Commercial seed selection and effectiveness of sanitization methods in preparation for plant growth experiments on the International Space Station

By Emma Boehm

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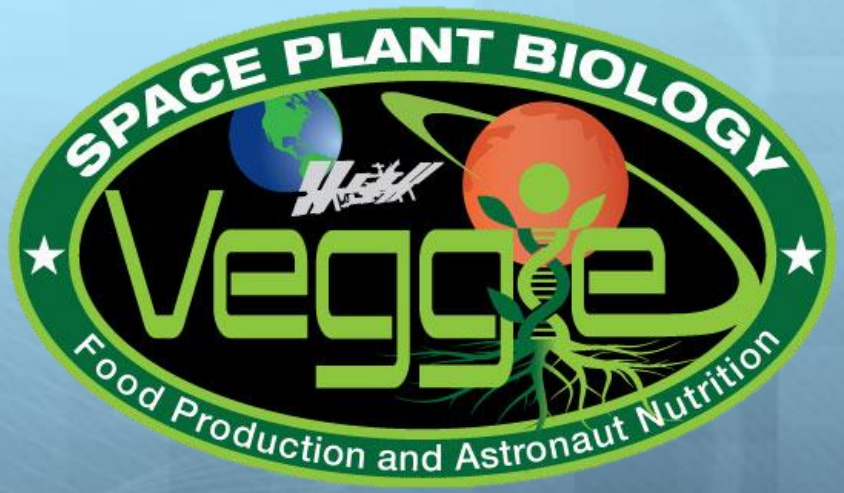
A closed-loop food production system will be important to gain autonomy on long duration space missions. Crop growth experiments in the 'Veggie' plant chamber aboard the International Space Station (ISS) are helping to identify methods and limitations of food production in space. Prior to flight, seeds are surface sterilized to reduce environmental and crew contamination risks.

A combination of hypochlorite fuming (30 mL: 0.5 mL) and 70% ethanol sanitization were used for previous test plants in 'Veggie.' Efficacy of these methods were compared for 'Mizuna' mustard and 'Waldmann's Green' lettuce, candidate crops for future ISS experiments (Veg03-D, E, F). Bleach fuming resulted in cleaner seed surfaces for 'Waldmann's Green' (bleach and ethanol respectively: 1.5 CFU/seed, 5.75 CFU/seed) and 'Mizuna' (bleach and ethanol respectively: 0.25 CFU/seed, 3.5 CFU/seed). Bleach fuming also showed 90%-100% germination whereas ethanol treatment impeded germination (20%-50% germination). The most common microbial genera on seeds surfaces include *Bacillus* and *Staphylococcus*, though no species pathogenic to humans were found. However, the same sanitization procedures failed to sanitize larger seeds like 'Red Robin' tomato and 'Pomepii' pepper. Due to the size of these seeds, more concentrated hypochlorite, additional surfactant, heat, or citric acid might be needed for effective sanitization.

Difference in seed vendors also contributed to variation in the microbial and fungal loads on seeds before and after sanitization as well as general growth. Commercial 'Waldmann's Green' seeds that originally had lower microbial and fungal loads tended to respond better to hypochlorite sanitization. To test seed vendor growth variability, 'Tokyo bekana' Chinese cabbage from four commercial seed vendors were grown in a 1:4 mixture of turface and arcillite combined with Nutricote 18-6-8 T70 slow-release fertilizer. Plants grew in a controlled chamber with conditions modeling average lighting, temperature, relative humidity, and carbon dioxide levels on the ISS. Harvest occurred after 36 days and 4 plants selected to grow to seed were excluded from fresh mass measurements. Johnny's seeds had the largest average leaf count (11 cm), plant height (110 cm), and plant width (30.2 cm), while West Coast seeds had the greatest average fresh mass (36.7 g). All plants showed some level of necrosis and chlorosis, though Johnny's displayed the lowest score for overall damage (1.8). Variability is likely due to genetic and phenotypic conditions of commercial parent crops. For future flight experiments, producing seed specifically selected for advantageous traits in microgravity conditions might reduce microbial and fungal contamination in addition to lessening variability in plant growth.



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Future of Space Exploration

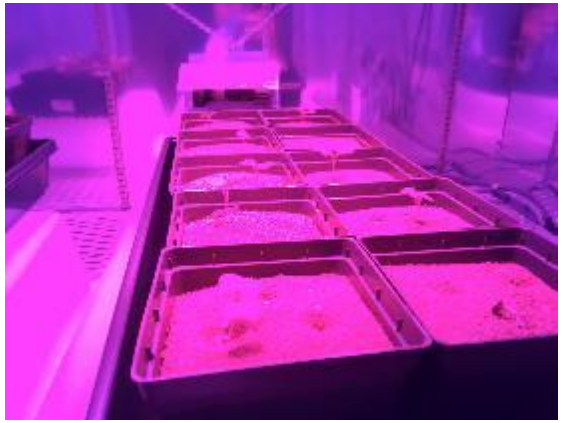
As space exploration expands to deep space and long term missions, crews cannot depend on resupply missions as a food source…

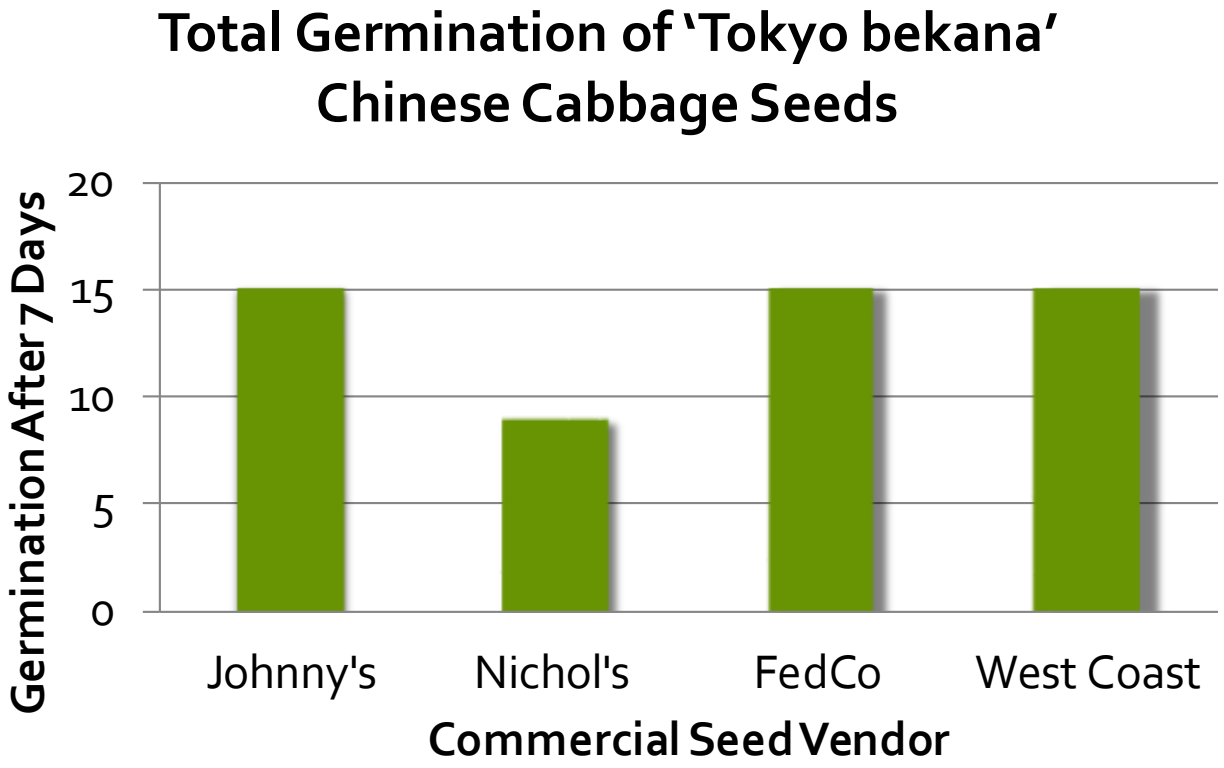
Future of Space Exploration

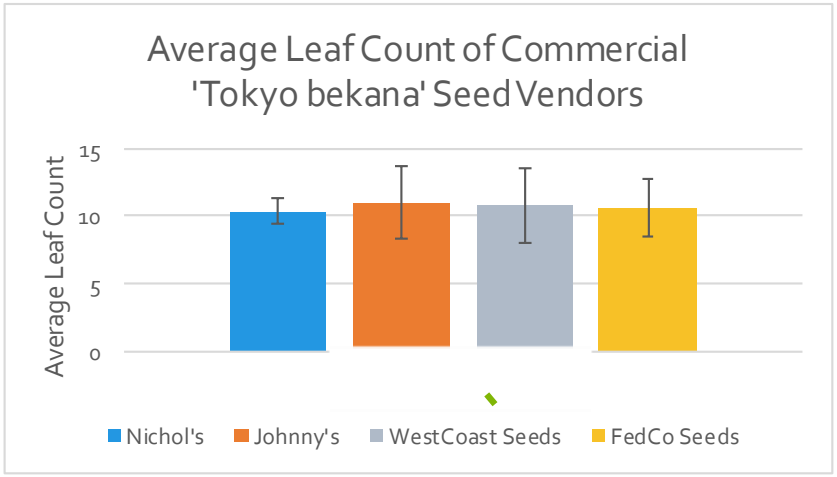
...and will need a closed-loop food production system.

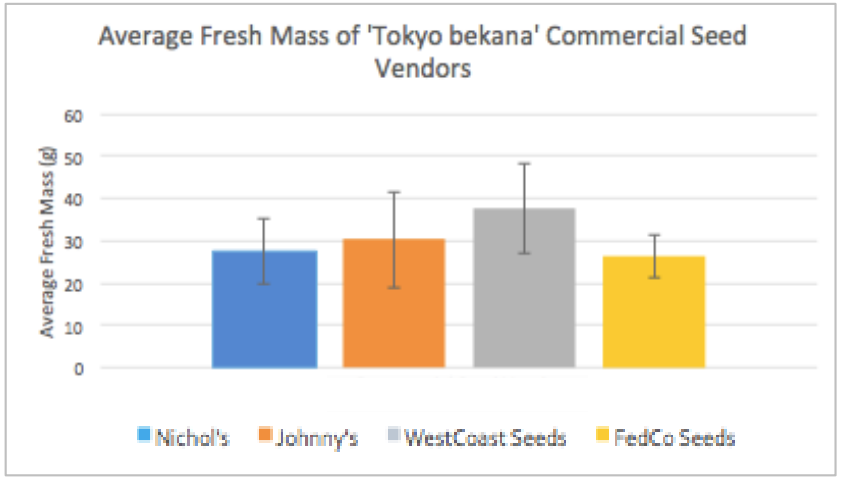
Commercial Seed Vendors

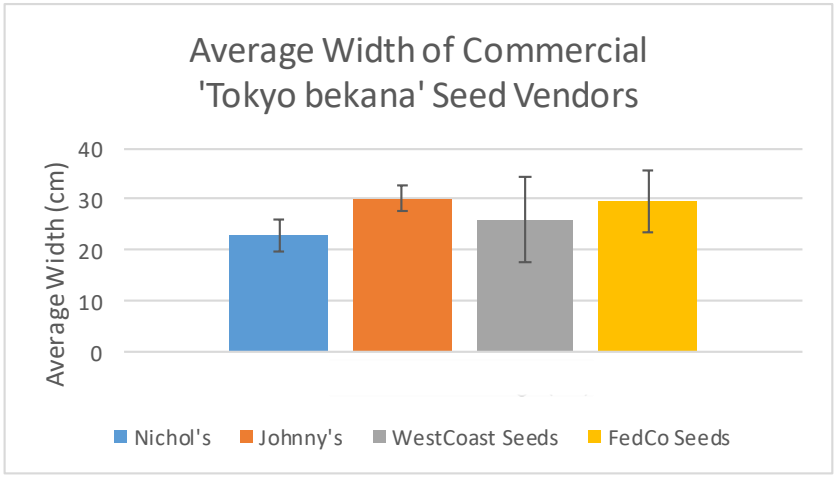
- Seed currently sourced from commercial supply
- Heirloom and organic growers
- Previous disparity in germination, morphology, biomass, health
- Test: 'Tokyo bekana' Chinese cabbage
- Expected result: if seed quality is affected by commercial growing practices, then fitness of the plants will vary among different seed vendors

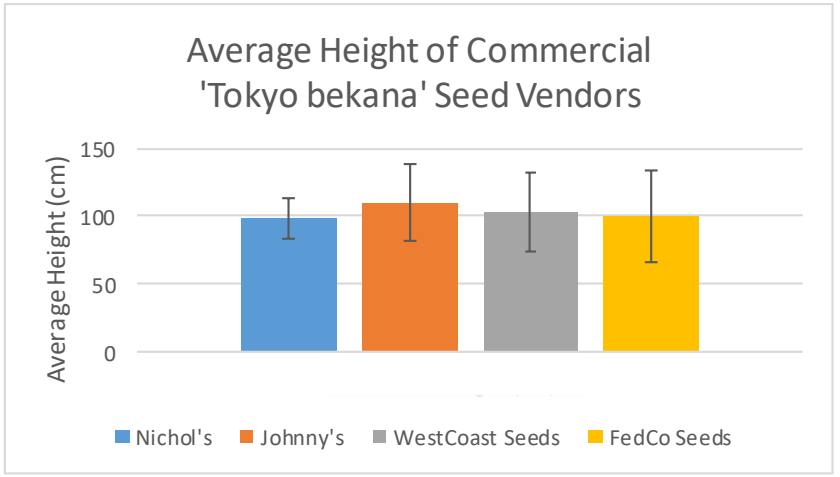



Total Germination of 'Tokyo bekana' Chinese Cabbage Seeds
Germination After 7 Days
20
15
10
5
0
Johnny's
Nichol's
FedCo
West Coast
Commercial Seed Vendor


Average Leaf Count of Commercial 'Tokyo bekana' Seed Vendors
Average Leaf Count
15
10
5
0
Nichol's
Johnny's
WestCoast Seeds
FedCo Seeds

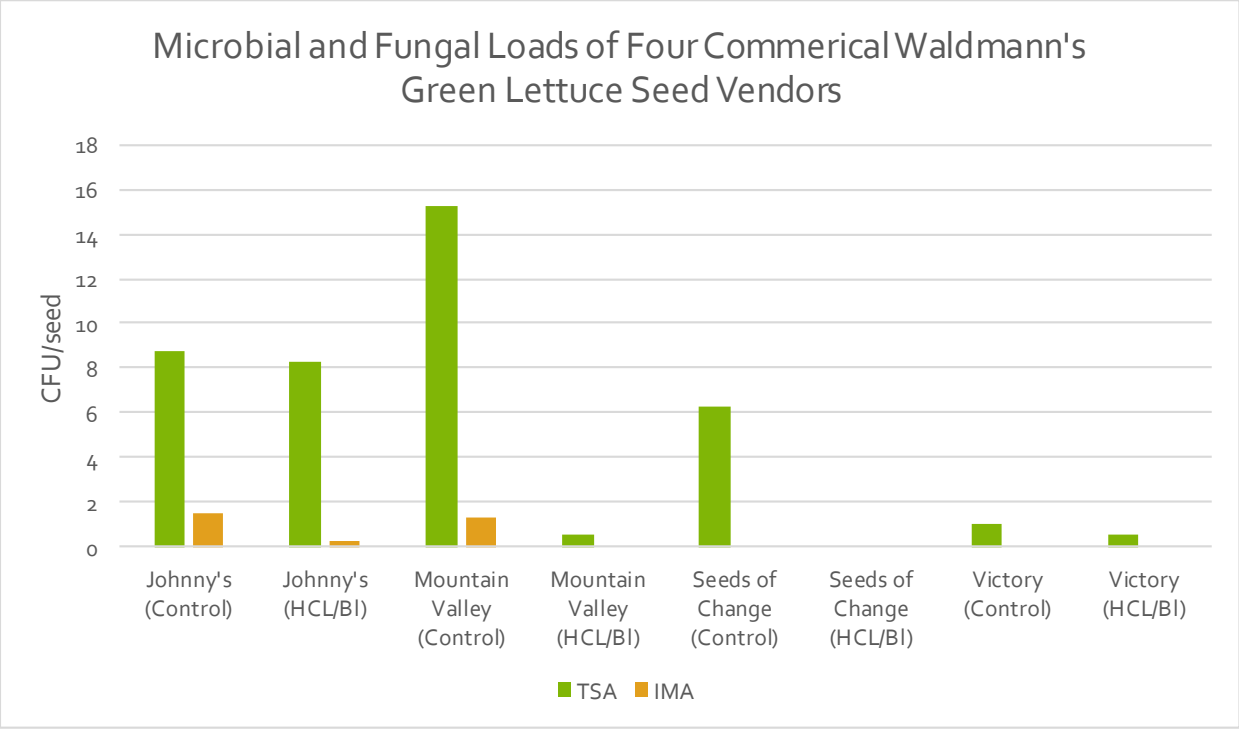

Average Fresh Mass of 'Tokyo bekana' Commercial Seed Vendors
Average Fresh Mass (g)
60
50
40
30
20
10
0
Nichol's
Johnny's
WestCoast Seeds
FedCo Seeds


Average Width of Commercial 'Tokyo bekana' Seed Vendors
Average Width (cm)
40
30
20
10
0
Nichol's
Johnny's
WestCoast Seeds
FedCo Seeds


Average Height of Commercial 'Tokyo bekana' Seed Vendors
Average Height (cm)
150
100
50
0
Nichol's
Johnny's
WestCoast Seeds
FedCo Seeds

Sanitization and Seed Vendor

- 'Waldmann's Green' lettuce

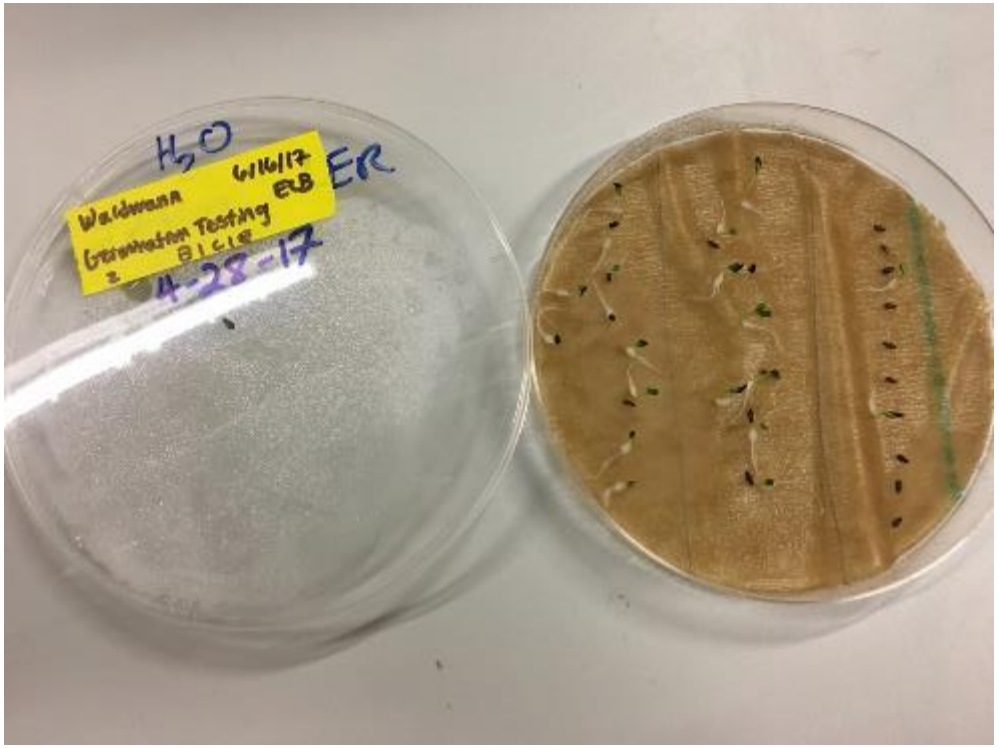


Seed Sanitization Methods

- Methods
  - Bleach + HCl fuming
  - 70% ethanol
- Seeds
  - 'Mizuna' Mustard (*Brassica juncea var. japonica*)
  - 'Waldmann's Green' Lettuce (*Lactuca sativa*)
  - 'Red Robin' Tomatoes (*Solanum lycopersicum var. red robin*)
  - 'Pompeii' Peppers (*Capsicum annuum var. pompeii*)

Germination Testing

- Results:
  - Bleach
  - Control
  - EtOH



Microbial and Fungal Analysis

Microbial

Fungal

With Sanitization

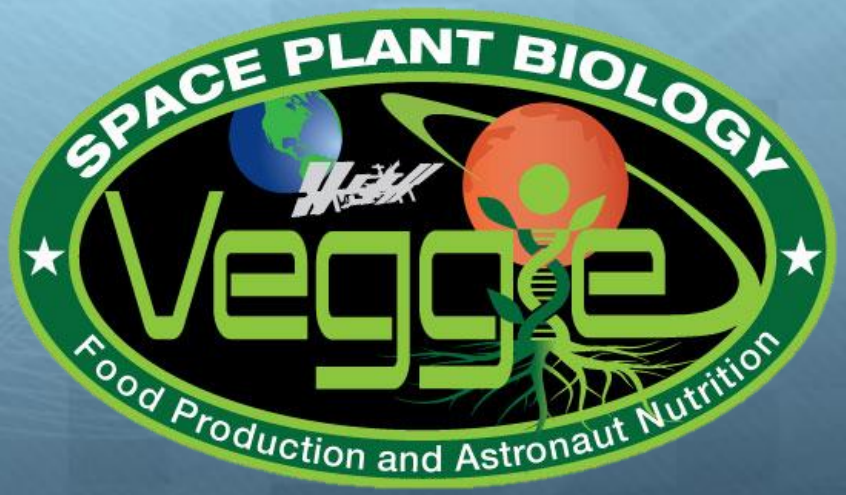
70% Ethanol

30 mL 6% Bleach + 0.5 mL HCl

What survives surface sterilization?

Future Directions

- Testing different sanitization methods
- Complete microbial and fungal analysis of flight seeds
- Effectiveness of sanitization methods
- Producing plants tailored for flight or outer planetary conditions


SPACE PLANT BIOLOGY
Veggie
Food Production and Astronaut Nutrition

Questions?