AMRL-TR-70-121

GERMINATION AND GROWTH OF SELECTED HIGHER PLANTS IN A SIMULATED SPACE CABIN ENVIRONMENT

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MARCH 1971

JOINT NASA/USAF STUDY

AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
Aerospace Medical Research Laboratory Aerospace Medical Div., Air Force Systems Command, Wright-Patterson AFB, OH 45433	UNCLASSIFIED
	2b. GROUP N/A

3. REPORT TITLE

GERMINATION AND GROWTH OF SELECTED HIGHER PLANTS IN A SIMULATED SPACE CABIN ENVIRONMENT

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Final Report (9 April 1970 - 30 August 1970)

5. AUTHOR(S) (First name, middle initial, last name)

Christopher T. Lind, Captain, USAF

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
March 1971	v + 18	11

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 6302	AMRL-TR-70-121
c.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	

10. DISTRIBUTION STATEMENT

This document has been approved for public release and sale; its distribution is unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
Prepared in part under NASA-Defense Purchase Request No. T-91350	Aerospace Medical Research Laboratory Aerospace Medical Div., Air Force Systems Command, Wright-Patterson AFB, OH 45433

13. ABSTRACT

Four species of higher plants including Raphanus sativus, Lactuca sativa, Brassica oleracea, and Capsicum frutescens were exposed to an environment simulating the conditions within the NASA Skylab. Seventy-two hundred seeds and four hundred eighty mature seedlings were placed in altitude chambers for a ten-day period. One chamber was held at 260 mm Hg total pressure (27,000 ft) and a duplicate chamber was held at 725 mm Hg total pressure and served as a control. Both chambers had equal partial pressures of oxygen and carbon dioxide. No significant differences in seed germination or seedling development were apparent between the control and reduced pressure treatments. All species obtained a high germination percentage during the ten-day exposure to the simulated space cabin environment.

Key Words: Skylab
Space cabin environment
Higher plants
Life Support
Altitude exposure
Seed germination
Seedling development

DD FORM 1 NOV 65 1473

Security Classification

FOREWORD

This study was conducted at the Toxic Hazards Division, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio, under Project 6302, "Toxic Hazards of Propellants and Materials." The research was initiated by the Preventive Medicine Division, National Aeronautics and Space Administration Manned Spacecraft Center, Houston, Texas, under NASA Defense Purchase Request T-91350. Dr. Charles H. Walkinshaw, Jr. was the contract monitor for the NASA Manned Spacecraft Center. The study was started in April 1970 and was completed in August 1970.

This technical report has been reviewed and is approved

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Commander
Aerospace Medical Research Laboratory

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

SECTION I

INTRODUCTION

Man's ability to journey into space for extended periods of time has been demonstrated by the Apollo flights and has led the way for longer space missions in NASA project Skylab. This program will utilize three men in an earth orbiting laboratory for as long as two months. At the end of this time, crews may be changed so that the laboratory will continue operation. Future manned space exploration will demand existence in the space cabin environment for greatly extended periods. A Mars mission, for example, will require more than a year in flight time.

To make these journeys, life support systems must be reduced in weight and volume but remain highly reliable. Thus far in the development of space technology, little effort has been made to develop bioregenerative life support systems that utilize multicellular higher plants. Most investigations utilizing biological organisms as components of life support systems have been concerned with algae (Myers 1958, Kraus 1962, Miller and Ward 1966). Multicellular higher plants are generally considered not to have the rapid-growth characteristics of unicellular algae. A few investigators, however, have demonstrated that higher plants under controlled conditions could be valuable components of bioregenerative systems (Pilgrim and Johnson 1962, Christensen 1963, Mansell et al. 1968, Wilks 1969).

There are many advantages to including higher plants in on-board space systems. The use of plants in space would contribute general scientific information on their physiological response to long-term reduced pressure and weightlesness. The greatest benefit of on-board plant systems, however, would be to the spacecraft crew, who could harvest fresh food to supplement their diet. Of equal importance would be the gas exchange properties of photosynthesis which would result in the regeneration of respirable oxygen and the utilization of carbon dioxide by the plants. Plant systems could also be designed to utilize waste effluent by incorporating wastes within the planting substrate (Christensen 1963). Finally, the inclusion of higher plants on long-term missions could be psychologically beneficial, since growing plants would offer a diversion from the more typical astronautical duties.

Skylab will offer a unique opportunity for applied bioscientific investigations. This study was, therefore, undertaken to determine the ability of seeds to germinate and seedlings to develop in an environment simulating that of Skylab.

SECTION II

METHODS

ALTITUDE EXPOSURE CHAMBERS

Two dome-shaped altitude-exposure chambers were used in the study (MacEwen 1965). Each chamber was designed to accomplish a high degree of fidelity in simulating the exotic, hypobaric atmospheric conditions encountered in space cabins. The chambers were further designed for uninterrupted, continuous-exposure studies. Each dome-shaped chamber is 2.65 meters (9 ft) in height and 3.65 meters (12 ft) in diameter and has a simulated altitude capability of 10,668 meters (35,000 ft). See Figure 1. An airlock system allows entry into the chambers without changing any of the environmental parameters. The top portion of the domes can be separated from the bottom for loading plants and equipment, and an airtight seal is achieved by a pressurized O-ring.

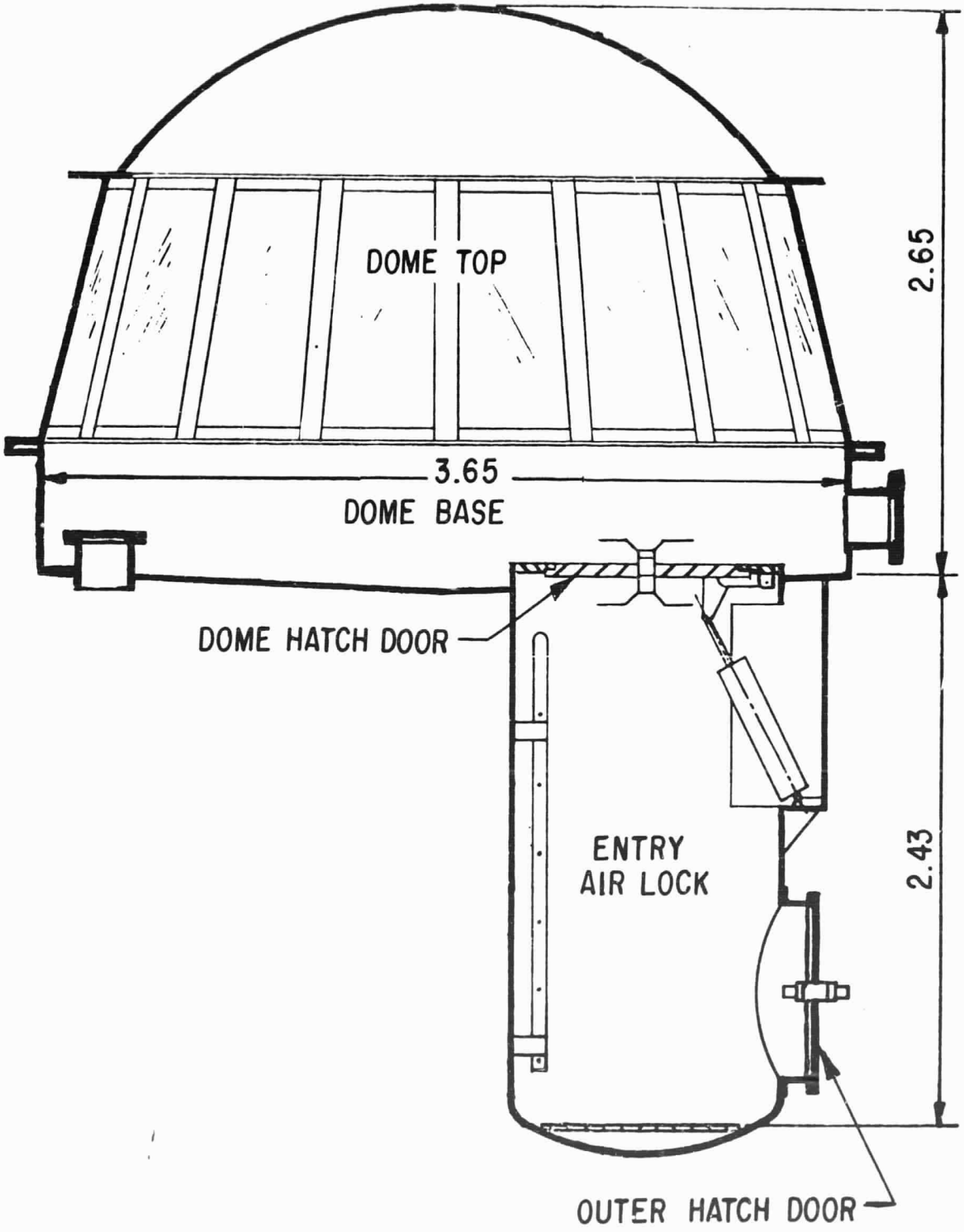
The main control console is fully automated, regulating and recording absolute pressure, gaseous flow rate, oxygen concentration, carbon dioxide concentration, temperature, and relative humidity. A three-way automated mixing valve is used to produce the desired concentration ratio of oxygen to nitrogen. Nitrogen is supplied by diluting oxygen with prefiltered air.

Both domes were enriched with carbon dioxide to a level of 3mm Hg. Artificial light was provided by eight 8-foot (2.44m) fluorescent tubes mounted thirty inches above the plants in each dome. Four Sylvania F96T12/CW/VHO tubes and four Sylvania F96T12/GRO/VHO/WS tubes were mounted alternately and gave approximately 750 footcandles (8,073 lux) of illumination.

EXPERIMENTAL DESIGN

The experiment consisted of two 10-day-exposure periods in which seeds and seedlings of four species were exposed to one of two treatments. These treatments consisted of identical environments except for the total atmospheric pressure and partial pressure of nitrogen. One altitude exposure chamber was held at 260mm Hg total pressure (8,230m or 27,000 ft) while the other was held at 725mm Hg total pressure and served as a ground level control. The plants in both domes were exposed to equal partial pressures of oxygen and carbon dioxide. A summary of the environmental parameters for each dome is given in Table 1.

Daily dome entries to water plants and collect data were made through the airlock. The investigator was required to prebreathe 100% oxygen for 1 hour before entry to prevent the possibility of bends.



DIMENSIONS: METERS

Figure 1. Altitude Exposure Chamber

TABLE I

ENVIRONMENTAL PARAMETERS AVERAGED FROM 237 HOURLY READINGS

EXPERIMENT I

Treatment	Pressure	Flow	Temperature	Humidity	CO_2	O_2
Altitude	260 mm Hg	10 cfm	89.9 F	68.9% RH	3.0 mm Hg	179.[illegible] mm Hg
Control	725 mm Hg	20 cfm	87.9 F	75.6% RH	2.9 mm Hg	173.6 mm Hg

EXPERIMENT II

Treatment	Pressure	Flow	Temperature	Humidity	CO_2	O_2
Altitude	260 mm Hg	10 cfm	94.6 F	73.8% RH	3.1 mm Hg	177.5 mm Hg
Control	725 mm Hg	20 cfm	89.6 F	87.0% RH	3.0 mm Hg	181.3 mm Hg

In the seedling test, 30 seedlings of four species were used in each dome per 10-day exposure. The four species were Raphanus sativus L. (radish, Cherry Belle #9458, Geo. J. Ball, Inc.) Lactuca sativa L. (lettuce, Burpee Bibb #6235, W. Atlee Burpee Co.), Brassica oleracea L. var. capitata (cabbage, Copenhagen Market #9063, Geo. J. Ball, Inc.), and Capsicum frutescens L. (pepper, California Wonder Select #9370, Geo. J. Ball, Inc.). The seedlings were started under greenhouse conditions in 10.16 cm (4") pots with vermiculite as a rooting substrate and were 40 days old at the time of initial exposure. Seedlings were watered with Hoagland's Nutrient Solution as needed (table II).

TABLE II

HOAGLAND'S NUTRIENT SOLUTION

IM Stock Solutions	ml Stock Solution/liter
$NH_4H_2PO_4$	1
KNO_3	6
$Ca(NO_3)_2$	4
$MgSO_4 \cdot 7H_2O$	2

Micronutrient Stock Solution	gm/liter	ml Stock Solution/liter
H_3BO_3	2.86	
$MnCl_2.4H_2O$	1.81	
$ZnSO_4.7H_2O$	0.22	1
$Na_2MoO_4.2H_2O$	0.025	
$CuSO_4.5H_2O$	0.08	
Fe Stock Solution	**gm/liter**	**ml Stock Solution/liter**
10% Iron Chelate (Sequestrene 330 Fe)	25.0	2

In each treatment, the seed germination test utilized 450 seeds of each of the four species. Radish seeds in this test were Early Scarlet Globe #655, Ferry-Morse Seed Co., Inc. rather than the Cherry Belle seeds used for the seedling test. Ten seeds were placed in each test unit, and there were 45 replicate units per species in each treatment. The germination test units were constructed of Plexiglas to facilitate handling and observation (figure 2, detail A). The front and back windows of the test units were 0.16 cm (1/16-inch) Plexiglas and were sealed to a 0.64 cm (1/4-inch) Plexiglas frame used as a spacer. Each unit had a small hole in the bottom to accommodate drainage. At the ends of the series of six tests units were stainless steel pivot pins which were supported by notches in a four-sided Plexiglas frame (figure 2). This frame held six series of the test units described above. All 36 units per frame could be observed by standing the frame on end so that the test units swung down and faced the observer. Each test unit was filled with BR-8 (a wood pulp product stabilized with acrylonitril resin and produced by the American Can Company) for a rooting substrate. This BR-8 contained no major nutrients and was pulverized in an electric blender before being packed into the seed germination units. The germination units, with 10 seeds per unit placed on top of the BR-8, were watered with distilled water and covered with aluminum foil for shading. Watering was done just before the domes were sealed and the pressure reduced. The shading was removed on the fourth day of the first experiment, on the sixth day of the second experiment. The seeds were watered only with distilled water throughout the first 10-day exposure but were watered twice with nutrient solution during the second exposure.

Data were collected from the seed germination units on days 2, 4, 6, 8, and 10, and included the number of seeds showing epicotyl emergence and seedling appearance. In addition, the final data included an evaluation of growth, stem collapse, stem formation, root formation, and

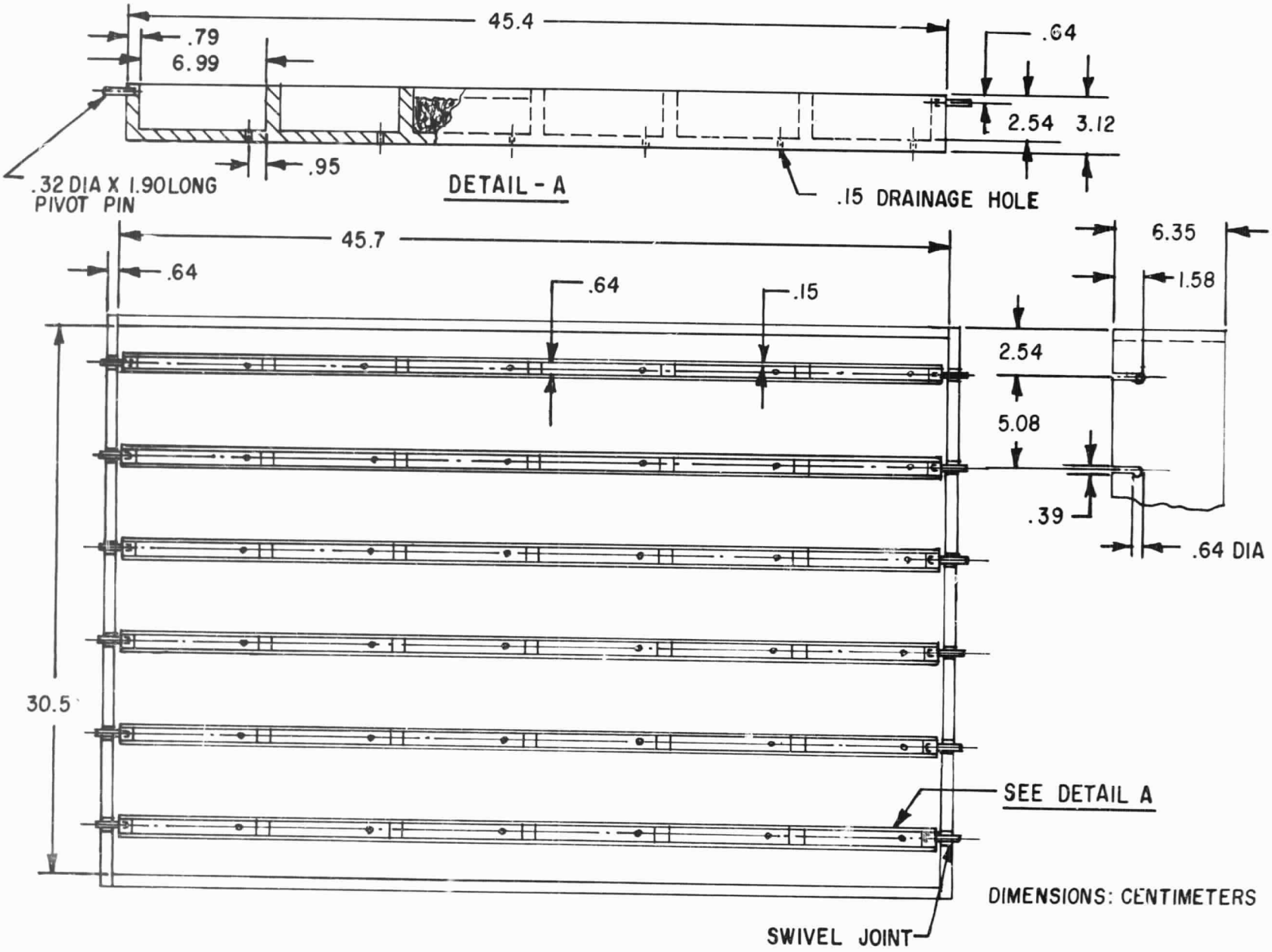


FIGURE 2. SEED GERMINATION TEST UNITS

pigmentation. Each of these characteristics received a rating of 1, 2, or 3 as shown in table III. The scores for these five characteristics were summed per test unit so that each unit had a total score of not less than 1x the number of characteristics being measured or not more than 3x the number of characteristics. The total scores were listed by the computer in a table for both of the treatments and for the 45 replicates according to species. Significant differences among total scores for the test units were determined by t-tests.

At the termination of the experiment, one seedling from each of the 45 germination test units per species was removed and fixed in 6% glutaraldehyde for histological examination under light and electron microscopy. These samples were taken while the plants were still in the reduced pressure environment. After the seed germination units were removed from the domes, each erect seedling was measured to determine the length of the shoot portion of the plant. Two hundred shoots per species were collected and dried to a constant weight at 80 C for dry weight measurements.

Data from the mature seedlings were also taken on the final day of both exposure periods. Color, greening, mass, health, injury, and configuration were the characteristics rated 1, 2, or 3 for each seedling (table III). Computer analysis of these values was the same as that described for the seed germination test. Histological samples of leaf tissue were collected and fixed in 6% glutaraldehyde on the final day of the experiments. In addition, plant heights for 30% of the seedlings were measured before and after the reduced pressure experiments.

TABLE III

EVALUATION OF PLANT CHARACTERISTICS

SEED GERMINATION TEST

Characteristics	Rating		
	1	2	3
Growth	No growth	Some growth	Most growing
Stem Collapse	Complete collapse	Some constriction	Normal appearance
Stem Formation	Severe stunt or malformation	Questionable stunting	No stunting or malformation
Root Formation	Severe stunt or malformation	Some effect on growth	No effect
Pigmentation	Marked variation from normal	Any change in color	No effective color change

SEEDLING TEST

Characteristics	Rating		
	1	2	3
Color other than green	Extensive coloration, flecks or spots	Any discoloration from normal	Normal color, no spots
Green Pigmentation (leaf)	Yellow green	Light green	Dark green
Mass of Plant	Stunted	Normal	Large
General Health	Sick	Questionable	Healthy
Mechanical Injury	Severe	Minor injury	None
Configuration	Any abnormal morphology	Questionable change	Normal

SECTION III

RESULTS

Radish, lettuce, and cabbage seeds germinated quickly in both treatments. Within 72 hours after the beginning of the experiments, these seeds showed greater than 75% germination. Pepper seeds germinated at a slower rate. Table IV gives the germination percentage on each sampling date for both experiments. T-tests showed that germination differences between treatments were not significant with the exception of the data collected on day 2 of experiment I (table IV). On this initial day of data collection the seeds in the ambient treatment (control) had a 6.1% greater germination than the seeds in the altitude treatment. This difference was significant at the .05 level. However, for the remainder of the experiment no significant differences between treatments resulted.

Table V summarizes final germination data for both experiments combined and indicates the percentage of seeds which formed true seedlings during the 10-day exposures. The average shoot lengths of all the true seedlings which developed during the seed germination test are given in table VI. Table VII gives the dry weight values for 200 of the above 10 day old seedlings of each species. Thirty percent of the mature seedlings in the experiments were sampled for shoot lengths before and after the 10-day exposures. The results of these measurements are given in table VIII. No significant differences between treatments resulted from rating the seedling appearance characteristics at the end of the experiments.

Histological examination of plant tissues revealed no abnormalities among the plants subjected to reduced atmospheric pressure.

TABLE IV

GERMINATION PERCENTAGE

EXPERIMENT I

Species	Treatment	Day 2	Day 4	Day 6	Day 8	Day 10
Radish	Altitude	93.3	97.6	97.6	97.6	97.8
	Control	97.8	97.8	97.8	97.8	97.8
Lettuce	Altitude	78.2	92.4	92.9	92.9	95.6
	Control	88.4	93.8	93.8	93.8	94.9
Cabbage	Altitude	64.7	87.6	90.4	91.8	93.1
	Control	74.7	86.0	88.7	90.7	91.1
Pepper	Altitude	0	14.2	90.4	95.8	96.0
	Control	0	21.1	93.1	97.6	98.0
All species	Altitude	59.1	72.9	92.8	94.5	95.6
	Control	65.2	74.4	93.4	95.0	95.5

EXPERIMENT II

Species	Treatment	Day 2	Day 4	Day 6	Day 8	Day 10
Radish	Altitude	90.4	95.1	96.0	96.0	96.0
	Control	95.1	96.7	96.7	97.3	97.3
Lettuce	Altitude	71.1	86.2	86.2	86.2	88.0
	Control	70.0	84.9	84.9	84.9	87.1
Cabbage	Altitude	51.7	68.0	68.0	68.0	69.3
	Control	60.4	66.0	66.0	66.0	66.0
Pepper	Altitude	0	20.4	82.9	89.3	95.1
	Control	0	24.7	88.4	91.3	96.2
All species	Altitude	53.9	67.4	83.3	84.9	87.1
	Control	56.4	68.1	84.0	84.9	86.7

TABLE V

PERCENTAGE OF GERMINANTS AND SEEDLINGS FOR BOTH EXPERIMENTS COMBINED ON DAY 10

Species	Treatment	% Germination	% Seedlings
Radish	Altitude	96.9	92.8
	Control	97.6	95.8
Lettuce	Altitude	92.2	84.7
	Control	91.0	83.1
Cabbage	Altitude	80.6	68.8
	Control	78.6	70.6
Pepper	Altitude	95.6	91.2
	Control	97.1	94.8
All Species	Altitude	91.3	84.4
	Control	90.8	85.6

TABLE VI

AVERAGE SHOOT LENGTH (SEED GERMINATION TEST)

Species	Treatment	Length (mm) Exp I	Length (mm) Exp II
Radish	Altitude	41.0	62.3
	Control	39.9	59.6
Lettuce	Altitude	23.4	39.7
	Control	25.2	47.2
Cabbage	Altitude	26.4	32.1
	Control	28.1	38.0
Pepper	Altitude	22.7	30.7
	Control	23.1	31.6

TABLE VII

DRY WEIGHT - 200 SEEDLINGS PER SPECIES

Species	Treatment	gm/200 Seedlings Exp I	gm/200 Seedlings Exp II
Radish	Altitude	1.95	1.87
	Control	2.47	1.65
Lettuce	Altitude	0.37	0.49
	Control	0.58	0.58
Cabbage	Altitude	1.08	0.78
	Control	0.99	0.96
Pepper	Altitude	0.45	0.71
	Control	0.53	0.67

TABLE VIII

AVERAGE INCREASE IN SHOOT LENGTH OF MATURE SEEDLINGS

DURING 10-DAY EXPOSURE

Species	Treatment	Average Increase (cm) Experiment I	Average Increase (cm) Experiment II
Radish	Altitude	11.9	9.0
	Control	9.1	7.6
Lettuce	Altitude	2.3	1.2
	Control	1.6	0.2
Cabbage	Altitude	1.8	2.8
	Control	1.3	3.7
Pepper	Altitude	10.6	7.4
	Control	14.2	10.3
All Species	Altitude	6.7	5.1
	Control	6.6	5.5

SECTION IV

DISCUSSION

Reduced pressure for a 10-day period had no effect on seed germination and the development of young seedlings. The percentage of germination that resulted was not significantly different between the altitude treatment and the control treatment. This result was reproduced in the second 10-day exposure. However, some differences in seed germination did occur between experiment I and experiment II. In the first experiment, all four species had high germination percentages (greater than 90% (table III). In the second experiment, two species obtained lower germination percentages. Cabbage seeds had 92% germination in the first 10-day exposure; but during the second 10 days, only 68% of the seeds germinated. Likewise, lettuce germination was reduced from 95% to 88% during the second experiment. This reduction in germination was evidenced in both the altitude treatment and in the ground level control treatment. The differences in germination between the 10-day exposures are the result of differences in environmental parameters. The second experiment incurred higher temperatures and humidity than the first, and germination conditions were less optimal during this second exposure experiment (table I).

The shoot lengths of 10-day-old seedlings in experiment I did not differ significantly between altitude and control treatments. In the second experiment, however, both cabbage and lettuce seedlings were longer in the control treatment (table VI). This difference was probably not caused by reduced pressure since no similar effect was seen during the first 10-day experiment; the differences in shoot lengths were again the result of temperature and humidity differences between the two domes. The altitude dome in experiment II became quite hot (94.6 F) and also had a lower relative humidity than the control facility (table I). The lengths of the seedling shoots in experiment II were greater than in experiment II (table VI). During the second experiment, the seed germination units remained shaded two days longer than during experiment I. This increased period of shading allowed greater elongation of the developing seedlings and resulted in greater shoot lengths during experiment II.

No general pattern of treatment effects was apparent from the dry weight data (table VII). In some instances, the altitude-treated seedlings obtained the greatest weight; in others, the control plants weighed the most.

The general appearance of these 10-day-old seedlings was similar in both treatments. All species formed healthy, vigorous, young seedlings during the period of the experiment (figure III). Near the end of the first exposure, radish and lettuce seedlings in the seed germination test developed a slightly chlorotic appearance. This chlorosis was due to nutrient deficiencies since the seeds received only distilled water. The chlorotic appearance was not apparent during the second experiment when the daily waterings were supplemented with nutrient solution. During both experiments, some plants developed necrotic leaf margins or spots. High temperatures and periodic drying of the BR-8 substrate were responsible for these discolorations. Complete drying of the substrate within twenty-four hours occurred in the altitude treatment. This rigorous environment caused a temporary wilting of some young seedlings in the seed germination test.

The mature seedlings also appeared relatively healthy after 10 days in both the altitude and control treatments, and no differences between treatments were observed. Lettuce plants in both treatments suffered from leaf necrosis at the center of the leaf rosette. This necrosis was probably a result of high temperatures and high humidity. Only radish and pepper plants increased in plant height during the experiments, while lettuce and cabbage plant heights remained nearly constant (table VIII). Radish and pepper plants grew approximately the same amount under both altitude and control conditions.

These results are opposed to the findings of Benjamin et. al. (1962). The results of their study on four vegetable species indicated an inhibition of germination and retardation of shoot growth under reduced atmospheric pressure and reduced oxygen partial pressure. Turnip, beet, bean, and carrot seeds achieved only 30% germination when exposed to a 260 mm Hg total pressure environment. However, in the present study greater than 90% of the seeds exposed to this reduced pressure germinated (table V), and the shoot lengths of these young seedlings did not vary significantly from control seedlings (table VI). It should be noted that Benjamin et. al. based their conclusions on an extremely small number of seeds. They used 10 seeds per species in bell jars at the reduced pressure and indicated that fungus and seed rotting were problems in their technique. The results reported in the present study represent 900 seeds per species and no fungal activity was apparent during the study.

Mansell et. al. (1968) also found little effect of reduced total pressure on plant growth and concluded that reduced pressure would not adversely influence the potential of plants in space. Their study of turnip (Brassica rapa L.) at 380 mm Hg total pressure indicated that the growth rate as measured by seedling dry weight was greater at 360 mm Hg than 700 mm Hg total pressure. The data presented in table VII does not show a similar trend indicating greater dry weight among 10 day old

individuals harvested from the 260 mm Hg altitude treatment. In the present study a subjective difference in water uptake between the altitude and control domes was noted. The substrate in the altitude treatment dryed at a faster rate causing a slight wilting between watering periods. Mansell also showed an increase in the percent dry weight with reduced pressure. This dehydration suggests a change in the rate of transpiration with reduced pressure.

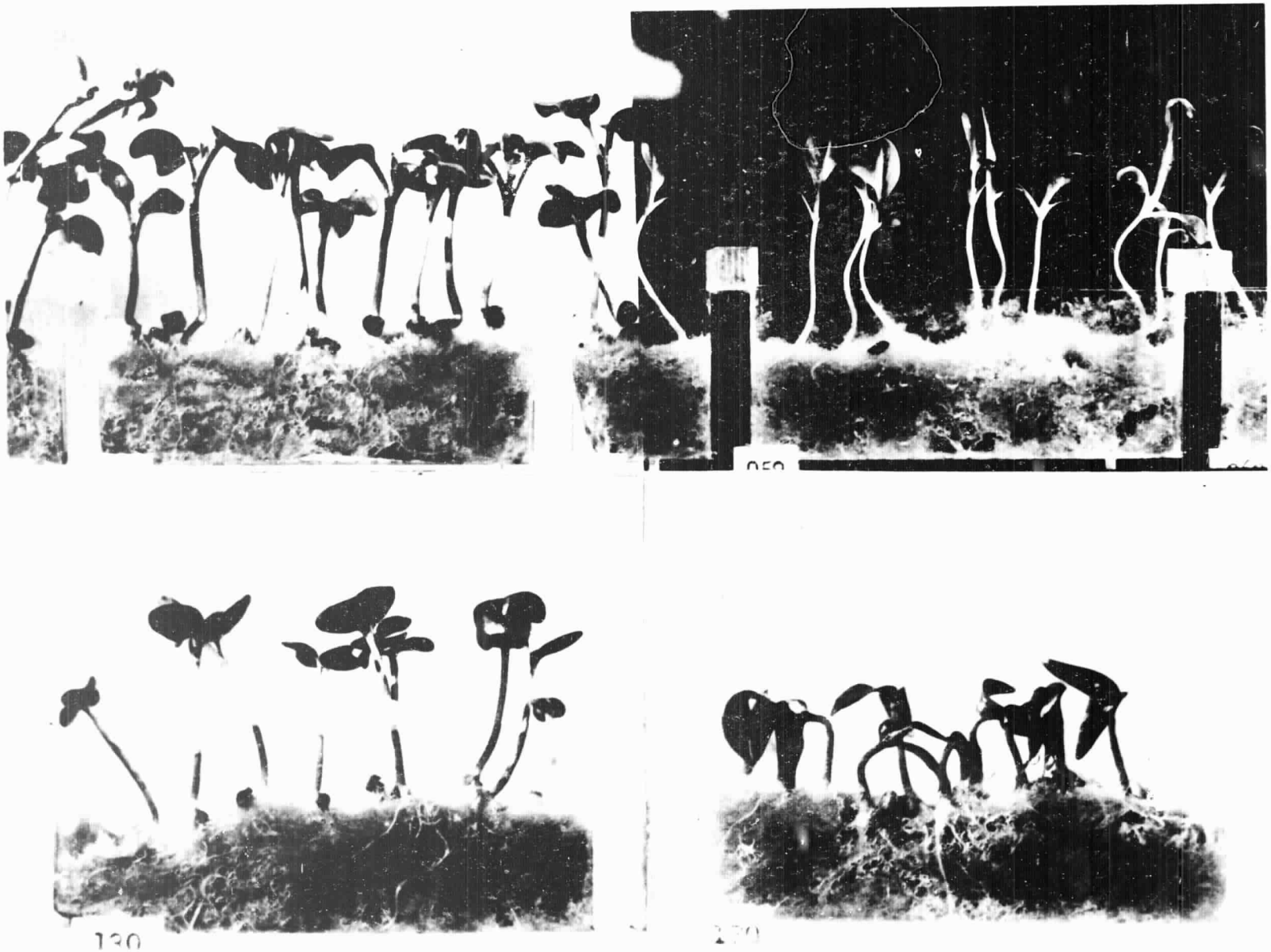


FIGURE 3. TEN-DAY-OLD, ALTITUDE-TREATED RADISH, LETTUCE, CABBAGE, AND PEPPER SEEDLINGS

SECTION V

CONCLUSION

Thirty-six hundred seeds representing four species were exposed to an environment which simulated the reduced pressure and mixed gas conditions of the NASA Skylab. An equal number of seeds at ground level pressure were used as a control for the reduced pressure experiment. Four hundred and eighty mature seedlings were also included in the experiment. No significant differences in seed germination or seedling development were apparent between the control and reduced pressure treatments. The high germination percentage and the development of healthy seedlings within 10 days in the simulated space cabin environment indicates that any of the species tested would not be adversely affected by the hypobaric environmental conditions of space flight.

These results show that plants can be expected to germinate and grow normally in the environment man carries into space. The inclusion of plants in a bioscientific Skylab mission will contribute practical information on the response of plants to a weightless, reduced pressure environment. This information is needed for developing closed ecological space systems in which higher plants can provide both food and respirable oxygen for the men within the spacecraft.

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