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Final Report of Research on

"EFFECTS OF PLANT GROWTH HORMONES ON PLANT DEVELOPMENT IN THE ABSENCE OF GRAVITATIONAL EFFECTS"

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Charles J. Lyon
Investigator

Department of Biological Sciences
Dartmouth College
Hanover, N. H. 03755

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1. Introduction

In spite of the need to devote most of our time to the developmental work of the wheat seedling experiment in the biosatellite Project, we have been able to complete some auxin studies with greenhouse plants. This work was expedited by the provision of improved facilities in the new Gilman Life Sciences Laboratory where we have modern equipment, use of controlled environment chambers for plant growth, and easier access to electronic instruments and the timesharing system of the Dartmouth G. E. Computer.

The auxin studies have resulted in the publication of 2 significant technical papers, additional evidence for some points reported in these and earlier papers on auxin transport, and an accumulation of evidence for a third paper to be submitted soon for publication. The scientific evidence for auxin control of orientation in wheat seedling organs with reference to gravity has also been published in a fourth paper that describes the method used in the biosatellite project to grow seedlings with their roots free of a dense substrate.

A by-product of difficulties encountered during the summer of 1966 while attempting to germinate wheat seeds within the flight-type hardware has opened up a new field in growth research. After a 2-month search for the cause of failure, electric heater blankets around the growth chambers were found to inhibit seed germination. The effect seems to be an interference with cell division. A less severe restriction of plant growth has also been demonstrated for low-gauss magnetic fields, an effect that may be related to that from the heater blankets. Both effects are unknown to science within the ranges of energies available for interference with growth processes.

2. Publication of Paper on Geotropism

Our paper on the downward transport of auxin (indoleacetic acid) by gravity during the formation of geotropic growth curvatures in a vascular plant was published in the January, 1965, issue of Plant Physiology. The title was "Auxin Transport in Geotropic Curvatures of a Branched Plant". Reprints were furnished with the semi-annual report of March 18, 1965.

3. Completion and Publication of Axial Curvatures Paper

The importance of gravity in maintaining an erect axis in most terrestrial plants was first noted by us in a short paper in Science in 1962. It described previously unreported curvatures that form in axial stems which grow while the plants are being rotated about their horizontal axes. During the subsequent 3 years we used our system of applying radioactive auxin (IAA-C^{14}), extracting the products of its degradation, and assaying the C^{14} with a liquid scintillation counter to establish the basis for the unequal growth on opposing sides of the axis.

We found that a large excess of the radioactive auxin was transported to the convex side of a curved stem. Sharper curvatures were correlated with greater imbalances of the growth hormone. Gravity corrects imbalances in auxin supplies and in uneven transport of auxin from a terminal supply. The method of correction is that reported for geotropic curvatures — lateral transport of the IAA to an even slightly lower side.

The downward transport action of gravity has the additional effect of moving auxin downward as far as the roots when the supply is so abundant that some auxin can reach the roots before it is degraded in the transport tissues. When gravity cannot act to assist the downward movement, as on a horizontal clinostat, some tissues in long, immature internodes get so little of the auxin that the growth zones fail to elongate as rapidly as corresponding tissues do in erect stems.

These lines of evidence for a significant effect of gravity on the growth of erect plants were combined in a paper on "Action of Gravity on Basipetal Transport of Auxin". It was published in the September, 1965, issue of Plant Physiology. Reprints of this paper have been in such great demand by students of auxins that the supply is now very low but copies were sent earlier to the NASA Office of Grants and Contracts.

4. Retarded Axial Growth on Clinostats

Our published evidence for retarded growth of internodes that develop on horizontal clinostats was obtained with seedling tomatoes and Torenias. Data used for the paper on basipetal transport included measurements from a group of 10 tomato plants that were kept horizontal for 6 weeks. We have since confirmed the stunting effect by 2 repetitions of the tomato experiment. The number of nodes and internodes remained the same in erect and clinostat plants for the 6-weeks period but extension of the growth period resulted in the production of more new internodes on the erect plants. The difference was probably due to much greater leaf surfaces on the control plants and the resultant more energy and materials for making new tissues.

Similar work with seedling Coleus plants for 3 months during the summer of 1965 failed to confirm the production of shorter internodes on clinostats. The plants were grown, however, from a mixed lot of seeds and longer internodes developed in erect seedlings of only a few varieties. The other strains grew with such short internodes that the endogenous mechanism for the basipetal transport was apparently sufficient to provide auxin to all levels of the immature internodes, regardless of the orientation to gravity.

A later test with seed of a "Red" variety of Coleus was conducted from Nov. 3, 1965 to Jan. 3, 1966. This variety was chosen because the plants had relatively long internodes during the early periods of growth from seed. Of 10 transplants at the 2-leaf stage, 5 were attached to a 1 rph clinostat and the other 5 left to grow as erect controls on the same greenhouse bench. The plants with horizontal stems grew more slowly and had developed pronounced axial curvatures after 2 months. The beneficial effect of gravity on basipetal transport in stems was thus confirmed with a third species.

Sections from some of the internodes from erect and clinostat plants were fixed in February, 1966, and preserved for anatomical study. When time permits we shall cut longitudinal sections of each position of growth and make microscopic measurements of cells in the internodal tissues. We shall be looking for quantitative differences in cell elongation between the longer internodes of the erect stems and the shorter internodes of the horizontal stems. We made a preliminary report of this condition in tomatoes (Plant Physiol. 40: 953-961. 1965) and have in storage more such tomato tissues as well as those from the "Red" Coleus.

5. Slow Transport of Radioactive Auxin in Horizontal Stems

To reinforce our evidence for a gravity effect on the movement of auxin through a stem, we have conducted a series of tests to trace the movement of radioactive auxin (IAA-C^{14}) through defoliated axes of tall Coleus plants. The auxin is applied to the decapitated end of the stem where the tagged compound enters from a cap of lanolin paste. The downward movement of the radiocarbon in the auxin is traced by assays of extracts from the upper nodes and internodes, each at a measured distance from the auxin source.

For a single test, 15 potted plants each are used to supply the auxin to Coleus stems erect or on clinostats. After 2 days in a dark room, the stem tissues are removed, weighed, diced and stored in a deep freeze for later extraction and radioassay.

The data and material from 8 such tests have been collected. Assays of part of the material have been made. The data have not been completely analyzed but they seem to agree in the demonstration of more auxin and its metabolic products in the stems that were horizontal during the 2-day periods of auxin transport toward the roots.

6. Tumbling vs. Horizontal Rotation

It is generally agreed that the continual horizontal rotation of a growing plant prevents the development of a geotropic curvature in a growth zone because the receptor tissue is turned to a new orientation with gravity before the chain of events of the presentation period has been completed. The constant interruption in reaction to a gravity vector prevents the multilateral stimulus from being effective for stimulation of unbalanced growth.

If this continual change in orientation is the cause of the failure to respond to the stimulus, the same rotation effect should be produced by tumbling the plant end over end about a point outside the regions of receptor tissue. Possible imbalance in illumination and other factors can be avoided with experiments in a dark room at a constant temperature.

We have used the growth curvatures of branch epinasty in Coleus for bioassay of comparative effects of the 2 methods of plant rotation. Plants were cultured for 2 branches each and selected in groups of 10 for branch length of 10 to 20 cm. The 20 branches were defoliated, the tips cut back to the first firm node, and a cap of 1% IAA in lanolin applied to the freshly cut end of each branch. The initial angular position of each branch was then recorded on paper by the system of shadowgraph tracings previously described for our study of epinasty.

The 10 plants were attached in 2 sets of 5 each to a clinostat that turned at 6 rph, with the axes of the plants either horizontal or at right angles to the axis of revolution. The potted plants in the second position were held in special cradles to avoid displacement as they turned end over end with the paired branches oriented either in the plane of rotation or normal to this plane.

Each set of measurements of epinastic curvatures in the branches was made after 12 hours of rotation in darkness at 24.5 ± 0.5° C. The second set of branch position tracings was made on the same sheet as the first, with the tracings of fixed reference stakes superimposed. The angle between tangents drawn to the initial and terminal position tracings was used as a measure of the imbalance of growth during the rotation.

After the plants had been left erect to gravity in the same dark room for 24 hours to permit growth adjustment of the branches to their plagiotropic positions, the 10 pairs of branches were used for a second test, either with a change of orientation or with the same positions of the plants for duplicate measurements. In all cases the number of 12-hour periods of rotated growth with epinastic curvatures was divided equally between day and night hours for any one position, since the growth rates of the branches were always greater during the period between 8 p. m. and 8 a. m.

Table 1. Epinastic curvatures on horizontal clinostats and tumble wheels. Data are means of 80 measurements for each test.

Rotation method	Mean curvature in 12 hours
Series 1 - Nov. 25 to Feb. 3	
Horizontal	78.2 ± 4.7°
Rotation tumble	78.5 ± 5.7°
Normal tumble	76.2 ± 5.6°
Series 2 - Feb. 8 to March 9	
Horizontal	87.2 ± 4.6°
Rotation tumble	81.6 ± 4.9°

The results of 2 series of experiments are shown in Table 1 above. The greenhouse plants of the first series were growing less vigorously than those of the second series for which the daylight hours were also longer. The similar results for the 2 orientations of tumbled plants showed a lack of polarity effects in the paired branches and left only the question of benefit from using a clinostat.

Since there were no significant differences among the 5 sets of test plants, the tumbling motion proved to be as effective as rotation in the horizontal position for preventing lateral transport of auxin by gravity. A tumbling system could be used either alone or in combination with the traditional clinostat but rotation about the horizontal axis of the plant remains the more convenient method for most studies of geotropism and the distribution of growth regulators.

This evidence and analysis will be submitted for publication in a suitable journal as soon as our time permits.

7. Biosatellite Work

An increasingly large proportion of our time has been devoted to refinement of the wheat seedling experiment in the Biosatellite Project. After the flight-type hardware was fabricated, the biocompatibility tests required practically all our attention.

The major part of the testing program was carried out at the Philadelphia facility of the General Electric Company during the Spring and Summer of 1966. In addition to proving the stability of the package and its capacity to contain the experiment within the spacecraft, the series of tests and the many delays between single tests provided an opportunity to work closely with members of the 2 other groups of experimenters who are using the wheat seedlings for their studies.

Our combined efforts developed dependable techniques for growing uniform seedlings. We worked out an acceptable set of steps (see the attached report for contract NAS2-1558) to follow in preparing the materials and planting the wheat seeds for the flight experiment. In the course of several simulated launches, we established a firm timetable for loading and closing the package at the correct time in the master plan for putting the payload in the spacecraft. Our "standing instructions" were developed and tested from the start for simultaneous delivery of the "flight" and "back-up" packages as required for the actual launch procedure.

A detailed description of this biosatellite work through the month of June, 1966, is attached as a supplement to this report. A copy of a published technical paper concerning the experimental method and auxin physiology of wheat seedling growth within the biosatellite package is also attached. This paper was published only recently (September, 1966) in the June issue of Plant Physiology.

Its analysis of seedling organ orientation, both erect to gravity and during rotation on a horizontal clinostat, supplies the physiological basis and considerable baseline data for judging the effect of weightlessness on a characteristic phase of plant growth as it will be tested in the coming orbital flight of the experiment.

The testing program for the months of July and August was enlivened but delayed by failure of the wheat seedlings to grow well during the first test of the experiments for 3 days within the first of 3 spacecraft built for the project. Since this was a thermal-vacuum test of the systems that maintain the environment of the vehicle, and because the very poor germination of the seeds was restored to a normal condition by subsequent return of the package to room temperatures, considerable time and effort was expended on confirmation of the temperature readings as reported from the thermal-vacuum tests. Secondary and repeated tests with carefully controlled temperatures produced only retarded growth of the seedlings at temperatures known from our earlier work to provide for the growth of much longer seedling organs.

In cooperation with the directors of the tests, we remained at the Philadelphia laboratory all summer to aid in the search for the obscure factor that inhibited the seed germination. Late in August we discovered that the seeds failed to germinate properly in our package only if the electric current was flowing within the heater blankets around the 4 growth chambers. The final thermal-vacuum test of the spacecraft was therefore conducted without activation of the heating units. The seeds germinated normally and thereby removed the last doubt of the readiness of our experiment for use in the spacecraft.

In the course of the search for the cause of the growth failures during the thermal-vacuum tests, we explored the possibility that seed germination can be inhibited by some magnetic field effect within the spacecraft. We obtained some evidence for retardation of growth in a field as low as 3 gauss. Since the field produced within the growth chambers by the electric heater blankets measures only about 1 milligauss, the cause of the severe retardation by activated heater blankets cannot be attributed to their magnetic field. It remains a mystery but the inhibition must be due to some unknown physical effect on the growth process. We suspect that the adverse effect is induced through an interruption of cell division processes, perhaps by preventing normal action of the spindle fibers during mitosis. Whatever the cause, the discovery of the effect and subsequent analysis of the cause and mode of action should provide a significant contribution to knowledge of the growth process in plant tissues. We hope study the problem further at a later date.

412 Gilman Laboratory
Dartmouth College
Hanover, N. H.
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Charles J. Lyon

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Charles J. Lyon
Principal Inv

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Department of Biological Sciences
Dartmouth College
Hanover, N. H.
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I. INTRODUCTION

The second year of work to develop reliable hardware and techniques for growing wheat seedlings within a 3-day satellite began with studies to improve the cultural techniques, introduced methods to study the physiology of organ orientation to gravity, and closed with critical tests of seedling growth within flight hardware installed in its place beside other units of the spacecraft's payload. Active cooperation between the 3 groups of experimenters who will use the plants that grow in orbit has produced a set of planting procedures that now yield uniform seedlings. Close cooperation and several meetings with the supervisors of the pre-flight and launch arrangements have also enabled us to prepare acceptable sets of procedures for the pre-launch and post-recovery operations.

Our method for germinating a grass-type seed with the coleoptile and root tips free of mechanical contacts and obstructions has enabled us to use our experimental data on orientation to analyze the auxin physiology of seedling development with reference to geotropism. A technical report of this study has been accepted for publication in the September, 1966, issue of Plant Physiology (actually used in June issue).

Biocompatability tests of the flight package, conducted outside the spacecraft, have established the adequacy of the hardware unit but a serious retardation of seed germination appeared when the package was used for a 3-day growth test within the spacecraft while the capsule was held within a thermal vacuum test chamber. The difficulty appears to involve either electrical field effects from equipment within the test chamber or temperatures much lower than those recorded by the system used at the General Electric facility in Philadelphia. It was finally traced to an unknown effect of the heater blankets.

II. REFINEMENTS IN TECHNIQUES

1. Lateral Curvatures of the Primary Root

In earlier reports we have described the use of the side-view angle of the primary root as a useful criterion for measuring the effect of gravity on the orientation of this root. The lateral deviations of the root have been noted to be appreciable when the seedling grows erect to gravity and very irregular when grown on a horizontal clinostat.

Analysis of these lateral variations from the 180° position (= straight down from an erect embryo axis) has revealed that the mean deviation from 180° is consistently much greater when the geotropic effect of gravity is lacking. Table I shows the difference for 2 sets of tests made during a 5-month period in 1965, with germination in darkness at 77° F. for 72 hours after soaking of seeds for 4 hours.

Table I. Variability in Lateral Deviation of Primary Root

Growth Position	No. of Tests	Mean Curvature ± S.E.	Range of Means
Erect	27	9.0 ± 0.4	5.4 - 13.1
Clinostat	30	39.6 ± 3.1	14.2 - 79.0

The use of this variability in lateral curvatures of the primary root corresponds exactly with the practice reported earlier for the use of such curvatures in the coleoptile. This new criterion holds even greater promise for analysis of the results of the orbital experiment. The coleoptile shows only small curvatures in the absence of a geotropic response unless the length exceeds 7 or 8 mm. but the primary root always grows long enough, even in 48 hours, to produce lateral displacement of the tip by many degrees from the 180° position.

The reason for the great variations in the growth rates on the sides of the primary root in the plane of the lateral roots must be sought in the growth controls within the central root. Since gravity reduces the growth curvatures to less than 5 degrees on the 2 sides of the vertical axis, appreciable imbalances of auxin transport in the absence of a gravity effect are the most probable cause for the curvatures. Variations in the imbalance between individual roots would account for the great range of mean lateral curvatures (cf. Table I). If the variations in auxin distribution occur within a single root during the course of its growth for 48 to 72 hours, they would account for the cases of changes in direction of growth of a root during such growth periods.

2. Growth Tests with Lower Temperatures and Long Holds

During the development work for growing wheat seedlings in the special seed stalks prior to July, 1965, we used a growth temperature of 77° F. as specified for the orbital experiment. The length of the 72-hour test period, however, was measured from the time of planting the soaked seeds. The result was to shorten the growth period in comparison with that of the 3-day flight which will start only after a hold of several hours while the rocket and spacecraft are being prepared for launch.

When the tentative 5-hour hold period was extended to a minimum of 8 hours, with uncertainties about the spacecraft and package temperatures before and during orbit, we carried out some tests with various temperatures and hold times. The lengths of seedling organs developed under several combinations of time and temperature conditions are shown in Table II on the following page.

Table II. 72-Hour Growth of Wheat Seedlings at Dartmouth for Effects of Temperature and Hold Time on Organ Length in Holders (lengths in mm.; new tests with vermiculite <40 & >80 mesh)

Growth	Hold		Erect	Seed.	Coleoptile	Primary	Left	Right
Temp.	Hrs.	Temp.	or Cl.	No.		Root	Root	Root
77	0 (old tests)		E or C	∞	ca. 8.0	ca. 31.0	ca. 18.0	ca. 18.0
72	0		E	11	6.3± .6	34.7±1.5	17.9±1.4	18.4±1.9
"	0		E	11	6.2± .8	33.7±1.9	16.2±1.6	14.5±2.0
"	0		C	15	9.9± .7	35.1±1.6	23.0±1.1	23.6± .8
"	0		C	11	10.7±1.0	33.9± .9	20.5±1.3	21.3±1.6
70	8	43	E	14	6.8± .5	35.5±1.4	19.2±1.8	19.3±1.7
"	"	"	C	15	6.7± .3	32.8±1.6	22.6±1.4	20.9±1.6
77	"	"	E	12	6.3± .9	30.8±2.0	18.9±1.3	18.8± .7
"	"	"	E	11	6.7± .5	31.0±1.4	18.9±1.1	14.4±1.2
"	"	"	C	15	8.2± .9	34.1±3.5	20.5±1.4	20.8±1.4
70	8	72	E	14	9.3± .8	37.0±2.6	25.5±1.6	28.4±1.5
"	"	75	E	14	6.4± .6	37.4±1.9	20.2±2.0	22.4±1.9
"	"	72	C	14	7.9± .8	39.3±1.0	24.6±2.1	24.5±1.8
"	"	75	C	14	7.3± .7	37.9±1.6	22.0±1.2	23.5±1.6
77	"	72	E	11	7.6± .9	32.2±2.8	23.5±1.3	23.3±1.4
"	"	75	E	11	7.5±1.3	34.2±1.6	21.8±1.6	21.4±1.3
"	"	72	C	13	8.4±1.0	32.5±1.4	25.0±1.2	22.4±1.7
"	"	75	C	13	10.0±1.5	33.3±2.4	23.5±1.3	25.3±1.7
70	12	70	E	15	8.8± .6	34.0±1.8	26.3±2.2	26.1±1.8
"	"	"	C	15	8.2± .6	33.6±1.8	22.1±1.9	22.7±1.7
77	"	"	E	12	8.2±1.1	31.7±2.0	18.4± .8	17.8± .9
"	"	"	E	12	10.0±1.3	31.9±2.0	21.3±1.8	22.3±1.5
"	"	"	C	14	14.4±1.5	27.4±2.5	18.9±1.1	19.3± .7

Notes: Seedling numbers of 11 or 12 represent growth in 12-unit holders.

A hold of 12 hours is intolerable because seedling growth has then started; retardation of growth by refrigeration will be impossible at the Cape.

The 5 tests of refrigerator hold (43° F.) for 8 hours showed that subsequent growth at either 70° (no benefit from heating blanket) or 77° was about the same as that without a hold period. However, this point is unimportant because the requirements for launch preparations include spacecraft and package temperatures like those of later orbit.

With an 8-hour hold at a temperature abovo 43° F. but somewhat below the projected 77° F., the subsequent 3-day growth produces longer roots and coleoptiles at either the spacecraft temperature of 70° F. or the possible package temperature of 77°. The greater increases are in the length of the lateral roots and coleoptiles. This is fortunate for orientation measurements since contact of these organs with the walls of the chambers rarely occurs. The increased growth after a hold of 8 hours should also help to produce significant orientation data from the fraction of the seed population that is slow in the early stages of germination. Seedlings from these slow starters are more likely to be of useful size at the time of 48-hour fixation if the hold time is at least 8 hours.

The increased length of the primary roots may produce more cases of root tips in contact with the side walls at 72 hours but the extra losses will not be serious if the orbital growth has a pattern close to that in clinostat experiments. The contact is made only if the side-view angle approximates 90° at the 48-hour stage with a face-view angle less than about 20° from the 180° position. A side-view angle either larger or smaller than about 90° permits considerable further elongation of the primary root without contact with a chamber wall.

Extension of the hold time to 12 hours did not increase the contacts of root tips with other objects when the hold temperature was 70°, even when the growth temperature for the following 72 hours was at 77°. The higher temperature for growth actually reduced the mean root growth in 2 tests (see Table II) but the data are insufficient for confidence on this observation.

Growth after a hold temperature of 77° was not tested because it cannot be attained before launch. Holds longer than 12 hours were also not tested; the seeds had been immersed in water about 4 hours earlier and germination had started in some seeds at 16 hours. Later work (see section IV below) showed that the presence of a very short primary root before launch will probably not prevent re-orientation according to the distribution of its auxin but the hold period must be kept to a minimum above 8 hours to prevent the germination time from exceeding 12 hours by a significant amount. Launch stresses on root tips must be avoided if possible lest they affect the form and orientation of roots that develop at zero gravity. The hold period cannot exceed 12 hours without compromising the experiment to some extent.

3. Soak and Hold Times

The extension of hold time from 5 hours to at least 8 hours after the seeds are placed in the flight package forced us to abandon the practice of soaking seeds for 4 hours before selection for planting. While our schedule required over an hour to plant a set of seed stalks, we reduced the preliminary soak time to 2 hours. Since this period of imbibition was insufficient to replace much of the water in the ripened, partially dehydrated seeds, it was difficult to select the seeds that would germinate promptly and produce perfect seedlings. The mean length of seedling organs was also reduced.

When the speed of planting was increased to a few minutes per seed stalk and to about a half-hour for each package, the soak time could be increased to 3 hours. Seed selection has now become dependable for a germination of about 96%. The number of imperfect seedlings remains high but the data for orientation of roots remains adequate after elimination of such seedlings from measurements. Coleoptile form is unaffected and the results of a 3-hour soak period are acceptable.

4. Surplus Water in Chambers

The growth of seedling roots in moist air requires the addition of water to each growth chamber at the start of an experiment. Experience has shown that a supply in excess of that required to saturate the air with water vapor serves as a special source of water to a plant with a primary root long enough and growing at the proper angle to touch the film or pocket of water. The result is abnormal length of its coleoptile and primary root. An excess must also be avoided in the orbital experiment because drops of water can collect anywhere in the absence of gravity.

Tests with our growth chambers have shown that only about 1 ml. of water is needed to saturate the air in a small chamber and that about 2.5 ml. will provide 100% relative humidity in the large chamber. These amounts are now being used in our tests. Records of growth in earlier tests had already been corrected by omitting data for a few oversize organs when computing mean lengths.

5. Rubber Caps for Seed Stalk Arms

The capping system for arms of the seed stalks has been developed as a thin sheet of rubber held tightly by a small ligature that presses the sheet into the groove near the end of the arm. The excess rubber dam is cut away with scissors before the capped stalks are filled with vermiculite.

In an effort to make the capping process less laborious, molded rubber caps were designed and supplied through the Ames Research Center. The problem was to prepare a cap with the thickness, tension and elasticity found by experience to be needed for planting and holding the soaked seed. Several models were made and tested but all failed to serve as adequate replacements for the older method of rubber patch and ligature.

6. Uniformity of Vermiculite Pack

A major factor in controlling the rate of wheat seed germination, with all water reaching the embryo through the endosperm, continues to be the supply of water inside the seed stalk. This supply is governed by the capillary capacity of the vermiculite and a sustained flow of water into the seeds through continuous paths of moist vermiculite that must maintain contacts with the seeds.

A joint study of the problem with our associates in the loading and injection of the stalks during the period of tests in Philadelphia (cf. Section VI) has developed an effective system for packing the vermiculite just firmly enough to retain and deliver water at all levels in the stalk. Uniformity of pack is attained by a combination of tapping and shaking the stalk as finely ground vermiculite is poured slowly into the upper end. The particles must be small enough to pass a #40 soil sieve and must be settled firmly against the rubber cap of each down-pointing arm as the powder is also firmed by tapping within the center tube. The only air spaces left within a stalk form a labyrinth of capillaries of nearly equal diameters throughout the loaded stalk.

7. Burning Slits in Rubber Caps

In place of a slit cut by fine scissors in each of the rubber caps, the hole for inserting a soaked seed is now being burned or melted by a hot iron tip, heated electrically. The tip was filed down from the broad end of a wood-burning tool.

Experience in cutting the slits with the new tool has shown an increase in accuracy and speed of operation. The holes can be made of uniform size and form and placed properly between the center and "upper" rim of each rubber cap. The melted edges allow easy insertion of a wet seed without danger of enlarging the hole except by stretching of the rubber that retains an even, firm grip on the seed.

8. Injection of Stalks

The column of evenly packed vermiculite is injected with distilled water with the aid of a 10-ml. syringe and steel needle. The stalk is set in a holder with the arms still pointing up after the slits have been made in the caps and the large Allen screw removed from the "lower" end, now uppermost.

Injection is started in the opposite end of the vermiculite column after inserting the needle deep into the stalk through the slit in the cap of the arm nearest that end. As air is forced slowly upward by the capillary water, the syringe needle is inserted progressively into the higher arm and finally into the open end of the stalk.

The injected stalk is then immersed, arms still pointing up, for a few hours in a cylinder of distilled water. The last few air bubbles will escape through the punctured rubber caps, leaving a continuous system of capillary water within the seed stalk.

The system is ready for planting the seeds in the slitted caps after the Allen screw has been replaced to close the end of the stalk.

9. More Lanolin in the Sealant

The groove in the wheat endosperm causes a gap in the contact between the seed and the rubber membrane, regardless of the size and form of the hole provided for seed insertion. Water can escape through the gap, with the danger of serious loss during vibration of the system.

Prior to the Philadelphia tests we had been using a mixture of 2.5 parts lanolin and 1 part beeswax to fill the gap. Some of the seals were dislodged during the 201 Vibration test and possible losses of water supply were indicated by reduced growth of the seedlings on some stalks. Since the lanolin-wax ratio was increased to 4:1, the seals have been very firm and losses of water have not been indicated. Retention of the seal has also been insured by more careful drying of the rubber at the points where the melted sealant is applied.

10. Twin Camera Racks for Photo Records

Our records of seedling size, form and organ orientation are made on 35-mm. color film, used later in projection to obtain data for analysis. Loss of a baseline experiment by loss of film or by damage done to it in processing would be inconvenient; loss of the only record of the orbital experiment and/or its controls would be catastrophic and must be prevented.

To guard against such a loss, 4 improved models of our camera stand have been made, with one Pentax camera, suitable lens and coupled strobe light attached to each when assembled for use. Two of the sets are being used to record the results of the tests at Philadelphia before shipment to Cape Kennedy. There they will be used to obtain duplicate records of the control experiments that grow while the biosatellite is in orbit.

The other 2 camera racks were used first to record the results of a special spacecraft test at Moffett Field. These sets of photographic equipment are being taken to the recovery laboratory in Hawaii for use at the end of the flight experiment.

The 2 sets of equipment at each terminal laboratory are duplicates except for the location of the 45° mirror that provides an image of the side view of a row of seedlings being photographed in face view in the same picture. On one camera rack the mirror is set to show the left side of the plants while the mirror on the stage of the other camera rack is on the right side of the seed stalk positioning pins. Views of both sides of each seedling are desirable because the position of a lateral root tip is sometimes screened from view from the opposite side of the plant.

Tests of the dual system of records for experimental plants have shown that the seedlings can be photographed so rapidly on each camera rack that even the root hairs are undamaged if the relative humidity of the room is at 75% or higher. The insurance feature of the duplicate pictures plan will be further preserved by developing the 2 films at separate times.

III. USE OF STALK METHOD FOR SEEDLINGS OTHER THAN WHEAT

1. Growth Tests With Oats

The use of the Avena coleoptile for the first bioassay of a growth hormone in 1926 plus the extensive work with this organ ever since for auxin studies has suggested that oat seedlings might be better than wheat and other grass seedlings for a biosatellite experiment. The advantages of wheat over rye and barley were described on our report of July, 1965. Similar tests with oats have now shown that wheat is decidedly better in every respect for quantitative experiments without being atypical in the physiological processes that are reflected in its growth responses to the environment.

Before soaking the oat seed and planting it in the wheat holders, the hulls were removed with some difficulty from the largest plump grains. The sets of seeds were allowed to germinate (without hold) in darkness at 77° F. for 72 hours. The growth was recorded and analyzed by the procedure used for wheat.

The data of the 4 tests with established usage are shown in Table III below. The coleoptiles were longer and their curvatures greater than for wheat but many of the side-view angles were negative on the clinostat. The roots were all relatively short and showed only small differences in orientation angles between erect and clinostat-grown seedlings. There is no distinct central root. The left and right roots of the table do not act as lateral roots and vary so much in their orientation angles that the standard errors of the means are prohibitive.

Table III. Avena Seedlings in Wheat Stalks

(mean lengths in mm: mean angles in degrees)

Position	Seed. No.	Coleoptile			Primary Root			Left Root			Right Root		
		Lgth	Face°	Side°	Lgth	Face°	Side°	Lgth	Face°	Side°	Lgth	Face°	Side°
Erect	11	9.8	6.9	5.8	17.0	176.3	13.9	11.9	204.9	24.8	13.8	165.9	19.9
"	12	11.4	6.6	7.6	14.5	184.9	16.2	14.0	188.6	19.9	12.6	161.4	31.8
Clinostat	11	12.1	16.8	21.1	14.7	*	27.9	12.0	181.9	53.3	12.0	134.2	42.8
"	9	12.6	34.8	18.6	17.6	*	20.7	16.3	190.4	23.7	13.0	128.2	39.9

* Not significant

Except for the strong response of the coleoptile to the elimination of a geotropic response, the oat seedling would be less useful than either rye or barley as a substitute for the wheat seedling. The same tendencies to growth curvatures appear in the 4 organs of all these cereal seedlings but the wheat seedling is superior as an experimental plant for quantitative studies.

2. Holder System for Dicotyledonous Seedlings

After earlier efforts to germinate good seedlings of the pea type in holders with rubber membranes around the cotyledons, we have returned to the hollow wick system which we had developed about 2 years ago for wheat. The cloth tubes must be larger to accommodate the Dicotyledonous seeds but a satisfactory source has been found in the form of white cotton laces for ski boots. Sections of them fit over the side arms of the plastic tube holders used for wheat just prior to the type made for the prototype package. The same fine vermiculite holds the water in the stalks and the cotton cloth keeps the cotyledons properly wet.

Good germination and growth of seedlings for several days has been obtained with vetch (Vicia villosa) and Canada field peas (Pisum sativum var. arvense). The seeds are sterilized chemically, soaked in water for several hours, selected for size to fit the cloth tubes, and inserted with the radicle side outermost. A steel needle is passed through the cloth and cotyledons without injury to the growing points of the embryo.

The largest vetch seeds produce seedlings with epicotyls and stems up to 10 mm. long. The root systems are shorter. When grown on a clinostat, the primary root and epicotyl make a characteristic angle of less than 90°, without strong curvatures in either. Secondary roots develop in a few days but the leaves and hooked tip of the stem are very small.

Canada field peas must be selected for the smallest seeds because the embryo swells so much during the imbibition phase of germination. Before planting in the wicks, the seed coats are excised from the radicle and epicotyl region. This part of the seed is left free of the cloth tube as the retaining pin is inserted. The axis of the embryo can be oriented as desired to permit growth of root and shoot with a minimum of contact with other objects.

The seedlings will grow in the present model of holder for at least 8 days. After about 5 days, the stem is 4 or 5 cm. long, bears a leaf and retains the plumular hook at the tip if the plant is turned on a clinostat. The primary root is shorter and bears secondary roots after the third day.

From a small number of tests with clinostats, we cannot describe a pattern for growth form beyond the development of growth curvatures in all epicotyls and primary roots. The diameters of these organs are large enough from the second day on to permit their use for experiments with chemical treatments and excision tests. We would like to continue experiments with the peas, using them for auxin studies as time permits. We have presented a proposal to grow them in an orbital experiment on some satellite, preferably one from which the seedlings can be recovered intact.

IV. PHYSIOLOGY OF SEEDLING ORGAN ORIENTATION

1. Apical Dominance of Primary Root

Whenever the primary root of an erect wheat seedling does not elongate normally, it also fails to respond as usual to the force of gravity. Growth curvatures always develop early in a short, thick organ. In these cases of atypical form and length, the seminal roots also fail to grow at their characteristic angles; they grow "downward", much as the branches of a stem tend to replace the leader when the terminal bud is injured or removed.

Along with other evidence that the presence of the indoleacetic acid type of growth hormone, and its distribution in response to the force of gravity, control the orientation of the seedling roots, the changes in the root pattern of these atypical cases suggest a dominance effect of the primary root during normal germination of wheat. If the auxin physiology is similar to that established for apical dominance in stems, growth hormone supplied by the elongating root may influence the auxin supply or distribution within the lateral roots.

Our attempts to influence the orientation of these roots by abscission of the primary root have failed completely to alter the growth position of lateral roots. The absence of abscission effects, however, does not preclude an endogenous hormonal relation between the primary and seminal roots. The internal defect responsible for the abnormal primary root may well cause the supply of auxin or auxin precursors to be diverted to the seminal roots. These roots appear to be longer than normal in the absence of a primary root from the start but the pathways of auxin supply are as yet unknown.

2. Treatments With Exogenous Auxin

In an attempt to modify the growth rate and/or the orientation of seedling organs, a 1% emulsion of indoleacetic acid in lanolin was applied to the faces of wheat embryos at the 48-hour stage in their germination. The treated seedlings were growing with controls on seed stalks that were either erect or turning on a clinostat. The sets of seedlings were removed from the growth chambers only long enough for pictures and applications of the paste with a glass rod.

From pictures of the seedlings before and 24 hours after the auxin application, analyses were made of root length and orientation. No influence of the exogenous auxin could be found. The work only served to confirm earlier observations that the orientation of a root is determined largely before the end of the second day of its growth. The tests must therefore be repeated with earlier applications of the auxin.

Negative results have also been obtained by applying the auxin to the surface of a young root. When a drop of 0.25% emulsion of the hormone in lanolin was placed on the "upper side" of a half-grown lateral root, it had no effect on the angle of root tip orientation. When applied in the same way to a root only a few mm. long, the root either grew at the same angle as before or growth was checked abruptly.

These tests must be repeated with young roots and weaker concentrations of auxin. The absence of an effect on older roots is probably due to impermeability or lack of good contact between the lanolin and the surface of the half-grown root.

3. Maintenance of Angular Orientation of Roots

Most of our measurements of root orientation have been made after germination has proceeded for 72 hours beyond the period of soaking and a subsequent hold time that is now established at about 8 hours. Since one set each of seedlings will be killed and fixed in orbit after growth periods of 48 and 60 hours respectively, the use of measurements from these dead seedlings requires a comparative study of changes in root orientation during the 3 days of germination. We have made this study and reported on it in detail in April, 1966.

For erect seedlings there is some fluctuation in the exact location of the primary root tip as this root grows downward with the face-view angle for any one root remaining either slightly larger or smaller than 180° during the third day of growth. The side-view angles vary very little but the face-view angles for the lateral roots change gradually in the direction of the 180° line as the increasing weight of the tissue pulls the root tip progressively lower.

When the 3 roots develop on a horizontal clinostat, the orientation angle for the face view of any one root usually remains close to the angle registered at the end of 48 hours. Some of the lateral roots show a weight effect on orientation as the plants are set erect before the camera for the later records but many other roots that elongate on clinostats have their tips raised farther from the 180° line after 72 hours than they were at 48 hours. This effect is due to strong root epinasty and seems to be associated with seedlings that are growing vigorously.

Analysis of the side-view angles for roots photographed at the 3 stages of growth shows small errors of the means for the measurements at 48, 60 and 72 hours. Exceptions occur when the lateral roots are too short at 48 hours to have developed curvatures in side view, or when long, heavy roots on older plants show the weight effect as the pictures are taken of the erect stalks of seedlings.

This good agreement in the orientation of roots grown without a directive effect of gravity makes it possible to combine the data for the 3 stages of germination. Data from the flight experiment can be assembled for comparison with the measurements obtained from the simultaneous clinostat control experiment. As an indication of distribution of auxin within the elongating roots in the absence of geotropic responses, the consistency of imbalance in the lateral roots during the period of most rapid growth adds further to our knowledge of the peculiar transport process that results in root epinasty.

4. Changes in Orientation with Alternation of Growth Position

As a critical test of the auxin hypothesis for control of root orientation in the wheat seedling, we have grown the plants under first one condition of exposure to gravitational force and then a second condition. The contrasting positions were growth erect to gravity vs. growth on a horizontal clinostat. If the orientation of a root tip is determined to a measureable degree by the distribution of its endogenous auxin during the period of elongation, rather than being predetermined by growth regulators that act within the embryo before or at the time of root emergence, a change in the effective gravity vector from the erect position to the conditions on a horizontal clinostat, or vice versa, should produce changes in the orientation of tissues formed after the tropistic effects of gravity are altered.

This alternation method has been used for 17 experiments, each with 12 or 15 seedlings grown in our holder system for 72 hours after initial seed immersion in water and 8 hours of hydration by contact of endosperm with wet vermiculite before organs were formed by germination. The 72 hours of subsequent growth were divided into an initial period of 38 hours and a second period of 34 hours, as indicated in

Table IV. The data for root orientation are there recorded as means of angles measured by methods described in earlier reports. The standard errors of the means show the variability that should be considered for each mean angle in evaluating the differences in orientation under altered conditions. Significant changes in orientation of the root tip after a change in position reflect gravitational effects on growth regulation during the elongation process.

The average position of the primary root could not be expected to change much from that reached in the first position, since about two-thirds of the elongation had been completed before the shift at the end of 38 hours. The most significant change was that produced in the mean side-view angle of about 55° after initial growth on the clinostat; during later growth in the erect position the roots grew downward enough to reduce greatly this angle of displacement from the seedling axis. The lateral deviations (from 180°) in the face-view angles were also smaller in erect plants.

Table IV. Effect of Gravity on Orientation Angles of Wheat Roots

Growth Positions	Erect →	Clinostat	Clinostat →	Erect
Hours of Growth	38	34	38	34
Prim. Root face view	182.2 ± 1.4	184.7 ± 2.4	171.3 ± 5.4	171.7 ± 4.0
" " deviation*	10.0 ± 1.0	14.6 ± 1.9	42.3 ± 3.7	30.5 ± 2.9
" " side view	21.4 ± 1.6	21.4 ± 2.9	54.6 ± 2.9	39.8 ± 2.4
Left Root face view	244.1 ± 1.2	257.8 ± 2.5	275.9 ± 2.0	252.2 ± 1.6
" " side view	12.1 ± 2.1	63.2 ± 5.5	96.5 ± 5.9	46.6 ± 3.5
Right Root face view	117.1 ± 1.1	105.8 ± 2.4	87.3 ± 1.5	109.5 ± 1.5
" " side view	15.3 ± 2.1	53.5 ± 5.2	96.4 ± 6.8	41.5 ± 3.5

* Deviation from 180° in face view

A similar direct effect of gravity during the elongation period of growth appears clearly in the data for both face- and side-view angles of the lateral roots. For change of position either from erect to clinostat or vice versa, the face-view angle between the root and a 180° orientation line was larger when the seedlings had just been growing on a clinostat. The side-view angle increased sharply if the shift was made to the clinostat and decreased when the plants were set erect to gravity after rotation for 38 hours in the horizontal position.

The larger face-view angle between the average lateral root and a 180° orientation after growth on a clinostat indicates the persistence of the root epinasty effect as the roots elongate; a different imbalance in growth regulation causes the root tip to be placed outside the plane of the 2 lateral roots, as measured by the side-view angle. Since the curvature which thus displaces the root tip to either side of this plane appears in both erect and clinostat seedlings, it probably represents variable imbalance in growth of suspended roots, imperfectly controlled by auxin distribution under the influence of gravity in erect plants and uncontrolled by gravity on a clinostat.

This proof of changes in orientation of roots when they are changed from the effect of gravity that may have induced a characteristic geotropic response in a newly formed root tip to a condition lacking in the effect of gravity has served to increase slightly the time that the wheat seeds can be held ater T-0 without degrading the results of the orbital experiment. A possible few millimeters of growth of the primary root during an enforced hold of a few hours can be tolerated without compromising the use of this root's final orientation as a criterion of the effect of weightlessness on growth regulation in a root.

5. Acceptance of a Paper by Editor of Plant Physiology

We reported in April, 1966, that we had submitted the manuscript for a technical paper on seedling physiology to the editor of Plant Physiology. That paper was accepted for publication and appeared in the June, 1966, issue. The title is "Orientation of Wheat Seedling Organs in Relation to Gravity" and Dr. Katsuyuki Yokoyama is a co-author.

This paper describes the techniques for growing grass-type seedlings in the seed stalks developed for the biosatellite experiment, reports the results obtained with wheat and certain other small grains, and presents an analysis of the action of growth hormones in relation to the force of gravity. [redacted]

V. INSPECTION TRIPS AND EXPERIMENTER MEETINGS

1. Philadelphia Conference and View of Flight Hardware

The conference on February 17-18th at the Space and Reentry Systems Division of the General Electric Company was valuable for the introduction to our flight hardware, the test conditions there, and the steps by which we shall learn to prepare our experiment on a tight schedule for incorporation of it into the payload of the spacecraft. We were instructed in the importance of writing out the precisely-timed steps to be followed in the drill to prepare experiments for delivery on schedule to the team of engineers who will load the vehicle.

In a group meeting of the experimenters with the wheat seedlings, we discussed the limitations and methods for the spacecraft tests, the use of our trailer laboratory, and the equipment needed for simulation of pre-launch and post-recovery operations. We also compared notes on special methods being used in our home laboratories to improve techniques for growing uniform seedlings.

2. Experimenters' Workshop in Los Angeles

During the period between similated launch and recovery of the March 20-24 qualification test at the North American Aviation facilities in Los Angeles, the experimenters took advantage of the opportunity to draft joint replies to requests from the Biosatellite Office for specific information and to prepare "standing instructions" for pre-launch and post-recovery operations.

We worked long hours by night and day to prepare outlines and timetables for all phases of the experiment. Secretarial service produced the necessary copies of our letters and S. I.'s. The times required for the tasks performed under the tentative plans for the Los Angeles test were used to develop a timetable that could be maintained by an experienced crew. Difficulties encountered through inadequate equipment and foresight were reviewed and noted as situations to be avoided in later tests. Personnel for all steps in the operations were designated for their skills, experience and availability.

3. Cape Kennedy Inspection and Conference

The April meeting of experimenters and others involved in launching the spacecraft from Cape Kennedy gave us a clear idea of the complex assignment to assemble a payload in cooperation with the rocket engineers. We were able to check on the laboratory space provided for loading the wheat seedling package and to arrange for such supplementary facilities as humidifier, work tables and office desks.

We worked at night to perfect our standing instructions and to draft joint replies to requests for details of materials, control experiments, personnel and technical points needed at once by the administrative officers of the project. The final stages in this joint study of working outlines and provisions for adequate equipment in the launch and recovery laboratories were completed at Emory University in Atlanta, Georgia, where the wheat seedling experimenters from the West and North gathered for one day's work on the return trips to their homes.

VI. TESTS AND TESTING PROBLEMS

1. Biocompatibility of Flight Hardware

A unit of the flight-type package, slightly modified from the prototype form, was given a complete qualification test of mechanical and biological compatibility in the facilities of North American Aviation in Los Angeles on March 20-24th. The test was conducted with a full complement of plants on a timetable of simulated hold before flight, estimated profiles of vibration and acceleration stresses at launch, 72-hour orbit (on a clinostat) with in-flight fixation of seedlings at 48 and 61 hours, reentry stresses, and a delivery time of 5 hours after recovery.

Three packages were loaded with seed stalks at hourly intervals, with seeds soaked for 2 hours and planted in about 30 minutes per package. The flight-type package was prepared first, before the workers had overcome some difficulties of new equipment and changes in the design at one end of the seed stalk. The clinostat control and erect control packages were better prepared, partly because the stalks were prototype and easy to load properly by our established methods. The data for number of seedlings and mean lengths of their organs are assembled in Table V below.

The serious problem of soft and uneven vermiculite packing in all flight hardware stalks and in some of the control package stalks was considered to be the cause of subnormal germination and growth throughout the flight package and in a few chambers of control packages.

Inspection of the seed pockets at the close of the test confirmed the fear that loss of contact between endosperm and vermiculite in the side arms had resulted in many ungerminated seeds and seedlings with suspended growth. The presence of mold on these seeds may have contributed to the poor or inhibited growth of the embryos but the poor packing of the side arms was probably the major reason for failures.

New methods were introduced for the post-recovery operation, such as 85% relative humidity in the disassembly laboratory, opaque cylinders with a basal supporting cup in each for dark storage of seed stalks between photography and seedling measurements, and an improved model of rack for camera, 45° mirror and seed stalk positioning pins. These new methods and others used for pre-launch tasks proved successful in meeting needs for improving our procedures but certain failures to apply them properly emphasized the need to have a second person check each operation as the work is being done.

Except for minor differences in a few cases, the coleoptiles and roots of the seedlings that grew during the test were oriented as we have found them to be in earlier growth tests. The stresses of launch and reentry do not appear to have affected the mechanism of orientation. The data of particular interest for this essential point are the mean values of the orientation angles for seedling organs grown in the new hardware and organs grown in prototype hardware in the clinostat control package, shown in Table V on the next page.

The first 2 entries in Table V show excellent agreement of the means within accepted statistical limits except for a small difference in the face-view angle of the right lateral root. Comparison of this measurement with that for the clinostat standard (third entry in Table V) shows that the plants in the flight package came closer to the standard than did the seedlings in the clinostat package. The larger mean angle for this measurement in both test packages was probably due to the less vigorous growth of the seedlings with the water supplies for the 77-hour growth period reduced by the sub-optimal conditions for loading the stalks.

Table V. Mean Orientation Angles of Wheat Seedling Organs
NAA Test of Flight Hardware - March 20-24, 1966

Package	Coleoptile		Primary Root		Left Root		Right Root	
	Face	Side	Face	Side	Face	Side	Face	Side
Flight	6.5	12.6	*	38.6	270.2	91.0	86.2	95.3
(new) S.E.	2.0	2.3		8.6	5.1	15.5	3.4	12.2
Clinostat	6.9	12.3	*	43.4	275.3	99.2	96.9	69.3
Control S.E.	1.2	1.6		7.3	4.0	11.7	3.1	10.2
Clinostat	11.4	12.8	*	44.4	285.1	116.7	75.1	123.7
Standard S.E.	.8	1.4		3.0	1.8	3.7	1.8	4.0
Erect	4.1	6.0	182.1	13.4	230.5	17.0	132.6	21.1
Control S.E.	.9	1.1	2.0	3.0	3.3	3.3	3.2	3.1
Erect	3.8	5.5	184.2	12.4	237.0	21.3	121.7	18.4
Standard S.E.	.4	1.8	.9	1.0	1.3	1.6	1.4	1.3

* Mean has no significance

The mean face-view angle for the left root in both clinostat and flight packages reflects the same reduction in the epinastic curvature over that of the clinostat standard (285.1°). The smaller side-view angles for lateral roots in the test packages and the reduction in face-view curvature of the (shorter) coleoptiles of the test, in contrast with the corresponding data for the clinostat standard, attest to the same lack of vigorous growth.

In spite of these quantitative differences in orientation of seedling organs and in vigor of the seedlings grown in the flight hardware for the first time, the test in Los Angeles proved the biocompatability of the new package for its use in an orbital experiment.

2. Vibration Test in 201 Spacecraft

Nature of Test

A severe vibration test in the General Electric Co. facility at Philadelphia was applied in an experiment that ran from seed soaking on May 27th at 0930 to May 30th at 1930. At this time the seedling organs were measured after a photographic record had been made in duplicate on separate films, one with the 45° mirror at the right and the other with the side-view image in a left-hand mirror.

The vibration was random and longitudinal with the axes of the embryos, applied for about 4 minutes of simulated launch but at the qualification level of 50% above the forces expected at launch. The seeds had been immersed in water about 12½ hours before vibration and were thus ungerminated.

Materials

The vibrated package was flight-type, without fixation squibs, and loaded with 78 seeds of the 1964 crop on 6 stalks that were numbered in the established pattern of $\begin{smallmatrix}1&3&2\\4&5&6\end{smallmatrix}$ where stalks 1 - 3 are in single cylinders and stalks 4 - 6 with 12 seeds each are spaced evenly in the large chamber. The control package was prototype and differed in seedling plan only by having 15 seed arms on stalk 3 rather than 12 seeds as in the flight-type hardware. Stalks 1 and 2 carry 15 seeds each in both types of packages.

Procedure

Following vibration of the spacecraft and its several experimental packages, the vehicle was held intact at unknown temperatures for mechanical checks before disassembly and delivery of the wheat seedling package to the experimenters at 1100 on May 28th. The seeds had then begun to germinate at the age of 25½ hours from initial soak time, with a few primary roots about 0.5 mm. long. Seeds in the control package had germinated to a slightly greater extent at the laboratory temperature of 74 ± 1° F.

Shortly before return of the vibrated seeds, the erect, non-vibrated stalks (with seeds 23 hours from initial soak time) were removed from the package in 2 groups and placed in jacketed cylinders to permit continued growth for the 72 hours of simulated flight time plus a few hours of simulated recovery period. Stalks 1, 3 and 4 were transferred to chambers mounted on horizontal clinostats for rotation at 1 rph. Stalks 2, 5 and 6 remained erect in a large cylinder to which stalks 1, 3 and 4 of the vibrated package were later brought and set erect until the close of the experiment.

The distribution of stalks of vibrated seeds was made as soon as they were returned from the spacecraft. Stalks 2, 5 and 6 were sealed in clinostat chambers and growth was allowed to proceed in all chambers at laboratory temperature of 74° F. until the test was closed with measurements at seedling age of about 82 hours.

Experimental Data

Mean Lengths of Seedling Organs (± S. E.)

Treatment	No. of Seedlings	Coleoptile	Primary Root	Left Root	Right Root
Vibrated					
Clinostat	14	5.9 ± 1.0	29.7 ± 3.1	16.9 ± 1.7	18.4 ± 1.3
	10	3.5 ± 0.3	22.0 ± 2.4	13.7 ± 1.8	13.2 ± 3.1
	12	4.8 ± 0.6	24.2 ± 2.4	17.9 ± 0.9	17.7 ± 1.1
	36 Mean	4.9	25.4	16.7	
Erect	14	3.4 ± 0.4	19.2 ± 1.7	14.1 ± 1.9	12.6 ± 2.5
	12	3.8 ± 0.3	23.9 ± 2.9	13.8 ± 2.7	14.5 ± 1.2
	12	3.2 ± 0.5	20.5 ± 2.0	15.0 ± 1.8	13.6 ± 3.2
	38 Mean	3.5	21.1	13.9	
Non-Vibrated					
Clinostat	15	7.6 ± 0.6	29.4 ± 2.9	19.7 ± 2.0	17.6 ± 1.2
	13	9.1 ± 1.0	41.1 ± 3.0	26.5 ± 1.7	23.3 ± 1.9
	12	4.0 ± 0.6	20.8 ± 3.0	13.0 ± 1.8	14.0 ± 1.5
	40 Mean	7.0	30.6	19.3	
Erect	13	3.5 ± 0.7	23.1 ± 2.8	13.3 ± 0.6	11.3 ± 1.7
	7	3.8 ± 0.3	23.6 ± 4.9	17.4 ± 2.5	13.3 ± 3.0
	12	4.5 ± 0.6	25.2 ± 1.8	16.4 ± 2.8	15.8 ± 2.4
	32	3.9	24.0	14.5	

Conclusions

1. Both vibrated and non-vibrated seeds grew slightly faster on clinostats than erect, the usual result that seems to be due to an even distribution of the water supply by reason of rotation of the seed stalk.

2. The slightly reduced growth of seedling organs from vibrated seeds over non-vibrated seeds with the same growth conditions after vibration time may be due to loss of water from the stalks during vibration but except for the lengths of coleoptiles that develop on clinostats, the differences are not statistically significant.

3. Measurement of the possible effect of vibration on the orientation of seedling organs has not yet been completed but study of the pictures taken at the close of the test suggests no effect.

3. Thermal-Vacuum Test of 201 Spacecraft

Nature of Test

To test the spacecraft systems for maintaining the biosatellite experiments within specified limits of capsule temperature and pressure during orbital flight, plus the behavior of the biological materials during the test, the fully loaded and instrumented vehicle was enclosed in a vacuum test chamber for 3 days of simulated orbit. The predicted range of temperatures was programmed by the use of electric blankets to replace solar radiation and an enclosing reservoir of liquid nitrogen to provide the low temperature of space. The actual environment was monitored and recorded from the time of assembly through simulated reentry by a system of telemetry like that to be used from the orbital path.

The wheat seedling experiment was included in the payload for the test of June 12-16, 1966. The period of simulated flight was June 13 at 1000 to June 16 at 1000, preceded by about 12 hours of pre-launch hydration of the seeds and followed by about 13 hours of check-outs and disassembly. The profile of temperatures for the seedlings during the test was characterized by 12 hours above 70° F., a cold phase in the test chamber with capsule temperatures in the low 60's, a warm phase of high 60's and low 70's, and 13 hours of temperatures from 66° to 70° F.

Materials

The test package was the one used for the 201 Vibration test but the fixation squibs had been installed for use on command at 48 and 60 hours after "launch". Control plants of the same number were grown in the same type of seed stalks set in lucite cylinders and held at a temperature of about 74° F.

Procedure

June 12 & 13	Three-hour soaking, selection and planting of seeds in 12 prepared stalks.
June 15	Fixation of plants in chamber I at 1000 and in chamber II at 2200, with simultaneous photography and measurement of seedlings from the corresponding stalks in control cylinders.
June 16	Close of test with pictures and measurements of organs at seedling age of 97 hours for unfixed test seedlings and 96 hours for stalks III-VI of control plants.

Experimental Data

				Lengths of Seedling Organs		
Test	Chamber	Seeds	Age	Coleoptile	Primary Root	Lateral Root
Package	I	15	60 hrs	mean=0.40 mm	mean=0.37 mm	mean= 0 mm
	II	15	72	mean=0.90	mean=0.57	mean=0.13
	III	12	97	total 7.0	total 9.5	total 0
	IV	12	"	" 14.0	" 24.5	" 2.5
	V	12	"	" 16.5	" 32.5	" 18.5
	VI	12	"	" 12.0	" 27.0	" 24.0
	III-VI	48	"	" 49.5	" 93.5	" 45.0
			97-hr mean	1.03 mm	1.95 mm	0.47 mm
Control	I	15	60 hrs	mean=2.4 mm	mean=18.3 mm	mean= 4.6 mm
Package	II	15	72 hrs	mean=3.7	mean=25.5	mean=11.2
	III	12	96	total 62.0	total 355.2	total 414.0
	IV	12	"	" 48.5	" 201.6	" 383.9
	V	12	"	" 58.0	" 339.0	" 399.0
	VI	12	"	" 36.0	" 167.0	" 211.0
	III-VI	48		" 204.5	" 1062.8	" 1407.9
			96-hr mean	4.26 mm	22.14 mm	14.67 mm

Conclusions

1. The growth of the seedlings in the test package was initiated but severely inhibited by something.
2. The normal growth in the control cylinders showed that the inhibitory factor was contained within the spacecraft.
3. If it was not within the test package, the retarded growth may have been due to low temperatures, since the small seedling organs had a normal appearance such as root hairs on roots long enough to bear them.

4. The unknown factor must be corrected before the experiment can be considered ready for use in the biosatellite project.

4. Efforts to Identify the Cause of the 201 T-V Failures

Toxicity in Hardware?

To test the 201 package for possible toxic compounds that might have been released or produced within the polycarbonate cylinders since the hardware had been used for the 201 Vibration test, a 72-hour growth test was conducted at once.

Two stalks of 12 seeds each were prepared and installed in the large chamber without cleaning it in any way. The same number of seeds were planted in 2 other stalks set erect in separate lucite cylinders. The growth of the seedlings from the two sources was measured at the end of 72 hours with a temperature of 73 ± 1° F. The means of seedling organ lengths were as follows:

Growth in	Coleoptile	Primary Root	Lateral Roots
Test Package Chamber	4.1 ± 0.7 mm	24.2 ± 2.2 mm	12.6 ± 1.3 mm
	3.7 ± 0.3	24.9 ± 2.1	12.7 ± 0.9
Control Cylinders	4.2 ± 0.3	26.5 ± 1.4	11.7 ± 1.0
	3.0 ± 0.2	23.1 ± 1.4	6.0 ± 1.2

The close agreement of seedling growth in the test package with the normal growth of the control seedlings removed all doubt that the cause of the retarded germination in the thermal-vacuum test might be inhibitory compounds within the test chambers. The seedlings grew well in both containers in the laboratory.

Errors in Report of Spacecraft Temperatures?

Since all temperature readings were obtained from thermistor data telemetered from within the payload compartment during the test, the

General Electric Company was asked to verify the accuracy of its data. In the absence of any other reasonable cause for the slow development of roots and coleoptiles that appeared to be healthy, a temperature close to the minimum for wheat germination, perhaps as low as 50° F., was indicated by the plant growth.

The directors of the thermal-vacuum test have insisted that their data are correct. Later checks with thermocouples beside their thermistors have confirmed the accuracy of their measurements as reported by telemetry.

Later Developments

The report period closed without a solution to the problem of retarded germination. This report could be prepared only after about 2 months of study and growth tests had been devoted entirely to the discovery of the cause for the inhibition. The non-toxic interference with seed germination was reported on August 25th to originate in the heater blankets around the growth chambers but the real cause for the inhibitory effect of the electric current within the heating units is still unknown.

Department of Biological Sciences
Dartmouth College
Hanover, New Hampshire

Charles J. Lyon