrow \) Orientation of microfibrils and their assembly in developing vessels are perturbed by \( \mu G \) at the beginning of wall deposition, while they are still able to orient and arrange in thicker and ordered structures at later stages of secondary wall deposition.
How does the Microgravity Environment affect Root Cell Structure? (R. Moore et. al.)

**TEM of Corn Cortical Root Cell on Earth**

**TEM of Corn Cortical Root Cell from μG**

Starch Storage in Amyloplasts Dominates

Amyloplasts contain less Starch and an abundance of Oil Droplets.

The space environment somehow disrupts normal carbohydrate metabolism.
Effects of Spaceflight on Mitosis and Chromosome Behavior
(A. Krikorian et al.)

Daylily Plants flown in $\mu$G as Somatic Embryos

Chromosomal Aberrations ($\mu$G Grown Daylily)

Left = Normal Daylily Plant
Right = Daylily derived from Space-Exposed Embryos

Unanswered Question:
Was the chromosomal damage observed due to $\mu$G or some other aspect of the space environment.

Upper Left: Normal Metaphase in GC Plants
Upper Right: Structurally Perturbed $\mu$G Chromosomes
Lower Left & Right: Deteriorated/Fractured Chromosome that signify serious damage to the integrity of the cell's genetic material. Cells as badly damaged as these would not survive to divide again.
Gene Expression Results

µG Grown Plants

- Usually + Significantly Altered Gene Expression (1)

Some µG-altered genes

- Related to Heat Shock
- yet Not easily Explained by Exposure to Elevated Temperatures (2)

Some µG Grown Plants (PESTO)

- Not in Agreement
  (µG Patterns = GC Patterns)

More Work Needed

- Tease Apart Why the regulation of certain genes are altered by spaceflight conditions.
μG → ± Altered Photosynthesis

Whole Canopy Calculation → Net Photosynthesis & Evapotranspiration Rates & Water Use Efficiency = GCs (33)

However even though single leaf measurements showed no differences in photosynthetic activity at moderate (up to 600 micromol m\(^{-2}\) s\(^{-1}\)) light levels, there was reduction in whole chain electron transport (13%), PSII (13%), and PSI (16%) activities observed under high (saturating) light & CO\(_2\) conditions (34).

Early study (35) μG wheat plants exhibited CO\(_2\) - saturated photosynthetic rates at saturating light intensities that declined 25% relative to GCs.

Also: Using thylakoids isolated from μG -grown plants light-saturated photosynthetic electron transport rate from H\(_2\)O through photosystems II and I was ↓ 28%

Therefore: Photosynthetic functions are affected by the space environment.
The use of plants for space-based life support presents multiple challenges, and there are numerous aspects of plant adaptation to spaceflight and closed environments that are not yet fully understood.

The ISS provides the opportunity to solve many of these issues, especially given the availability of new hardware that can provide more precise environmental control and sustain larger plants for multiple production cycles.

The solving of these challenges will be critical for the establishment of long-term extraterrestrial colonies that will become practical only when plant-based bioregeneration is utilized.
Thank you for your attention.

Questions?
References

(1) Salmi ML, Roux SJ. Gene expression changes induced by space flight in single-cells of the fern Ceratopteris richardii. Planta. 2008 Sep 20. [Epub]


