

## MIXED VS. MONOCULTURE HYDROPONIC PRODUCTION OF SALAD CROPS AT THREE CO<sub>2</sub> CONCENTRATIONS

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### ABSTRACT

Development of cropping systems for use on the International Space Station, long duration transit missions, and lunar or Mars habitats has been a part of NASA's Advanced Life Support (ALS) research efforts for many years. Growth of multiple crops on a common solution will increase efficiency, but may result in allelopathic responses and decreased yields. Three candidate ALS salad crops, radish (*Raphanus sativus* L. cv. Cherry Bomb II), lettuce (*Lactuca sativa* L. cv. Flandria) and bunching onion (*Allium fistulosum* L. cv. Kinka) were grown hydroponically in either a monoculture (control) or mixed cropping (MC) arrangement in a walk-in growth chamber at three CO<sub>2</sub> concentrations (400, 1200 and 4000  $\mu\text{mol mol}^{-1}$ ) to determine if allelopathic responses were occurring. The chambers were maintained at 22°C, 50% RH, and 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPF with a 16-h light/8-h dark photoperiod using cool-white fluorescent lamps (17.3  $\text{mol m}^{-2} \text{d}^{-1}$  PAR).

### INTRODUCTION

NASA's Advanced Life Support (ALS) program has studied a variety of salad crops for dietary supplementation on short-term space missions and as a component of bioregenerative life support systems on long-term missions (1). In addition to the physiological and nutritional benefits of providing fresh food for a crew on long duration space missions, positive psychological benefits may be achieved as well (2). A salad crop growing system or "salad machine" on ISS and early Mars missions would require optimal use of limited area (volume) and electrical power. A MC arrangement could provide a more efficient use of resources, a greater satisfaction in dietary variety, a greater crop biomass production per unit of growth area, and a greater overall yield stability through crop diversity. However, MC could also have a negative impact through competition for nutrients, the production of allelopathic compounds (3), or a more complicated mineral nutrition and irrigation management routine. In developing a MC system with a common root and aerial environment, it is critical to understand the crop interactions under a range of environmental conditions. Lettuce, onions, and radishes are being grown under baseline environmental conditions that would be encountered aboard the ISS to determine if mixed cropping impacts their growth and yield (4, 5 and 6).

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## MATERIALS AND METHODS

### Environmental Parameters

All experiments were conducted in 48 ft<sup>2</sup> (4.5 m<sup>2</sup>) walk-in controlled environment chambers at Kennedy Space Center's Controlled Environment Laboratory. Lighting was provided from VHO cool white fluorescent (CWF) lamps cycled to provide a 16-h light/8-h dark photoperiod.

Daily light integrals were maintained at 17.3 mol m<sup>-2</sup> d<sup>-1</sup> (300 μmol m<sup>-2</sup> s<sup>-1</sup> PAR) of photosynthetic photon flux (PPF) as measured by a handheld quantum sensor (model LI-250A, Li-COR, Lincoln, NE, USA).

Air temperature was maintained at a constant 22± 0.2°C for both light and dark cycles. Relative humidity was adjusted to 50% to simulate the relatively dry environments of spacecraft. Carbon dioxide was maintained at 400, 1200, or 4000 μmol·mol<sup>-1</sup> (0.04, 0.12, or 0.4 kPa).

### Plant Cultural Conditions

Seeds were planted in plastic tray inserts (0.30 m<sup>2</sup> growing area) that were inserted in NFT hydroponic trays. Three of the trays were planted as monoculture controls of radish, lettuce and onion in the following manner: Radish (*Raphanus sativus* L. cv. Cherry Bomb II) and lettuce (*Lactuca sativa* L. cv. Flandria) were double seeded into evenly spaced holes in 0.3 m<sup>2</sup> plastic growing trays. Green onion (*Allium fistulosum* L. cv. Kinka) was seeded by evenly dispersing 250 seed between 14 - 1 X 50 cm parallel slits in a 0.3 m<sup>2</sup> plant support insert. The other three trays were planted as MC with the three species combined in different arrangements (R, O, L; O, L, R; and L, R, O). Each hole was fitted with a nylon Nitex® wicking material to support germination and growth (5, 6). Plant support inserts were then placed in 0.3 m<sup>2</sup> hydroponic trays. Seeds were sprayed with distilled water twice daily and covered with translucent covers for 5 days. After 5 days, covers were removed.

### Nutrient Management

Plants were grown hydroponically using NFT (nutrient film technique) with a recirculating modified ½ strength Hoagland's solution (7). The flow rate was adjusted to 1 L·min<sup>-1</sup>·tray (~3L min<sup>-1</sup> m<sup>-2</sup>). The pH was automatically controlled to 5.8 with 0.4 M nitric acid. Evapotranspirative water loss was measured and replaced daily for 35 days to maintain the constant 12 L of nutrient solution per reservoir. The electrical conductivity (EC) was maintained at 120 mS m<sup>-1</sup> via manual daily additions of a concentrated stock of Hoagland's nutrient solution (8). Root zone temperatures were maintained at the chamber air temperature by cooling the nutrient solution reservoir with a submersed stainless steel cooling coil containing recirculating chilled water.

## RESULTS AND DISCUSSION

### Mixed Vs. Monoculture Cropping Method

In lettuce (Table 1), both edible and total DM plant<sup>-1</sup> was equal to or higher than control in MC at 1200 and 4000 μmol mol<sup>-1</sup> CO<sub>2</sub>. Harvest index (HI) was lower in MC plants at all three CO<sub>2</sub>

levels, due to an increase in root mass. Radish (Table 2) grown in MC were not significantly different than the control in edible DM, total DM or Harvest Index (HI) at any CO<sub>2</sub> level. Mixed cropping of onion (Table 3) significantly reduced total plant DM at 400 and 1200 µmol mol<sup>-1</sup> CO<sub>2</sub> over the monoculture treatment.

### **CO<sub>2</sub> Effects**

In onion, the only significant difference in growth between CO<sub>2</sub> levels occurred in the edible DM of monoculture plants when the concentration was increased from 400 to 1200 µmol mol<sup>-1</sup>. In both radish and lettuce, edible and total DM increased significantly when CO<sub>2</sub> was increased from 400 to 1200 µmol mol<sup>-1</sup> in both MC and monoculture conditions. The only affect seen in radish grown in either MC or monoculture arrangements between 400 and 4000 µmol mol<sup>-1</sup> CO<sub>2</sub> was a decrease in HI at 4000 µmol mol<sup>-1</sup> CO<sub>2</sub>, due to an increase in root mass.

Increasing CO<sub>2</sub> from 1200 to 4000 µmol mol<sup>-1</sup> in radish resulted in a significant decrease in edible DM and HI for both MC and monoculture plants. For lettuce there was a significant increase in growth between 400 and 1200 µmol mol<sup>-1</sup> with MC and between 400 and 4000 µmol mol<sup>-1</sup>. Increasing concentration of CO<sub>2</sub> from 400 to 4000 µmol mol<sup>-1</sup> resulted in a decrease in HI. Monoculture lettuce plants experienced the same increases in growth between 400 and 1200 µmol mol<sup>-1</sup>, and 400 and 4000 µmol mol<sup>-1</sup>, but the change between 1200 to 4000 µmol mol<sup>-1</sup> CO<sub>2</sub> resulted in a significantly higher HI.

**Table 1:** Growth measurements for lettuce at 28 days in mixed (MC) versus monoculture (Mono) trays at three CO<sub>2</sub> levels. Data represents means, n=24 for MC and n=16 for Mono.

Cropping Method	CO <sub>2</sub> (ppm)	Edible DM (g plant <sup>-1</sup> )	Total DM (g plant <sup>-1</sup> )	Harvest Index (%)
Monoculture (Mono)	400	1.24 (0.11)	1.38 (0.12)	89.44 (0.92)
	1200	1.94 (0.10)	1.98 (0.16)	91.69 (0.62)
	4000	2.02 (0.09)	2.15 (0.10)	94.27 (0.52)
Mixed (MC)	400	1.48 (0.08)	1.68 (0.09)	88.14 (0.38)
	1200	1.92 (0.09)	2.14 (0.10)	89.32 (0.68)
	4000	2.43 (0.10)	2.73 (0.12)	88.99 (0.84)
Contrasts				
	Mono 400 vs. MC 400	ns	ns	ns
	Mono 1200 vs. MC 1200	ns	ns	ns
	Mono 4000 vs. MC 4000	***	**	***
Interaction				
	CO <sub>2</sub> vs. Cropping Method	ns	ns	*
Mono				
	400 vs. 1200	***	**	ns
	400 vs. 4000	***	***	ns
	1200 vs. 4000	ns	ns	*
MC				
	400 vs. 1200	**	**	ns
	400 vs. 4000	***	***	***
	1200 vs. 4000	***	***	ns

<sup>z</sup>ns,\*,\*\*,\*\*\* = non-significant or significant at p<sub>≥</sub> 0.05; 0.01 or 0.001 respectively using ANOVA. Numbers in ( ) are S.E.

**Table 2:** Growth measurements for radish at 28 days in mixed (MC) versus monoculture (Mono) trays at three CO<sub>2</sub> levels. Data represents means, n=24 for MC and n=16 for Mono.

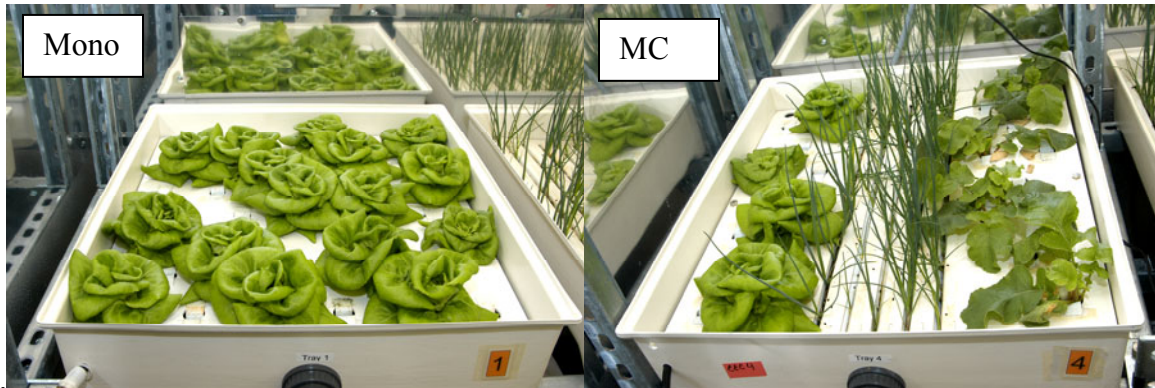
Cropping Method	CO <sub>2</sub> (ppm)	Edible DM (g plant <sup>-1</sup> )	Total DM (g plant <sup>-1</sup> )	Harvest Index (%)
Monoculture (Mono)	400	2.08 (0.11)	3.11 (0.15)	66.6 (1.0)
	1200	3.01 (0.24)	4.36 (0.30)	68.5 (1.4)
	4000	2.07 (0.15)	3.26 (0.19)	63.0 (1.3)
Mixed (MC)	400	2.17 (0.10)	3.29 (0.15)	65.8 (1.0)
	1200	2.80 (0.17)	4.16 (0.21)	66.6 (1.3)
	4000	2.21 (0.09)	3.65 (0.14)	60.4 (0.6)
Contrasts				
		ns	ns	ns
		ns	ns	ns
		ns	ns	ns
Interaction				
		ns	ns	ns
Mono				
		***	***	ns
		ns	ns	ns
		***	***	**
MC				
		**	**	ns
		ns	ns	***
		**	ns	***

<sup>z</sup>ns, \*, \*\*, \*\*\* = non-significant or significant at p<sub>≥</sub>0.05; 0.01 or 0.001 respectively using ANOVA. Numbers in ( ) are S.E.

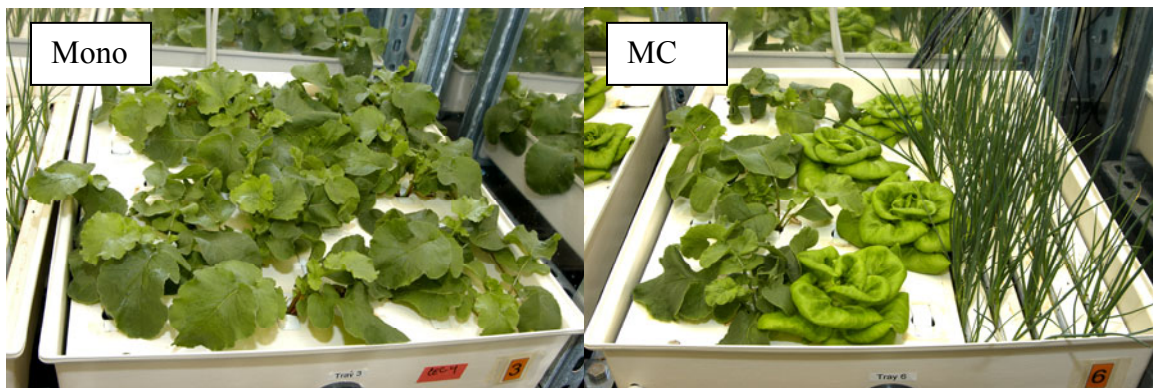
**Table 3:** Growth measurements for onion at 28 days in mixed (MC) versus monoculture (Mono) trays at three CO<sub>2</sub> levels. Data represents means, n=24 for MC and n=16 for Mono.

Cropping Method	CO <sub>2</sub> (ppm)	Edible DM (g plant <sup>-1</sup> )	Total DM (g plant <sup>-1</sup> )	Harvest Index (%)
Monoculture (Mono)	400	0.11 (0.01)	0.13 (0.01)	85.5 (1.38)
	1200	0.15 (0.01)	0.16 (0.01)	90.5 (1.16)
	4000	0.17 (0.01)	0.19 (0.01)	86.8 (2.75)
Mixed (MC)	400	0.12 (0.01)	0.15 (0.01)	85.9 (0.52)
	1200	0.12 (0.01)	0.13 (0.01)	88.9 (0.76)
	4000	0.14 (0.01)	0.17 (0.01)	85.7 (1.38)
Contrasts				
		ns	ns	ns
		**	*	ns
		*	ns	ns
Interaction				
		**	**	ns
Mono				
		*	ns	ns
		***	***	ns
		ns	*	ns
MC				
		ns	ns	ns
		*	*	ns
		***	***	ns

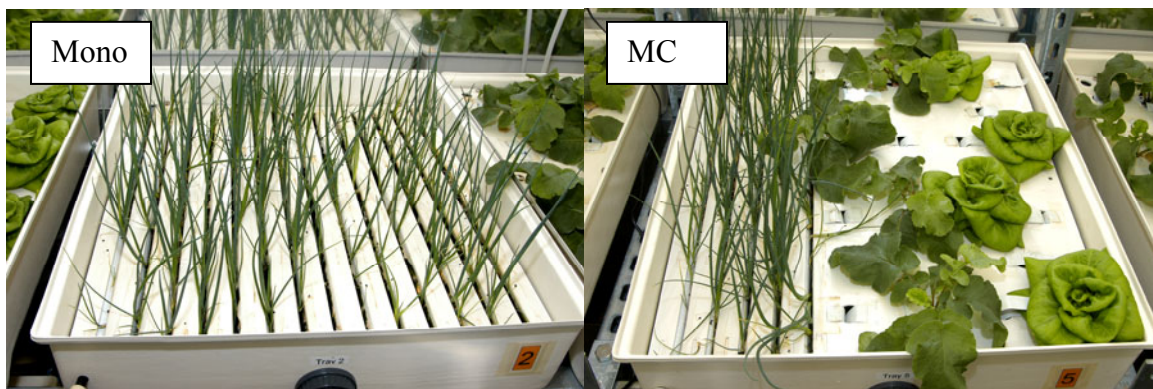
<sup>z</sup>ns, \*, \*\*, \*\*\* = non-significant or significant at p<sub>≥</sub>0.05; 0.01 or 0.001 respectively using ANOVA. Numbers in ( ) are S.E.



**Figure 1.** Lettuce: monoculture tray (left) ,and mixed crop (MC) tray (right) at 28 days



**Figure 2** Radish: monoculture tray (left) ,and mixed crop (MC) tray (right) at 28 days.



**Figure 3.** Onion: monoculture tray (left) ,and mixed crop (MC) tray (right) at 28 days.

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## CONCLUSION

Intercropping of salad crops is one way to increase diversity of vegetables within a small spaceflight growth chamber. Determining if there are any negative effects of this arrangement on species being grown is important in developing the design and horticultural management of such chambers. This series of experiments has shown that for onion, there is no significant difference in the harvest index between plants grown in either a mixed or monoculture arrangement at all three CO<sub>2</sub> levels. For radish, there was no significant difference between the different cropping arrangements at any of the CO<sub>2</sub> levels. Lettuce grown in a mixed cropping arrangement only showed a significant decrease in HI from the monoculture plants at 4000 μmol mol<sup>-1</sup> CO<sub>2</sub>, due to an increase in root biomass.

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