SEED SPROUT PRODUCTION: CONSUMABLES AND A FOUNDATION FOR HIGHER PLANT GROWTH IN SPACE

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ABSTRACT
Seed sprouts can be produced as a source of fresh vegetable material and higher plant seedlings in space. Sprout production was undertaken to evaluate the mass accumulations possible, the technologies needed and the reliability of the overall process. Baseline experiments corroborated the utility of sprout production protocols for a variety of seed types. The automated delivery of saturated humidity effectively supplants labor-intensive manual soaking techniques. Automated humidification also lends itself to modest centrifugal sprout growth environments. Under unprotected growth conditions, bacterial and/or fungal contamination occurred in about 10% of the growth cups. A small amount of ultraviolet irradiation effectively suppressed the contamination and the sprouts were suitable for consumption. These experiments have provided the foundations for designing an automated sprout production facility for space use. Scaled from the laboratory experiments, the sprout production needs of a crew could be handled in a volume equivalent to that of a mid-deck locker.

INTRODUCTION
Seed sprouts have been seen as an opportunity to produce fresh consumable biomass in zero gravity for use as both a dietary supplement and a source for higher plant seedlings (1, 2). Many technical, methodological and reliability questions are raised when biological systems are considered as major components for space life support systems. Space experience and research with most biological systems has been too limited to provide suitable answers to such questions. Opportunities to remedy this situation, however, do exist. The production of seed sprouts in space is a means to satisfy two NASA needs. Sprouts, from a
variety of seeds, can be used to produce fresh consumables in space. At the same time, sprout production can be used to answer a variety of questions raised regarding the use of biological systems in space to provide life support (3).

Sprout production in space can meet the austere volume and power budgets typical of current space missions. The technology is relatively simple and the production is quite high. Ground-based studies have been undertaken to assess ground production. These studies quantify (1) the amount of biomass accumulated (wet weight) for each of a variety of seeds, (2) the methods through which human interaction and effort can be minimized, and (3) the methods through which contamination (bacterial, fungal) can be minimized. Sprout production and consumption in space would document the utility of biological systems in an overall life support scenario. Suitable measures taken during production would extend our understanding of space environment effects on biological systems and plant germination, particularly. The tests undertaken here allow the specification of design parameters needed for space sprout production.

METHODS
Baseline:
Standard sprout production techniques were used to establish a data base against which a variety of technique modifications could be compared. Seed soaking times and water delivery schedules were evaluated for impact on sprout mass accumulation. Using alfalfa (0.715 g, approximately 300 seeds), mung bean
(2.464 g, approximately 40 seeds), and spicy clover/radish mix (0.924 g, approximately 200 seeds), individual plastic cups (32 ml volume, 3 cm bottom diameter, 4 cm top diameter, 3.3 cm height) were prepared with the indicated seed amounts for these experiments. Small holes (2 mm diameter) penetrated the bottom of the cups to permit draining of excess water. With eight holes in each cup, the bottom of the cup remaining unpenetrated was approximately 6.82 cm². When not being watered, weighed or otherwise treated the cups were maintained in a low light environment at 22-24 degrees C with 60-80% relative humidity levels. Ambient air was not forced to move across the openings to the cups. For soaking, approximately 20 volumes of tap water (22-24 degrees C) were added to the seed volumes: soaking times were evaluated hourly for one to twenty-four hour soak periods. The seeds were weighed prior to and following these soaking periods. Prior to weighing, the excess water was "wicked" from the seed cups using absorbant paper towels. Wicking was permitted for five minutes. After the initial soak period, all seeds were sprayed with 30 ml per cup of distilled water at eight hour intervals. Daily, after three intervening sprayings, all seed cups were "wicked" and weighed. These procedures were followed until the fifth day following initial soaking. At this time, the formation of secondary leaves and a bitter taste factor had begun.

Humidity:
Automatic, controlled humidification was considered as an option for watering that required minimal human interaction. For this
system, all that was required was to fill the humidifier with water daily. The seeds were weighed before soaking as in the above baseline studies using the following amounts of seeds: mung (2.464 g, approximately 40 seeds), alfalfa (0.715 g, approximately 300 seeds), spicy (0.693 g, approximately 150 seeds). A smaller amount of spicy seeds were used in the humidity experiments because, in the baseline studies, it was determined that 200 seeds caused severe crowding in the growing sprouts. Following the initial weighing of the seeds and the cups containing them, all of the seeds were soaked for eight hours. The wicking system used in baseline was employed to drain the seeds after soaking, at which time they were weighed. Then they were placed into a humidity chamber (53 cm x 24 cm x 12 cm) and humidified for the following amounts of time: three hours (0.25 gallons of water delivered daily), six hours (0.5 gallons of water delivered daily), twelve hours (1 gallon of water delivered daily) and twenty-four hours (2 gallons of water delivered daily). The humidity chamber was a cast acrylic box in which the humidity was delivered at the top center portion of the lid. The humidity cycles were determined by an automated timer assembly so that the sprouts would get humidity at regular, predetermined intervals. During the times in the cycle when humidity was not being administered, a fan circulated air (50-70% relative humidity) across the sprouts. For five days, the sprouts were weighed daily after draining. The sprout growth was compared with that achieved in baseline experiments.
Bacterial and Fungal Suppression:
Broad spectrum ultraviolet light was used to suppress the contamination of sprouts with bacteria and fungus. Treatments ranged from 2 minutes to 24 hours daily. Two sources of radiation were used: (1) 12" Sylvania Germicidal Lamps (8W each) at 10" above sprouts and (2) Black Ray UV Lamp using 100W mercury spot bulb at 14" above the sprouts.

RESULTS
Baseline:
After the variations in soaking periods, the seeds weighed at 24 hours after the beginning of soaking showed mass accumulations directly related to soaking times (Fig 1). The accumulated mass appeared to slowly approach asymptotic values as soaking times extended beyond about eight hours. Clearly, however, most mass was accumulated during the first hour of soaking.

Not all of the seed types responded in the same ways to soaking time variations. The alfalfa seeds clearly showed asymptotic accumulations whereas the mung seeds showed less evidence for asymptotic accumulations.

Interestingly, the large differences in seed size (and volume to surface ratios) were not reflected in differential mass accumulation. The three varieties of common sprout seed examined here all showed mass increments of about 200% regardless of soaking times.
When total mass accumulation is plotted for five days, the sprout production continues to reflect slightly higher accumulated masses for those seeds exposed to longer initial periods of soaking (Fig. 2). The effect is most pronounced in spicy seeds and is almost nonexistent in alfalfa seeds. In any event, the spicy seeds yielded an eight-fold accumulation in mass compared to initial starting mass of seeds. Alfalfa accumulations were just below a six-fold level, and mung at approximately a four-fold level.

Humidity:
Provision of water for sprout production can be achieved by direct humidification systems. Such systems eliminate labor intensive steps and assume uniform water delivery. Also, humidification systems are readily automated to handle a variety of water and gas transport requirements of the sprouts.

The results of different humidification delivery schedules are shown in Figs. 3, 4, and 5 for mung, alfalfa, and spicy sprout production, respectively. Baseline values are shown, as well, for comparative purposes.

The mung sprouts (Fig. 3) clearly required almost continuous humidity delivery to assure maximum mass accumulation. It is interesting that 12 and 6 hour humidification times yielded mass accumulations that approached the 24 hour and baseline values on days 4 and 5. Clearly, 3 hours of humidity delivery was insufficient for significant mass accumulation in mung seeds.
Alfalfa sprouts (Fig. 4) were even more dependent on high levels of humidification delivery. Despite the apparent variance in the data, it seems clear that neither 3 or 6 hours of humidification adequately supported alfalfa sprout production.

The spicy seeds (Fig. 5) were similarly dependent on high levels of humidification. Interestingly, both the 12 and 24 hour humidification treatments yielded spicy mass accumulations in excess of those recorded in baseline studies.

Table 1 compares the total mass accumulation of the mung, alfalfa, and spicy sprouts over the whole five day growth period in percent form of the humidity and baseline experiments (as compared with dry seed weight).

The Table 1 baseline and humidification data suggest that, whereas the seed volumes do not have a systematic influence on water uptake during soaking, the volume to surface ratios may be critical to seed drying and thus to overall mass accumulation. The small seeds may benefit, accordingly, more from water delivered by saturated humidification than the large seeds.

Bacterial and Fungal Suppression:
Two kinds of suppression information were sought in using ultraviolet radiation during sprout production. First, what amounts of ultraviolet light were needed to suppress sprout contamination during the five days of production and, second,
what amounts of ultraviolet irradiation would be tolerated by the sprouts during growth such that neither mass accumulations nor taste would be adversely affected.

Under baseline production conditions fewer than 10% of the sprout production cups exhibited either bacterial or fungal contamination. With a single, two minute period of ultraviolet irradiation daily, the contamination either did not develop or was sufficiently suppressed to permit full term sprout production in the absence of contamination levels that would make the sprouts unacceptable for consumption. Shorter periods of irradiation (tested at 30 second intervals) were only partially effective. Longer periods yielded no significant improvements in the observed suppression. It should be noted that no attempt was made to disturb the sprouts during irradiation. Thus, shadowed areas and residual water undoubtedly accounted for decreased ultraviolet effectiveness.

To examine sprout tolerance to ultraviolet irradiation, the sprouts were exposed to continuous ultraviolet light. Such irradiation, at the levels used in the present studies, did not inhibit sprout mass accumulation. Since these sprouts were not grown to mature plants, effects on growth remain to be determined.

These studies demonstrated that modest levels of ultraviolet irradiation readily controlled sprout contamination. The required ultraviolet light levels were two orders of magnitude
less than those demonstrably tolerated by the sprouts. These observations demonstrate an effective means for controlling contamination and, accordingly, the reliability of sprout production in space.

CONCLUSIONS

The baseline studies of sprout production undertaken here show that any of several sprout producing seeds can be used to reliably yield consumable vegetable materials using little space and specialized equipment. A couple of 32 ml cups yield sufficient fresh biomass for a sprout salad. Given the production times of 4-5 days, the daily salad needs of an astronaut might be met with 10 of these small cups and the needs of a crew could be met within the volume of a mid-deck locker. Although the systematic results shown here involve only three seed types, more than 15 types of sprouts have been produced under similar conditions. Thus, a good deal of sprout variety is assured.

To deal with the labor intensive watering demands of sprout production, tests were done using a saturated humidification delivery system that was fully automated. With sufficiently long periods of humidification, the measured accumulation of fresh biomass actually exceeded that produced by conventional sprout production methods.

Finally, the occurrence of contamination raised issues of sprout production reliability. With small amounts of irradiation by
ultraviolet light, the contamination problems were readily suppressed. These treatments were much less intense than those having any observable consequence on sprout production.

The goal of producing fresh biomass can be reached through experimentation using either the humidity chamber and/or baseline methodology. These studies and follow up studies will lay the foundation for sprout production and consumption in space. As indicated, many other seed types are being investigated. Humidity delivery has proven to be effective, and centrifugal systems are being evaluated for use in both soaking cycle needs and in gravitational needs of the spouts. All of these technologies are being developed to serve the two NASA needs cited above: the production of fresh edible biomass and the production of plants in space as a subsystem for CELSS.
BIBLIOGRAPHY

(1) GRAY, STEPHEN W. AND EDWARDS, BETTY F. The Effect of Weightlessness on the Growth and Orientation of Roots and Shoots on Monocotyledonous Seedlings. The Experiments of Biosatellite II: 123-165.


TABLE AND FIGURES

Table 1

<table>
<thead>
<tr>
<th>Hours of humidity</th>
<th>Mung</th>
<th>Alfalfa</th>
<th>Spicy</th>
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<tr>
<td>3</td>
<td>81%</td>
<td>206%</td>
<td>229%</td>
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<tr>
<td>6</td>
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<td>1147%</td>
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<tr>
<td>Baseline results</td>
<td>340%</td>
<td>560%</td>
<td>762%</td>
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(average)

Fig. 1 Graph of 24 Hour Mass Accumulation

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Fig. 1 Linear regression summary of the accumulation of seed mass based on soaking times ranging from 1 to 24 hours in duration. Triplicate sample cups were soaked at each of the one hour intervals. The 72 samples yielded significant correlation coefficients for each of the three regression lines representing alfalfa, mung and spicy seeds, respectively. All data show results at 24 hours.
Fig. 2  Graph of Five Day Mass Accumulation

Dotted Line: Spicy Sprouts  $m = 35$ mg
Solid Line: Alfalfa Sprouts  $m = 14$ mg
Dashed Line: Mung Sprouts  $m = 220$ mg

Fig. 2  Linear regression summary of the accumulation of mass based on soaking times. Conditions as in Fig. 1. Total sprout masses for measurements obtained after five days of baseline treatment.
**Fig. 3** Graph of Mass Accumulation vs. Time

Mung Sprouts

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**Fig. 3** Summary of the accumulation of seed mass based on a soaking time of eight hours and humidity cycles of three, six, twelve, and twenty-four hours with baseline data shown for comparative use. Four sample cups were used for each experiment iteration.

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**Fig. 4** Graph of Mass Accumulation vs. Time

Alfalfa Sprouts

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**Fig. 4** Summary of the accumulation of seed mass based on a soaking time of eight hours and humidity cycles of three, six, twelve, and twenty-four hours with baseline data shown for comparative use. Four sample cups were used for each experiment iteration.
Fig. 5  Summary of the accumulation of seed mass based on a soaking time of eight hours and humidity cycles of three, six, twelve, and twenty-four hours with baseline data shown for comparative use. Four sample cups were used for each experiment iteration.