Growing Plants for NASA – Challenges in Lunar and Martian Agriculture

Fred T. Davies Jr., Chuanjiu He, Ron Lacey and Luis Cisneros

Dept of Horticultural Sciences
Texas A&M University
College Station, Texas
Humans Have Always Wanted to Fly!
"Are you sure about this Stan? It seems odd that a pointy head and long beak is what makes them fly."
Humans Have Always Had the Urge and Spirit of Adventure

⇒ Some 500 years ago (around the time of Columbus), common knowledge was that the earth was flat and sailing ships & their crews would fall to their deaths.

⇒ 105 years ago (Dec 17, 1903) in Kitty Hawk, N.C., the Wright Brothers first successful powered air flight (37 m in 12 sec –clocked 48 kph in a 32 kph tailwind).

⇒ Pushing the Envelope – The “Right Stuff”.

Chuck Yeager broke the sound barrier (Mach 1 -1086 kph) in Bell X-1 jet in October 1947.
39 Years Ago Man Landed on the Moon: Apollo 11 - July 1969
Near-Term Opportunities for Plants in Space—Intl. Space Station
Plants for Human Life Support

Food

(CH₂O) + O₂ → CO₂ + H₂O

Clean Water → Waste Water

Humans

Metabolic Energy

PLANTS

Light

(CH₂O) + O₂ → CO₂ + H₂O

Clean Water ← Waste Water
Chemical & Physical Systems

Space Station Regenerative ECLSS
Flow Diagram (Current Baseline)
KSC Focus
Large Systems, Staple Crops
Onion cv. Comparison—HPS vs. CWF Lighting

- *Allium fistulosum*
- Eight cultivars
- NFT culture
- 42 days
- 23°C
- 16 h light / 8 h dark
- 1200 ppm CO₂
- 300 μmol m⁻² s⁻¹ PPF
Crop Growth Under LED Lighting

Light Emitting Diodes

+ good electric efficiency
+ long operating life *
+ no IR output
+ no Hg or arc discharge
- narrow spectrum

Red
Photosynthesis
Phytochrome

Blue
Photomorphogenesis
Phototropism
Stomatal Control

Green
Human Vision
Canopy Penetration ?
effect of adding green light
Psychological Effects of Plants in Space?

- Fresh Foods
  - Colors
  - Texture
  - Flavor
- Bright Light
- Aromas
- Gardening Activity
The Problem *(con)*

- Travel to Mars & Return to Earth is a 3-year process, i.e. you can’t cram enough food into a space ship.

- Need to incorporate Biogenerative Life Support Systems (recycling using PLANTS & physical-chemical systems), rather than just depending on “Resupply”.

- Plants also supplement physical/chemical methods of removing Carbon Dioxide (CO$_2$) and producing Oxygen (O$_2$).

- Plants are being used as supplemental food source: “NASA’s Salad Bar program”
The Problem

⇒ Tough Environment for Plants - Lunar & Martian Agriculture

⇒ Atmospheric Pressure: Moon-0; Mars-1/100\textsuperscript{th} earth’s.

⇒ Gravity: Moon –1/6\textsuperscript{th}; Mars 2/5\textsuperscript{th} earth’s.

⇒ Day length: Moon 29.5 days—14.8 days in dark Mars-24 hr 39min.

⇒ Mars –Atmosphere is 95% CO\textsubscript{2}; ½ light as on earth.

⇒ No carbon on Moon (needed for photosynthesis).

⇒ Light- can be captured, but will need to be produced artificially.
Solar Collectors for Crop Production

Buried Plant Growth Chambers
The Currency of Space

- **POWER** – Energy costs to produce power, i.e. generating light for plants

- **MASS** – **WEIGHT** – very expensive to ship stuff into Space;

- **Vacuum of space makes large transport structures difficult to build**
Plant Growth at Sub-Ambient Atmospheric Pressures

Advantages of Low Pressure System

⇒ Less structure needs to be shipped into space.

⇒ Less gas leakage from low pressure crop production to vacuum of Moon or near vacuum of Mars.

⇒ Crew could tend crops without suiting up.

⇒ Won’t have to ship or produce as much Nitrogen gas
ALS Candidate Crop Testing--KSC

Mars Greenhouse Concepts

Increased gaseous diffusion
Gas Composition

- Provided by pressurized N₂, CO₂ and O₂ cylinders.

**Perfect Gas Law:** PV = nRT
- P = pressure; V = volume; n = moles; T = temperature

<table>
<thead>
<tr>
<th>Total Pressure (kPa)</th>
<th>pN₂ (kPa)</th>
<th>pO₂ (kPa)</th>
<th>pCO₂ (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>80</td>
<td>21</td>
<td>100</td>
</tr>
<tr>
<td>25</td>
<td>13</td>
<td>12</td>
<td>100</td>
</tr>
</tbody>
</table>
Low Pressure Plant Growth (LPPG) System

- 6-chambered, clear acrylic, modular LPPG system which uses weak-acid electrolyte oxygen sensors, non-dispersive infrared CO₂ sensors and a pressure transducer.

- Controls the partial gas pressures of oxygen, nitrogen and carbon dioxide from 20 kPa to 200 kPa (101 kPa = ambient).

- Has cross flow heat exchanger to control humidity and condensation.

- Changes in CO₂ tracked during the light and dark periods on a whole canopy basis.
Research Objectives

• Characterize the influence of hypobaric (low pressure) conditions on plant growth of lettuce (Lactuca sativa).

• Characterize influence of hypobaric conditions on plant gas exchange

• Separate the effects of hypobaria and hypoxia (low oxygen).
**CO₂ Assimilation & Dark-Period Respiration**

- 10-day study; 25kPa/12kPa pO₂; 101kPa (ambient)/21kPa pO₂
- Setpoint of 100 Pa pCO₂ during light period.
Fig. 7.
Fig. 5

Partial Pressure of O₂ (pO₂)

Dark-Period Respiration (CO₂ Pa min⁻¹)

Day 1

Day 5

Day 10
Fig. 8
### Effect of Total Pressure and Partial Pressure of Oxygen (pO$_2$) on Lettuce Plant Growth, Chlorophyll and Relative Water Content (RWC)

<table>
<thead>
<tr>
<th>Total pressure (kPa)</th>
<th>pO$_2$ (kPa)</th>
<th>Leaf area (cm$^2$)</th>
<th>SLA (cm$^2$ g$^{-1}$)</th>
<th>Leaf DM (g)</th>
<th>Root DM (g)</th>
<th>Total plant DM (g)</th>
<th>Chl (µg. cm$^{-2}$)</th>
<th>RGR (mg.g$^{-1}$. day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>6</td>
<td>857</td>
<td>44.5</td>
<td>2.7</td>
<td>0.4</td>
<td>3.2</td>
<td>42.1 ± 1.7</td>
<td>0.18</td>
</tr>
<tr>
<td>12</td>
<td>1190</td>
<td>50.1</td>
<td>3.6</td>
<td>0.8</td>
<td>4.4</td>
<td>39.9 ± 0.5</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>1349</td>
<td>54.5</td>
<td>3.5</td>
<td>0.8</td>
<td>4.3</td>
<td>37.3 ± 0.5</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>101</td>
<td>6</td>
<td>832</td>
<td>37.6</td>
<td>2.3</td>
<td>0.3</td>
<td>2.7</td>
<td>37.8 ± 1.4</td>
<td>0.16</td>
</tr>
<tr>
<td>12</td>
<td>1172</td>
<td>49.9</td>
<td>3.4</td>
<td>0.8</td>
<td>4.2</td>
<td>37.8 ± 0.7</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>1250</td>
<td>54.2</td>
<td>3.5</td>
<td>1.0</td>
<td>4.4</td>
<td>34.2 ± 0.7</td>
<td>0.22</td>
<td></td>
</tr>
</tbody>
</table>

**Signif.**
- Pres.(P) O$_2$:
  - NS
  - ***
- P × O$_2$:
  - NS
  - NS

- **10-day studies; seedling transplants 20-days old.**
- **Setpoint of 100 Pa pCO$_2$ during light period.**
A. 25/12 pO$_2$
B. 101/21 pO$_2$
C. 25/12 pO$_2$
D. 101/21 pO$_2$

Day 5
Day 10

A. 25/12 pO₂

B. 101/21 pO₂

C. 25/12 pO₂

D. 101/21 pO₂
Summary

• Growth was comparable between low (25kPa) and ambient (101kPa) pressure lettuce plants during 10-day studies.

• Low pO$_2$ (6 kPa) reduced plant growth compared to 12 and 21 kPa pO$_2$. (oxidative phosphorylation limited)

• Low pO$_2$ (6 kPa) caused greater growth reduction & stress with ambient (101 kPa) than low (25kPa) pressure plants — trend in lower SLA, leaf + total plant DM

• Leaf chlorophyll was higher at 25 than 101kPa; RWC was unaffected by total pressure or pO$_2$. 
**Summary (con)**

- 25/12 kPa pO$_2$ had comparable CO$_2$ assimilation and 25% lower dark-period respiration than 101/21 kPa pO$_2$ (ambient) plants.

- Greater efficiency of CO$_2$ assimilation/dark-period respiration (ratio) with low pressure plants (6 kPa pO$_2$). [↑diffusion rate]

- Hypobaria $\neq$ Hypoxia.

  (Paul et al., 2004; less half genes up-regulated or down-regulated; response to hypobaria is unique)

  (Richards et al., 2006; no effect 5 photorespiratory enzymes – Rubisco; hypobaria – no altered regulation photorespiratory pathway)
**Elevated Ethylene Levels**

- Elevated levels of ethylene occur in CEA and microgravity-spaceflight environments, leading to adverse plant growth & sterility (Wheeler et al., 1996; Bugbee, 1999; Stutte, 1999).

- Russian Space Station Mir: ethylene ranged from 1000 to 1700 nmol mol$^{-1}$ (ppb) (Campbell et al., 2001).

- International Space Station (ISS): 50 nmol mol$^{-1}$ (ppb) ethylene.
Research Objective

- Characterize influence of ethylene on plant gas exchange (CA, DPR) and growth of lettuce (*Lactuca sativa*) under ambient (101 kPa) and hypobaric (25 kPa) conditions
### Effect of Total Atmospheric Pressure and Ethylene [(C$_2$H$_4$), either scrubbed or endogenously accumulated] on CO$_2$ Assimilation (CA), Dark-Period Respiration (DPR) and the CA /DPR ratio

<table>
<thead>
<tr>
<th>Total Pressure (kPa)</th>
<th>C$_2$H$_4$ Treatments</th>
<th>C$_2$H$_4$ level in Chamber (nmol mol$^{-1}$)</th>
<th>CA (CO$_2$ Pa min$^{-1}$)</th>
<th>DPR (CO$_2$ Pa min$^{-1}$)</th>
<th>CA /DPR Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>Accumulated</td>
<td>1119$^a$</td>
<td>2.45$^b$</td>
<td>0.50$^{bc}$</td>
<td>4.85$^b$</td>
</tr>
<tr>
<td></td>
<td>Scrubbed</td>
<td>11$^b$</td>
<td>3.40$^a$</td>
<td>0.62$^a$</td>
<td>5.46$^{ab}$</td>
</tr>
<tr>
<td>25</td>
<td>Accumulated</td>
<td>936$^a$</td>
<td>2.30$^b$</td>
<td>0.46$^c$</td>
<td>5.03$^{ab}$</td>
</tr>
<tr>
<td></td>
<td>Scrubbed</td>
<td>8$^b$</td>
<td>3.12$^a$</td>
<td>0.53$^b$</td>
<td>5.83$^a$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Significance</th>
<th>Pressure (Pres)</th>
<th>NS</th>
<th>NS</th>
<th>**</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C$_2$H$_4$</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Pres x C$_2$H$_4$</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>
### Effect of Total Atmospheric Pressure and Ethylene [(C\textsubscript{2}H\textsubscript{4}), either scrubbed or endogenously accumulated]; Lettuce (*Lactuca sativa* L cv. Butter Crunch)

<table>
<thead>
<tr>
<th>Pres (kPa)</th>
<th>C\textsubscript{2}H\textsubscript{4} Treatment</th>
<th>Leaf area (cm\textsuperscript{2})</th>
<th>SLA (cm\textsuperscript{2} g\textsuperscript{-1})</th>
<th>Leaf DM (g)</th>
<th>Root DM (g)</th>
<th>Total Plant DM (g)</th>
<th>RGR (mg g\textsuperscript{-1} d\textsuperscript{-1})</th>
<th>RWC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>Accumulated</td>
<td>211\textsuperscript{b}</td>
<td>52.3\textsuperscript{b}</td>
<td>6.04\textsuperscript{b}</td>
<td>0.97\textsuperscript{b}</td>
<td>7.02\textsuperscript{b}</td>
<td>0.173\textsuperscript{b}</td>
<td>94.2</td>
</tr>
<tr>
<td></td>
<td>Scrubbed</td>
<td>297\textsuperscript{a}</td>
<td>56.4\textsuperscript{a}</td>
<td>7.31\textsuperscript{a}</td>
<td>1.22\textsuperscript{a}</td>
<td>8.53\textsuperscript{a}</td>
<td>0.195\textsuperscript{a}</td>
<td>94.7</td>
</tr>
<tr>
<td>25</td>
<td>Accumulated</td>
<td>230\textsuperscript{b}</td>
<td>52.9\textsuperscript{b}</td>
<td>6.19\textsuperscript{b}</td>
<td>0.98\textsuperscript{b}</td>
<td>7.17\textsuperscript{b}</td>
<td>0.178\textsuperscript{b}</td>
<td>94.1</td>
</tr>
<tr>
<td></td>
<td>Scrubbed</td>
<td>321\textsuperscript{a}</td>
<td>56.6\textsuperscript{a}</td>
<td>7.42\textsuperscript{a}</td>
<td>1.14\textsuperscript{a}</td>
<td>8.56\textsuperscript{a}</td>
<td>0.201\textsuperscript{a}</td>
<td>94.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Significance</th>
<th>Pres</th>
<th>NS</th>
<th>NS</th>
<th>NS</th>
<th>NS</th>
<th>NS</th>
<th>NS</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C\textsubscript{2}H\textsubscript{4}</td>
<td>***</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>***</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Pres x C\textsubscript{2}H\textsubscript{4}</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>
A  Ethylene Accumulated
B

C  Ethylene Scrubbed
D

101 kPa

25 kPa
Summary

- Ethylene reduced $C_A$, DPR and plant growth of both ambient and hypobaric plants.

- Negative, linear correlation of increasing ethylene up to 1000 nmol mol$^{-1}$ (ppb).

- Hypobaria had no significant effect on endogenous ethylene production.
**Phytochemicals in Plants**

- Phytochemicals in plants can act as anti-carcinogenic and antioxidant compounds - neutralizing free radicals for protection DNA, lipids, proteins from oxidative damage (Morris et al, 2002)).

- Phytochemical-rich crops are relevant for long-term exploration & habitation – astronauts exposed to ionizing cosmic radiation – oxidative stresses & increased carcinogenesis.
## Effect of Oxygen Stress on Phytochemicals & Nutritional Quality of Lettuce ‘Red Sails’

<table>
<thead>
<tr>
<th>Oxygen Pressure (kPa)</th>
<th>Leaf dry mass (g)</th>
<th>Anthocyanin conc (mg g(^{-1}))</th>
<th>Total Phenolic conc (mg g(^{-1}))</th>
<th>Ratio of Anthocyanins to Phenolics</th>
<th>Free** Radical Scavenging Activity (µmol g(^{-1}))</th>
<th>Carbohydrate conc (mg g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>5.2 a</td>
<td>2.2 b</td>
<td>32.0 b</td>
<td>6.8 b</td>
<td>208 b</td>
<td>178.4 b</td>
</tr>
<tr>
<td>12</td>
<td>5.2 a</td>
<td>3.5 a</td>
<td>36.8 ab</td>
<td>9.3 a</td>
<td>252 a</td>
<td>197.4 b</td>
</tr>
<tr>
<td>6</td>
<td>4.5 a</td>
<td>4.2 a</td>
<td>39.0 a</td>
<td>10.5 a</td>
<td>265 a</td>
<td>288.3 a</td>
</tr>
</tbody>
</table>

**Antioxidant activity – DPPH frsa in Trolox equivalents (Yamaguchi et al, 1998).**

(Rajapaske, He, Cisneros-Zevallos, Davies, unpublished).
101/6 kPa pO2 ‘Red Sails’ lettuce, reduced biomass but greater anthocyanin pigmentation.

101/6 pO2 (last 3 days) – last 3 days hypoxia – maintained biomass, greater anthocyanin pigmentation.
Hypoxia (Oxygen Stress)

- Enhanced anthocyanin levels, total phenolic compounds, carbohydrate levels and free radical scavenging capacity.

- Oxygen Stress induces production of protective pytochemicals in plants -- enhanced nutritional & functional value.
Identify Selected Andean Crops Grown at Higher Altitude with Greater Tolerance to Low Pressure (Hypobaria, Hypoxia) – Abiotic Stress

- Low fertility & chemical inputs, high vitamins, micronutrients, starch & CHO

- Potential to manipulate for enhanced nutrition & phytochemical production.
- **Ahipa**
- **Arracacha (Arracacia)** - young stems – salads, cooked veg,; starchy root,
- **Maca (Lepidium)** – 4400 m, immune system; capsules of dehydrated roots;
- **Mashua (Tropaeolum)** – isothicyanates, companion plant with potatoes; flavor?
- **Mauka (Mirabilis)** – succulent, edible stems; roots high in CHO, protein; rediscovered 1965
- **Oca (Oxailis)** – tubers, hardier than ulluco -4100 m.
- **Ulluco (Ullucus)**, edible, cooked leaves high protein, Ca, carotene; tubers.
Acknowledgements

- Ron Lacey
- Chuanjiu He
- Luis Cisneros
- N. Rajapaske
- Denise Brown
- Jody Worthington
- Leslie Scheuring

NASA- NAG-9-1067 — Plant Growth and Metabolism at Sub-Ambient Atmospheric Pressures.

NASA- NAJ04HF31G — Plant Growth at Sub-Ambient Atmospheric Pressures with Control of the Partial Pressures of Constituent Gases.

http://aggie-horticulture.tamu.edu/faculty/davies/research/nasa.html