

• • • • EFFECT OF ATMOSPHERIC PRESSURE, GRAVITY, AND PARTIAL PRESSURE OF NITROGEN ON PLANT GROWTH

F. B. Benjamin
W. I. Taufman
W. M. Helvey

Space Environment and Life Sciences
REPUBLIC AVIATION CORPORATION • • • • •

INTRODUCTION

For the past several years Republic Aviation has been actively engaged with the problem of determining the influence of extra-terrestrial environment on the growth of higher plant life. As most of the technical barriers to outer space exploration are overcome, this problem will undoubtedly assume greater importance and increased efforts will be devoted to obtaining solutions. A survey of the literature shows little information in regard to what effect low pressure, decreased gravitational field, and reduced nitrogen pressure will have on the growth and health of plants.

Procedures and results obtained involving these factors are the subject matter of this paper. Each of the three experiments was carried to conclusion separately. However, a combination of these and other factors involved will be necessary before a final evaluation can be made.

Influence of Decreased Atmospheric Pressure

The atmospheric pressure on the lunar surface has been estimated to be in the order of 1/10,000 of our own. Therefore any plants of the higher order have to be grown in an artificially created environment, where pressure, composition of the air, radiation, light cycle and intensity, humidity, temperature, nutrients, etc., will be under constant regulation and control. Our approach to the problem is by means of simple experiments, utilizing the bell jar technique as shown in Figure 1.



Figure 1 - Bell Jar Technique

In this particular setup three bell jars were used with a wall mercury manometer, a bank of three 40 watt fluorescent tubes, two 25 watt incandescent lamps, a vacuum pump, and individual 12" x 12" aluminum base plates. Difficulty with moisture condensation on the inside surface of the bell jars was experienced. This was later remedied by the addition of coiled quarter inch aluminum tubing introduced through the rubber stoppers on top. Tap water at approximately 60°F was allowed to circulate through these coils continuously. In this manner the temperature inside the jars was brought down from an average of 82°F to 72 - 74°F and relative humidity from 100% to 85%. Condensation was transferred from the glass surface to that of the coils, thereby leaving the glass completely clear for the transmission of light. This also helped to eliminate some of the difficulty experienced with fungus growth and rotting of seeds.

For the first experiment four vegetable plants were used, dwarf tomato plants, eggplants, radishes, and snap beans. The eggplants were planted 40 days prior to the experiment. The radish and snap bean plants were grown from seeds. A representative of each group was placed under the bell jars, and the jars were then evacuated to absolute pressures of 2, 5, and 10 psi. After eight days these plants were removed from the bell jars. It was observed that growth of the dwarf tomato and eggplant was considerably retarded in the 2 psi bell jar, and that the radish and snap bean seeds which had been planted in vermiculite did not germinate. Relatively normal germination and growth was observed in the 5 and 10 psi bell jars. Therefore, the 2 psi environment was eliminated and four higher pressure levels: 5 psia, 8 psia, 11 psia, and 14.7 psia (sea level control) were used.

Because of the size limitations of the bell jars as well as the inability to adequately control temperature and humidity, only vegetative growth was obtained, and considerable difficulty was experienced with fungus and rot. Despite this, the plant weights obtained at the various pressures were not significantly different, although the stems were thicker, and the plant heights were reduced at the lower pressures. When the same plants (at all pressure levels) were removed from the bell jars and field grown, the yields were normal. Table I summarizes the growth results of experiments in low pressure, indicating an inhibition of germination and growth, with lowered atmospheric pressure.

In a follow-up series of experiments the aforementioned condenser coil arrangement was utilized, but fungus still remained a problem. A spray nozzle arrangement was installed (Figure 2) for watering and providing a liquid nutrient supply. This accomplished both foliage, and root feeding. Fungus was controlled partially by the addition of a fungicide (Orthocide) and the washing effect of the spray. This demonstrated adequate methods of experimentally growing plants under low pressure conditions, and permitted the planning of a larger experiment in the 64 cubic foot American Research Altitude Chamber shown in Figure 3. The inverted jars were used to withdraw excess water from the chamber due to the operation of the water spray.

TABLE I
 GERMINATION AND GROWTH DATA ON FOUR TYPES OF VEGETABLES
 FOR DIFFERENT AMBIENT PRESSURES
 (BEETS, BEANS, TURNIPS AND CARROTS)

	Nominal Pressures	Number of Seeds Planted		Per Cent Germinated (After 23 Days)	Average Height In Inches		Average Temperature for 17 Days (°F)	Average Pressure for 17 Days (psia)
		Germinated	Not Germinated		After 5 Days	After 10 Days		
1. <u>Beets</u>	5 psia	10	4	40	1/8	5/8	82.9	5.098
	8 psia	10	9	90	1/2	3/4	83.5	7.99
	11 psia	10	8	80	3/4	1	83.6	10.74
	14.7 psia	10	9	90	3/4	1-1/4	82.1	14.64
2. <u>Beans</u>	5 psia	5	2	40	2	2-3/4	82.9	5.098
	8 psia	5	4	80	3-7/8	4-1/2	83.5	7.99
	11 psia	5	3	60	3	3-7/8	83.6	10.74
	14.7 psia	5	4	80	4	5-7/8	82.1	14.64
3. <u>Turnips</u>	5 psia	10	4	40	0	3/8	82.9	5.098
	8 psia	10	6	60	3/4	3/4	83.5	7.99
	11 psia	10	6	60	1-3/4	2	83.6	10.74
	14.7 psia	10	6	60	1-3/4	2	82.1	14.64
4. <u>Carrots</u>	5 psia	10	0	0	0	0	82.9	5.098
	8 psia	10	6	60	0	3/8	83.5	7.99
	11 psia	10	8	80	1/2	1/2	83.6	10.74
	14.7 psia	10	6	60	1/8	1	82.1	14.64



Figure 2 - Spray Nozzle Arrangement

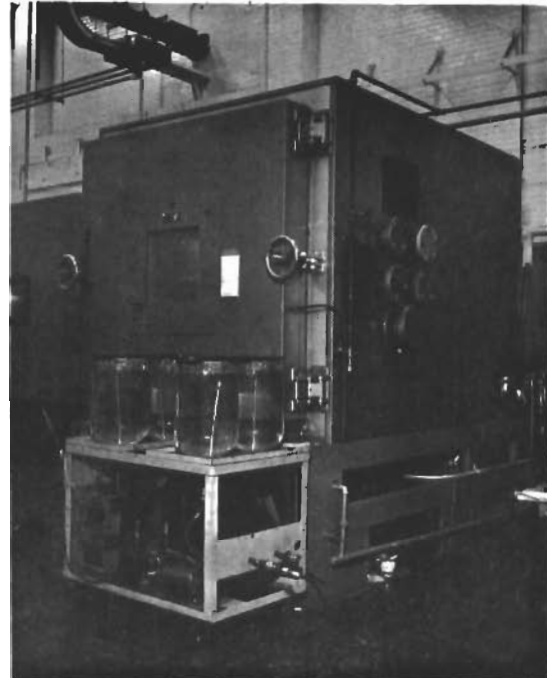


Figure 3 - American Research Altitude Chamber

Figure 4 is a schematic of the complete environmental control system for the plant growth experiment. Temperature control was achieved by use of heating elements in the walls, and a freon refrigeration system mounted on a frame at the rear. Pressure control was obtained by means of a positive displacement vacuum pump. Humidity was controlled by steam injection. Water supply and nutrients were provided through the spray system. Six nozzles were mounted above the plant boxes in a pattern designed to assure uniform distribution. Water was admitted through a solenoid valve controlled by an anemostat. When nutrients were needed (periodically) the hydraulic reservoir was filled with solution pressurized by a compressed air supply, and connected to the spray system. Water was sprayed for 20 seconds at five minute intervals around the clock. This provided a total flow rate of 0.4 gallons per hour, or about 10 gallons per day. Light was supplied by incandescent lamps and fluorescent tubes, controlled by a timer cycle (16 hours on, 8 hours off). Temperature, pressure, and humidity were continuously recorded on a clock chart.

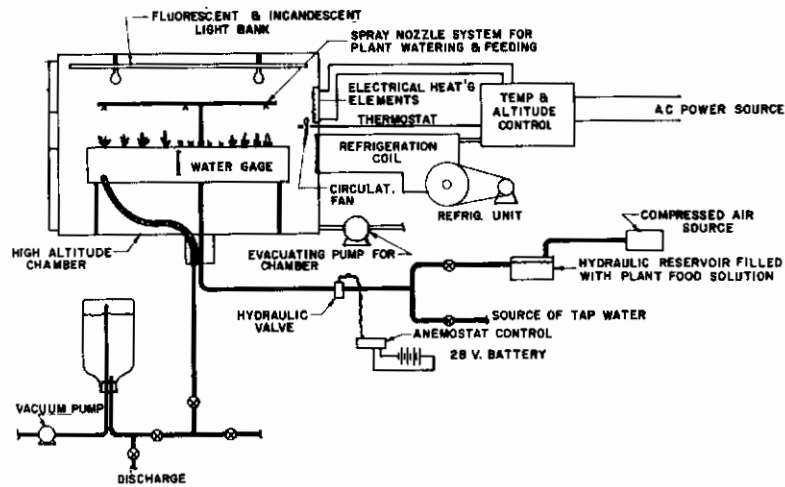


Figure 4 - Schematic of Complete Environmental Control System

Some comments might be in order for people intending to use this type of chamber for plant experimentation. The temperature control system was "on-off" in response to thermocouple sensors, and refrigerator start-up vibration made it difficult for climbing plants to physically support themselves. Air circulation was required at high velocity over the cooling coils, which made it necessary to provide internal baffles in order to avoid wind damage. The heating coils in the wall provided local radiant heating, which was not necessary and might have been detrimental. Humidity control was adversely affected by the spray system providing huge peaks every five minutes. Perhaps the most important limitation of this type of chamber is the inability to perform simple internal tasks such as plant staking or measurements such as leaf area.

Figure 5 shows the interior of the chamber about two weeks after planting with the chamber set for a simulated altitude of 18,000 feet. The soil in the boxes on the left is powdered perlite. The right side was planted in vermiculite.

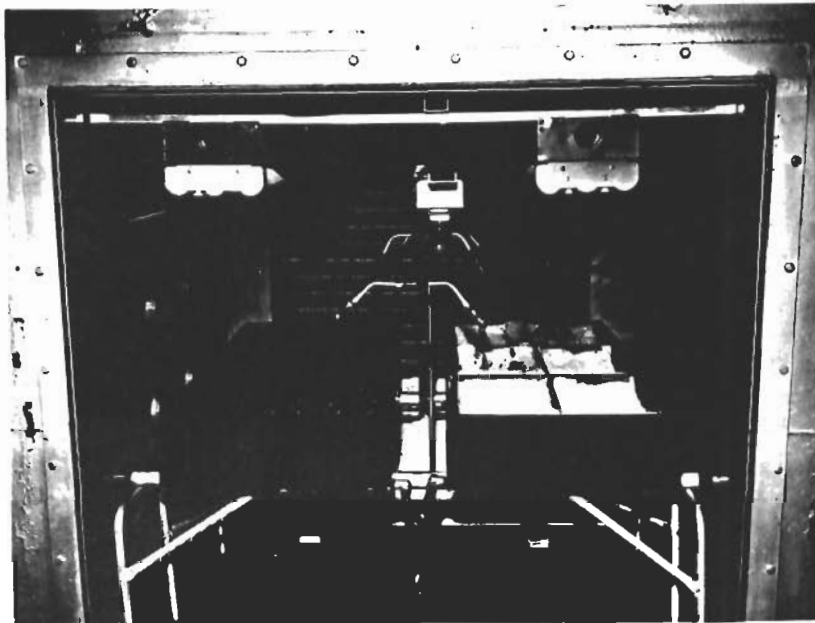


Figure 5 - Chamber Interior (Simulated Altitude of 18,000 feet)

No significant differences were observed between the growth in the two media. The vegetables growing are the common snap bean (*phaseolus vulgaris*), which reached maturity, and an Andean grain crop (*chenopodium quinoa*), which failed to survive. The failure of the Andean grain crop, which normally grows at an altitude of 10,000 to 15,000 feet, was probably due to the high relative humidity (60%) and low light intensity (500 foot candles) at the "soil" surface.

Another possible reason why many of these seeds did not germinate may be illustrated by another experiment in which seeds of a wild pineapple plant (*puya rainondii*), obtained from an altitude of 12,000 feet at La Paz, Bolivia, by Dr. Bassett Maguire of the New York Botanical Garden were planted. These seeds were divided into four groups and treated as follows: Group I received no treatment and was planted at 14.7 psia; Group II received 30 seconds of red light and was planted at 14.7 psia; Group III received no treatment and was planted at 18,000 feet (simulated); Group IV received 30 seconds of red light and was planted

at 18,000 feet (simulated). The only seeds to germinate and grow were those in Group IV, where all four of the seeds survived. Improper treatment of the seeds may, therefore, be the reason why chenopodium quinoa failed.

The growth of the snap beans under these conditions may be seen in Figure 6, which shows the interior of the chamber with the fully matured plants. In an area of about two square feet, a yield of 19.2 grams of edible material was obtained, which is about 39% of the average field grown crop yield. The difference may be explained by the lower than normal light intensity as well as the inability to provide normal care. Further efforts are necessary before any final judgement on the advisability of plant growth at reduced atmospheric pressures can be made.



Figure 6 - Snap Bean Growth

Effect of Decreased Gravitational Factor

Studies on the physiological effects of zero and reduced gravity on plants might yield pertinent information on the possible effects of weightlessness or near weightlessness on biological growth and development. Such data could possibly be used to determine the feasibility of using the higher plants in the life support system of an extraterrestrial environment, either as a primary or secondary source of gas exchange. In addition, the bonus of a fresh food source for the astronaut is a very strong and attractive consideration.

Since the stimulus-response time of plants is characteristically slow, it appeared feasible that rotation of the plants about their long axis in a horizontal plane, and with a cycle period less than the response time, could essentially nullify geotropic response. The experiments lasted two to six weeks. Figure 7 illustrates the technique used in modifying the effect of gravity in our experiments. The plant on the left represents the control in the normal one "G" position. The inverted plant next to it is under a negative one "G" force. The zero "G" condition is illustrated by the plant shown in the horizontal position. By slowly rotating the plant in its horizontal attitude each of the plants organs; roots, stems, leaves, etc., is subjected alternately to equal negative and positive gravitational influences within the geotropic response time. In summing the "G" force acting on each individual cell a zero "G" effect is created in the physiological sense.

Lunar gravity was simulated by rotation also, but with the stems inclined at $9-1/2$ degrees from the horizontal, which resulted in a vector force of $1/6$ "G" along the stem* axis. This is based upon the simple premise that the vertical "G" force can be resolved into two components, one component along the axis and the other perpendicular to the axis of rotation. The perpendicular component is effectively nullified by the slow rotation of the axis, thereby leaving just the remaining component to act on the plant.

* Any reference to stems is taken here to mean the total portion of the plant which is above the root.

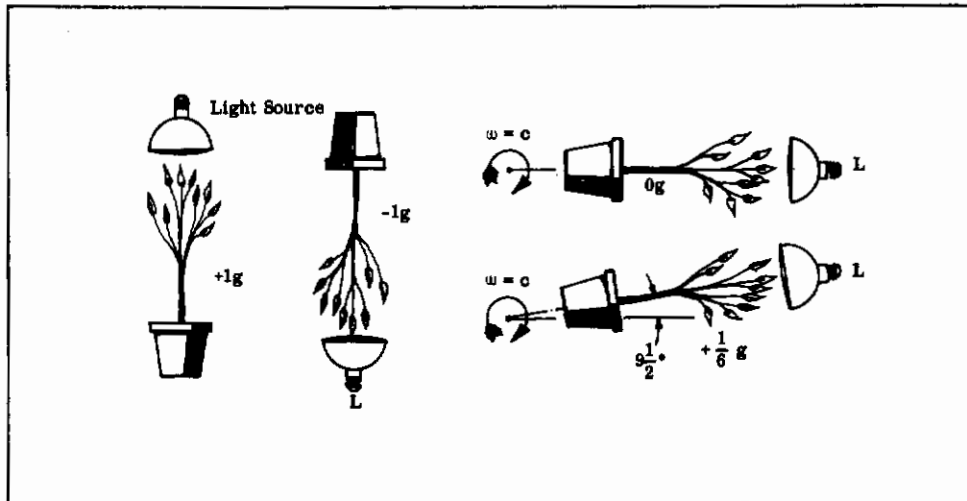


Figure 7 - Schematic Presentation of Modification of Gravitational Force for Plants

Controls in each case were vertical non-rotating plants grown simultaneously with the experimental plants and provided with identical temperature, water, and light conditions.

This method is applicable to plants only because of their slow response rate to gravity. Dolk, 1936, measured the curvature of different zones of a plant as a function of time and established that it took minutes for curvature to appear. His findings have been confirmed by other investigators.

A series of five 4-inch clay pots were mounted on cantilevered shafts which were, in turn, supported by bearings imbedded in aluminum blocks. The axis of each shaft was rotated at 1.2 rpm by a gear chain mechanism driven by a constant speed electric motor.

Figure 8 shows the mechanical arrangement employed to impart rotary motion to the five clay pots. For this particular experiment counter-clockwise rotation was maintained, looking from the base of the plant toward the top. The electric motor used to drive the mechanism is located in the lower left side of the picture. The mechanism is shown in the vertical position for illustration.

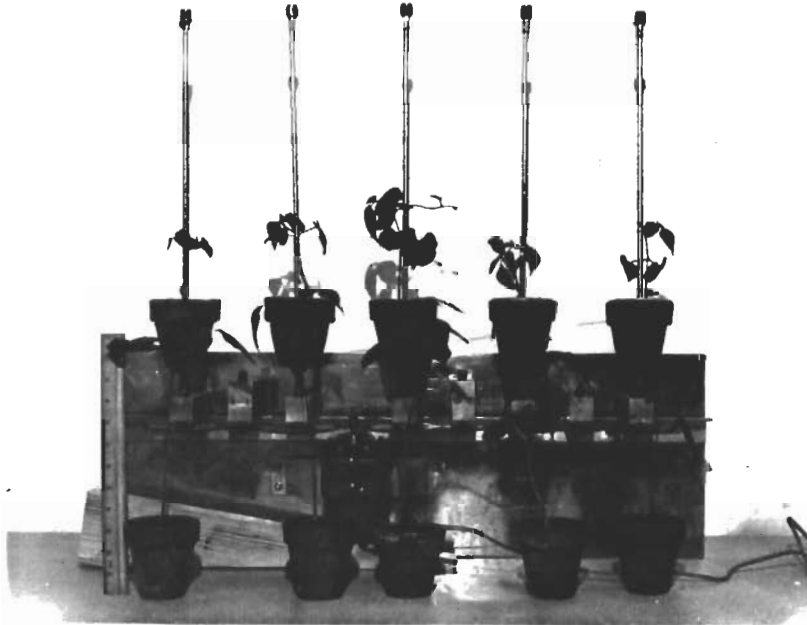


Figure 8 - Mechanical Arrangement for Rotary Motion

The series of five plants in the top row (Figure 8) were rotated continuously over periods from two to six weeks in the simulated zero "G" position. The five plants located directly below the ones mounted on the gears were the controls.

For purposes of comparison the ruler on the left side shows the difference in the stem heights. The tubing extending out from the center of each pot was found to be necessary to furnish support because of the cantilever action on the base of the stem. This was partially alleviated by fastening the tops loosely to the tubing.

Contrails

All plants were exposed to continuous light. Three cool white fluorescent tubes, rated at 40 watts each, were arranged in a bank and placed 15 inches away from the top surface of the soil. Light intensities measured at the soil level ranged from 250 foot candles on the sides, to 300 foot candles in the middle, measured with a Leeds & Northrup Macbeth Illuminometer. Figure 9 shows the lighting arrangement in relation to the rotating mechanism. The entire unit was encased in a plywood box, the inside of which was painted black to eliminate light reflections. The plants located on the left side of the fluorescent lights are horizontal controls.



Figure 9 - Lighting Arrangement

A commercially available all-purpose type of plant food, soluble in water, was utilized for feeding. Water was given in equal amounts to all plants. A mixture of peat moss and sterilized potting soil was selected for a soil medium.

Figure 10 shows a close-up of one of the bush bean plants which had been subjected to simulated zero "G" for a four week period, and shows the abnormal thickening in the base of the stem which was a characteristic of the plants subjected to this condition.

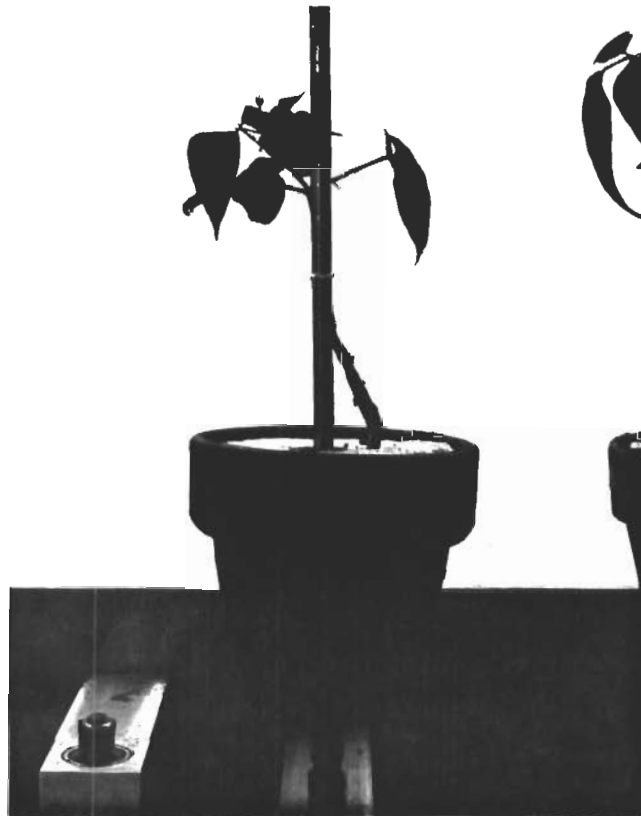


Figure 10 - Bush Bean Plant After Four Weeks at Zero Gravity

Figure 11 shows bush bean plants growing in an inverted position. The bracket supporting the pots can be raised or lowered by means of a pulley arrangement. This photograph shows the inhibition of growth when plants are inverted, and the geotropic curvature in spite of phototropic influence.

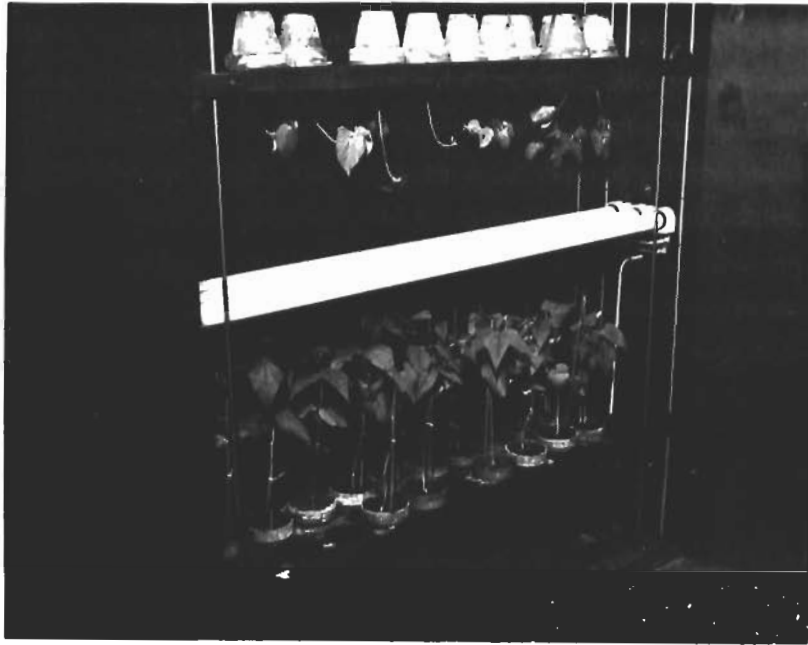


Figure 11 - Bush Bean Plants Growing in an Inverted Position

The results of the experiments are given in Tables II - V inclusive, and in Figure 12.

TABLE II
RESULTS FROM THE INVESTIGATION OF PLANT GROWTH
UNDER SIMULATED ZERO "G"

	<u>Water Distribution</u>			<u>Water (%)</u>	
	<u>Control</u> <u>(grams)</u>	<u>Experimental</u> <u>(grams)</u>	<u>% of Control</u>	<u>Control</u>	<u>Experimental</u>
Roots	0.18	0.03	16.7	9.4	8.3
Stems	1.14	1.14	100.0	55.1	70.4
Total	1.32	1.17	88.6	33.1	45.3

TABLE IIa

PLANT GROWTH UNDER SIMULATED ZERO "G"

(weight in grams)

Species: Bush Bean (Asgrow Black Valentine)

Growth Period Two Weeks

	<u>Wet Weight</u>						<u>Dry Weight</u>					
	<u>Control</u>			<u>Experimental</u>			<u>Control</u>			<u>Experimental</u>		
	No.	Mean	s.d.	No.	Mean	s.d.	No.	Mean	s.d.	No.	Mean	s.d.
Roots	5	0.82	0.42	5	0.41	0.31	5	0.58	0.32	5	0.38	0.31
Stems		1.92	0.67		0.99	0.61		0.20	0.05		0.09	0.61
Total		2.74	1.02		1.40	0.71		0.78	0.38		0.48	0.71

Growth Period Four Weeks

Roots	13	1.99	1.05	10	1.06	0.38	13	1.07	0.56	10	0.72	0.32
Stems		2.78	1.96		1.77	0.79		0.63	0.52		0.22	0.25
Total		4.77	3.21		2.83	1.10		1.70	1.09		0.94	0.56

Growth Period Six Weeks

Roots	5	2.85	1.18	4	1.40	0.42	5	2.69	0.30	4	1.25	0.68
Stems		2.50	1.40		2.11	1.02		0.54	0.12		0.39	0.16
Total		5.35	2.82		3.52	1.91		3.23	0.46		1.64	0.91

TABLE III

RESULTS FROM THE INVESTIGATION OF PLANT GROWTH
UNDER SIMULATED LUNAR GRAVITY

<u>Controls</u>				<u>Experimental</u>			
<u>Weeks</u>	<u>No. of Plants</u>	<u>Mean (grams)</u>	<u>s. d.</u>	<u>Weeks</u>	<u>No. of Plants</u>	<u>Mean (grams)</u>	<u>s. d.</u>
2	7	0.38	0.13	2	4	0.39	0.09
3	4	0.38	0.10	3	3	0.40	0.13

TABLE IV

RESULTS FROM THE INVESTIGATION OF PLANT GROWTH
UNDER SIMULATED LUNAR GRAVITY

(2 Weeks)

	<u>Wet Weight (grams)</u>						<u>Dry Weight (grams)</u>					
	<u>Control</u>			<u>Experimental</u>			<u>Control</u>			<u>Experimental</u>		
	<u>No.</u>	<u>Mean</u>	<u>s. d.</u>	<u>No.</u>	<u>Mean</u>	<u>s. d.</u>	<u>No.</u>	<u>Mean</u>	<u>s. d.</u>	<u>No.</u>	<u>Mean</u>	<u>s. d.</u>
Roots	6	1.61	0.65	4	1.89	0.59	6	0.77	0.32	4	0.45	0.46
Stems	6	2.06	0.14	4	2.00	0.82	6	0.20	0.08	4	0.23	0.09
Total	6	3.67	0.73	4	3.89	1.41	6	0.96	0.35	4	0.68	0.31

TABLE V

RESULTS FROM THE INVESTIGATION OF PLANT GROWTH
UNDER SIMULATED LUNAR GRAVITY
 (2 Weeks)

Water Content
(grams)

	<u>Control</u>		<u>Experimental</u>	
	<u>No.</u>	<u>Mean</u>	<u>No.</u>	<u>Mean</u>
Roots	6	1.16	4	1.12
Stems	6	1.83	4	1.80
Total	6	2.99	4	2.93

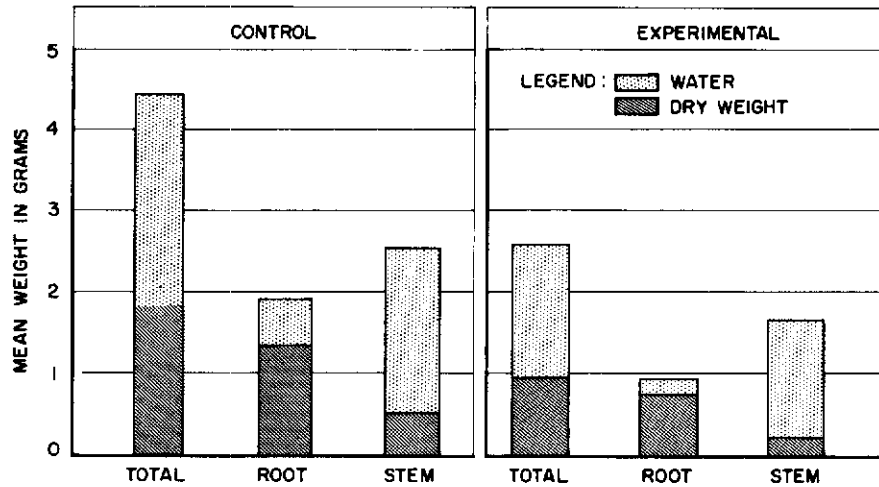


Figure 12 - Effect of Simulated Zero "G" on Weight and Water Distribution
 Bush Bean Plants: 23 Controls and 19 Experiments
 Growth Period: 2-6 Weeks

Germination development is apparently no different in controls and simulated zero and lunar gravity, however the number of experiments is too small to determine the effect on per cent of germination.

In the zero "G" studies there is a marked decrease in the weight of the plant. This is in contradiction to the results obtained by Dolk, 1929. The difference may be due to his having used cultivated oat seedlings whereas bush bean seeds were used in our experiments.

A climbing type of plant was decided upon for these experiments because it was assumed that it would be more sensitive to gravity than one of the grasses.

There is a change in water distribution between the roots and the stems, i. e., in zero "G" the proportional water content in the stems is increased by 8%, and that of the roots is decreased by 8%.

In the lunar gravity study there was no significant difference in weight between experimental and control plants. In both zero and lunar gravity simulations it was observed that the angle between the two secondary stems increased considerably, from about 60° to 120° or more. The differences in weight obtained from the two types of experiments indicate that even a small gravitational force can neutralize the detrimental effect of zero gravity upon growth.

A survey of the literature indicates that change of the gravitational field produces changes in:

1. Distribution of growth hormones
2. Distribution of nutrient materials (sugar)
3. Temperature gradient
4. pH
5. Electric potential

Our experiments indicate two additional factors.

6. Water distribution
7. Total growth

In view of the results of these studies, further investigation seems to be warranted to determine the effects of reduced gravity on other plant and animal systems.

Effect of Low Partial Pressure of Nitrogen

In previous work it has been shown that; 1) if gas composition remains constant, decreasing atmospheric pressure inhibits plant growth; and 2) if atmospheric pressure is constant, plant growth varies with the oxygen concentration. However, one factor has never been determined, at least as far as we know, and that is plant growth under various atmospheric pressures while the partial pressure of oxygen remains constant. This means that the only variable is the partial pressure of nitrogen.

Due to the prevailing circumstances these experiments had to be limited in number, scope, and the degree of control.

Methods: Bell jars were used, as described previously. Figure 13 shows the experimental setup.



Figure 13 - Bell Jar Technique

The jars were filled with the appropriate gases at the beginning of the experiment and subsequent adjustments were made every second day. The atmospheric control (Bell Jar IV) was left open to the outside, which was not a good method, as it made it difficult to consider the atmospheric experiment a true control. Therefore, evaluation of results will be limited to the three experimental jars.

Results: Table VI gives the wet weights. There is considerable variability, but the total weights show an increase, with a decrease of the partial pressure of nitrogen. The dry weights of Table VII bring this factor out more clearly. The only section of the plant where this does not seem to apply is the leaves. The stems, while showing the same gradient, have a total growth considerably in excess to the control plant. The total amount of water in the plant as shown in Table VIII shows the same trend. However, the percentage of water in the plant increases with the partial pressure of nitrogen.

Figure 14 is a graphical representation of these findings. It raises two problems, one of which is the need of a better control, as the present control values are clearly inadequate. The other problem is the continuation of the curves toward a pure oxygen atmosphere.

Figure 15 is a graphic summary of the results, showing that the partial pressure of nitrogen has an inverse relationship to plant growth and total water, and a direct relationship to percentage of water. As an incidental finding rhythmic changes of metabolic activity were observed which appear to be independent of diurnal rhythm, light, and temperature. Further work is needed before these findings can be explained adequately.

TABLE VI

AVERAGE GROWTH RESULTS OF SEVEN EXPERIMENTS TO DETERMINE
EFFECT OF VARIED GAS PARTIAL PRESSURES ON PLANTS

	Gas Composition, Partial Pressures in mm of Hg					Wet Weight in Grams			
	N ₂	O ₂	CO ₂	H ₂ O	Ar	Leaves	Stems	Roots	Total
B. J. I	385	195	7.0	18	5.1	1.299	1.09	0.805	3.195
% of Control (24 plants)	65.6					85.6	96.5	46.1	72.8
B. J. II	250	200	7.0	14	3.4	1.263	1.046	1.306	3.583
% of Control (24 plants)	42.6					83.3	92.6	74.8	81.6
B. J. III	149	200	7.0	11	1.9	1.383	1.157	1.134	3.674
% of Control (22 plants)	25.4					91.2	102.5	65.0	83.7
B. J. IV	587	159	7.0	21	7.0	1.517	1.129	1.749	4.390

TABLE VII

AVERAGE GROWTH RESULTS OF SEVEN EXPERIMENTS TO DETERMINE
EFFECT OF VARIED GAS PARTIAL PRESSURES ON PLANTS

	Gas Composition, Partial Pressures in mm of Hg					Dry Weights in Grams			
	N ₂	O ₂	CO ₂	H ₂ O	Ar	Leaves	Stems	Roots	Total
B. J. I	385	195	7.0	18	5.1	0.156	0.140	0.120	0.415
% of Control (24 plants)	65.6					69.3	97.9	30.7	54.6
B. J. II	250	200	7.0	14	3.4	0.205	0.184	0.199	0.588
% of Control (24 plants)	42.6					91.1	128.7	50.9	77.4
B. J. III	149	200	7.0	11	1.9	0.165	0.222	0.239	0.625
% of Control (22 plants)	25.4					73.3	155.2	61.1	82.2
B. J. IV	587	159	7.0	21	7.0	0.225	0.143	0.391	0.760

TABLE VIII

**AVERAGE GROWTH RESULTS OF SEVEN EXPERIMENTS TO DETERMINE
EFFECT OF VARIED GAS PARTIAL PRESSURES ON PLANTS**

	Gas Composition, Partial Pressures in mm of Hg					Water Content in Grams			
	N ₂	O ₂	CO ₂	H ₂ O	Ar	Leaves	Stems	Roots	Total
B. J. I	385	195	7.0	18	5.1	1.143	.950	.685	2.780
% of Total Weight (24 plants)	65.6					88.0	87.2	85.1	87.0
B. J. II	250	200	7.0	14	3.4	1.058	.862	1.107	2.995
% of Total Weight (24 plants)	42.6					83.8	82.4	84.8	83.6
B. J. III	149	200	7.0	11	1.9	1.218	.935	.895	3.049
% of Total Weight (22 plants)	25.4					88.1	80.8	78.9	83.0
B. J. IV	587	159	7.0	21	7.0	1.292	.986	1.358	3.630

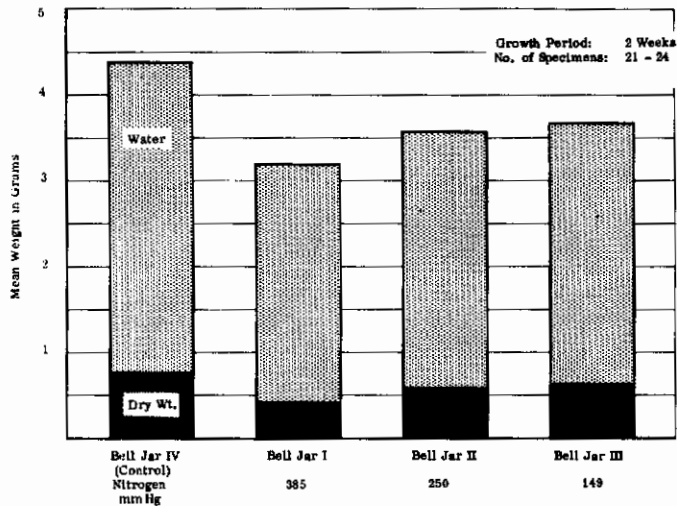


Figure 14 - Comparison of Total Growth, Wet, and Dry Weights (Bush Bean Plants)

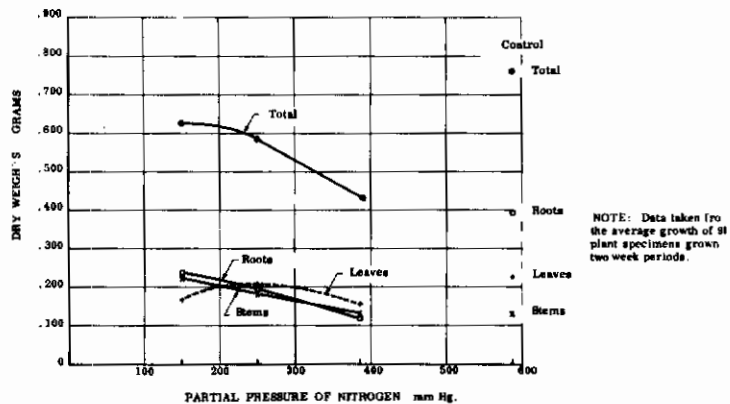


Figure 15 - Effect on Plant Growth of Partial Pressure Nitrogen