Short Communication

Wheat Seedling Growth in the Absence of Gravitational Force[1]

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The orientation of wheat seedling organs has been shown by Lyon and Yokoyama (6) to be controlled by the interaction of geotropism and root epinasty. A weak form of plagiotropism keeps the primary root from growing straight down in a gravitational field. The first pair of lateral roots are strongly plagiotropic as they grow in a plane on opposite sides of the primary root.

The epinastic tendencies produce growth curvatures in the roots if the geotropic responses of the seedling organs are eliminated by rotation of the developing seedling in vertical planes about the axis of a horizontal clinostat. Growth curvatures form in the coleoptile during its growth on a clinostat when this organ is elongating fast enough to show the effects of unequal basipetal transport of its auxin.

This information about the growth form of a wheat seedling in relation to gravity was obtained primarily as baseline data for an experiment in which wheat seedlings were to be grown in an earth satellite and returned for analysis of the effects on germination and growth. The successful launch of NASA's Biosatellite II in September, 1967, its controlled orbital flight at an altitude of about 170 nautical miles for nearly 2 days, and the return of the experiments by parachute and airplane has provided unique experimental evidence in the area of growth physiology of seedlings. The results also provided the first basis for testing the reliability of the rotary clinostat for critical studies of geotropism and related aspects of plant growth.

Materials and Methods

Seeds of winter wheat (*Triticum aestivum* L.), selected for uniform weight and good embryos, were planted in holders devised by a group of experimenters to produce sets of seedlings with organs free of mechanical disturbance of their tissues and orientation. These experimenters shared the use of the seedlings which grew in the orbital experiment package and in 4 identical packages planted and managed for ground control experiments.

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The preparation of the sets of planted seed holders and their installation in 4 chambers of each sealed package was carried out according to a rigid plan and timetable under the supervision of qualified inspectors. The system provided for accuracy in timing the growth periods and a maximum of uniformity in the growth conditions for the 78 seeds in each experiment, flight and control. The form, structure and use of the seed holders were described in our earlier papers (3,6). Each seedling developed with its organs growing in moist air, free from contact with solid materials.

The length of each coleoptile and root was measured at the close of an experiment, after a picture on color film had been taken of each row of seedlings, as in figure 1. As explained and illustrated in the 1966 paper, each combined face- and side-view picture of the plants was used later in projection on a wall. There the orientation of the tip of an organ could be measured by the angles formed between the longitudinal axis of the seed and a line drawn from the tip to the base of the root or coleoptile. The 2 angles for each organ were computed from a set of X, Y, and Z coordinates, measured by dividers and scale from the projected, enlarged image of each organ and a vertical line through its base. A computer program supplied the angles with an accuracy of 0.1° as they are used in the data for means in table II. Each face-view angle was measured clockwise from the vertical 0° position while the side-view angle described the third-dimensional displacement of the organ from the same 0° to 180° line through the origin of the organs.

An experiment package consisted of 4 plastic cylinders mounted on a metal base and each fitted with a thermistor for periodic records of internal temperature (3). One large cylinder provided space and a moist, sea-level atmosphere around 3 sets of seedlings. Of the other 3 chambers with 1 seed holder each (5), 2 were equipped to spray-fix their seedlings with formalin-acetic acid-alcohol before the package was returned from orbit. This fixation was performed in each package about 58 hours after initial hydration of the seeds and 43 hours after launch of the spacecraft. The other seedlings grew 2 hours longer before return of the experiment to gravity while on the parachute. They continued to grow with seed axis erect to gravity for about 5

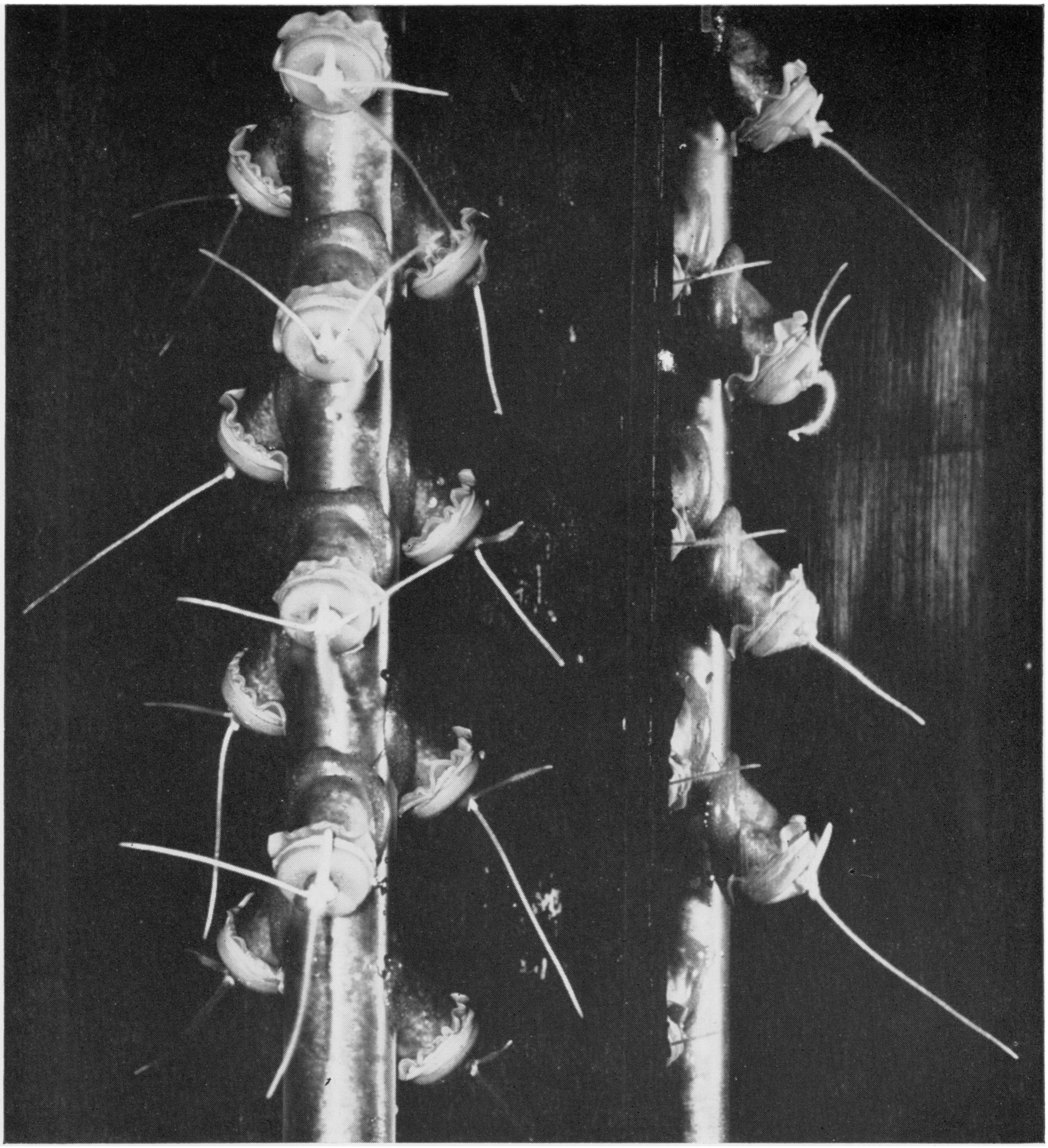

Fig. 1. Wheat seedlings that grew during 45 hours of orbit and 5 hours of recovery period. Side views in 45° mirror at right of face views.

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hours, until their removal from their growth chambers at the recovery laboratory in Hawaii.

The time required for assembly of the various experiments and spacecraft parts resulted in the earliest steps of seed germination being subject to the influence of gravity for about 15 hours. Post-flight tests have shown that germination during this period goes no farther at most than ruptured seed coats over the radicle end of the embryo. The culture system produced no organs of measurable length before the partially hydrated seeds were carried into the free fall conditions of orbit. Possible effects of vibration and acceleration during launch had been pre-tested and found to have no detectable effects on growth rate or form of seedlings grown thereafter on horizontal clinostats.

Of the 4 ground controls for the orbital experiment, the Erect Control I package was held at 70° F with the seed holders and embryos erect to gravity. The temperature of the Erect Control II package was made to vary narrowly around a mean of 67.5° F, following that of the flight package as reported by telemetry. Clinostat Control II seedlings grew for 60 hours at a mean temperature of 70.6° F, with the package attached to a horizontal clinostat that rotated the plants at 6 rph for 45 hours while the flight experiment was in orbit. Clinostat Control I seedlings grew on a similar clinostat for the same period, then erect for 5 hours, with the package at a mean temperature of 68.7° F. Because of a conflict with the closing time of this ground control experiment after the same 65 hours of growth as in the flight experiment, the seedling organs of Erect Control II were not measured until about 66.2 hours from the time of immersing the seeds in water.

Results and Discussion

Seedlings grown in the orbital experiment of Biosatellite II resembled those from the clinostat ground controls in every respect. They differed from seedlings grown erect to gravity only in the orientation of their organs, notably the roots. The germination rate was about 96 % in all experiments and only 3 seedlings were eliminated as too defective for use in calculations of mean lengths.

Organ Length. The mean lengths of the organs from the 5 test packages are shown in table I. The fixation of some plants with only 43 hours of growth in free fall and the return of the capsule from orbit 2 hours later found many of the seedlings with lateral roots less than 2 mm long. Since the extent of growth of all organs was the same for the orbital and control experiments, the average lengths were computed without selection or adjustment of data.

The essential agreement of measurements for 25 seedlings fixed in orbit at growth age 58 hours and for the 48 plants recovered alive after growing about 7 hours longer, with comparable measurements from the control experiments, is apparent from the entries in table I. The statistical analysis of the data for length of organ was directed specifically toward significant differences at the 1 % level of confidence. The computer program for means, variance and T-ratio was based on the Kurtz method (2) for pairs with possible unequal variance. The exact probability was computed by the Veldman (7) 2-tail formula for use of known T-ratio and degrees of freedom.

The few cases of differences in organ growth appear mostly among the means for the lateral roots of the control experiments and in the measurements at age 58 hours. This is understandable from the differences in temperatures and in the variability of initial germination stages under the conditions for water supply to the embryos. Except for 1 instance of slightly shorter coleoptiles at 65 hours in an erect control and for the longer lateral roots at the higher temperatures of a clinostat control test after 58 hours, the organs that developed in the orbital experiment were neither longer nor shorter on the average than those in the ground control packages.

The absence of gravitational force within the organs of a seedling seemed to have no effect on the basic growth processes and biochemical reactions which control the rates of meristematic activity.

Table I. *Mean Lengths of Wheat Seedling Organs*[1]
Flight and ground control experiments.

	Mean	Coleoptile		Primary root		Lateral roots	
58 Hrs	*F°*	(*n*)	*mm*	(*n*)	*mm*	(*n*)	*mm*
Flight	67.5	(25)	3.2	(25)	8.6	(50)	2.4a
Clino. control I	68.7	(25)	2.7a	(25)	7.7a	(50)	2.0cd
Clino. control II	70.6	(30)	3.3a	(30)	11.0a	(60)	3.8ade
Erect control I	70.0	(27)	2.8	(28)	8.8	(56)	1.8bc
Erect control II	67.5	(27)	2.9	(27)	10.4	(54)	4.0bc
65 Hrs[2]							
Flight	67.5	(48)	4.1a	(48)	21.5	(96)	10.1
Clino. control I	68.7	(46)	4.0	(46)	21.3	(92)	10.6
Erect control I	70.0	(45)	3.6a	(46)	21.5	(92)	9.7

[1] Comparable pairs of means are significantly different statistically only if coded with the same subscript letter.
[2] Organs for Clinostat Control II and Erect Control II were not measured at 65 hours.

Table II. *Mean Orientation Angles of Seedling Organs*

Flight experiment and ground controls.

Organ angle data No.	Coleoptile (n)	Face[1] + Side	Primary root Face[1]	(n)	Side	Left root Face	(n)	Side	Right root Face	(n)	Side
Flight	(73)	15.2a[2]	21.2a	(71)	34.7ac	279.7a	(45)	135.8a	79.7a	(51)	112.8a
Clino. control I	(71)	13.1ab	20.2a	(69)	26.1a	272.1a	(47)	94.5c	83.1a	(50)	102.6a
" " II	(72)	15.7a	28.0a	(72)	43.8c	277.9a	(61)	114.2ac	76.7a	(58)	118.3a
Erect control I	(67)	10.6b	7.8b	(70)	12.5b	242.0b	(48)	18.1b	116.4b	(53)	16.7b
" " II	(73)	11.1b	8.5b	(71)	10.3b	239.8b	(63)	21.1b	116.8b	(64)	16.5b

[1] Angle measures deviation from the 0° to 180° line, left or right.
[2] Pairs of means in any column are not significantly different statistically if coded with the same subscript letter.

There was also no detectable disturbance in the production and transport of growth regulators during the germination stages. The limited use of seeds in Russian satellites (1) did not test these effects because the conditions for germination were not supplied until after the seeds were returned to earth.

Orientation of Organs. The criteria for determining possible effects of orbital growth on the orientation of seedling organs appear in comparisons of face- and side-view angles at which the organs grew in the flight and ground control experiments. Marked differences were known to occur between organs produced on clinostats and those which develop from embryos erect to gravity (6). The critical test of special effects from germination during free fall is comparison of orientation angles in the flight and clinostat control seedlings.

The data for these angles in table II show close agreement of the mean values for all organs, as indicated by the coding of statistically significant differences. The methods used for the analyses of data were the same as those used for length of organs. There are very few differences among the comparable angles which describe the orientation of organs that developed without a geotropic effect of gravity. With only 1 exception, these angles are significantly different from the corresponding mean values for seedlings held erect to gravity.

The limited growth of the coleoptiles in the curtailed experiment made it necessary to combine the measurements for displacement of the tips in the face-view and side-view planes. The average imbalance in growth from the initial orientation of this organ was predictably smaller than that for a root either in free fall or on a clinostat, since a growth curvature of a coleoptile was due only to an unequalized longitudinal transport of auxin (4) from the tip of a short organ. The much larger growth curvatures of roots produced without a gravity vector in flight or a geotropic effect on clinostats were mediated by the unequal supplies of growth hormone to opposing sides of an organ in which an epinastic tendency (6) is responsible for the plagiotropic orientation when the plant grows erect to gravity. The symmetry of the epinastic force in the lateral roots of the wheat seedling and its like effect during seedling growth on a clinostat or in the orbital experiment can be seen in the mean face-view angles for the 2 lateral roots as displayed in table II.

Clinostat Confirmation. The essential identity of the small growth curvatures in the coleoptiles and of epinastic responses in the roots of seedlings grown in orbit, with the corresponding orientation pattern of seedling organs which developed in plants grown on a horizontal clinostat has confirmed the reliability of this device as an instrument for terrestrial studies of geotropism and related aspects of growth physiology. The absence of differences in size of organs between those developed on a clinostat rotating at 6 rph and those grown either in free fall or erect to gravity also indicates a lack of disturbance of the basic growth processes in such a plant. The limitation of the experiment, however, to small plants grown in darkness from stored foods does not preclude possible secondary effects on morphogenesis in the absence of an effective gravity vector within a plant that normally grows with an erect axis.

Acknowledgments

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Literature Cited

1. Il'ina, G. V., *et al.* 1966. Effect of spaceflight conditions on wheat seeds and plants grown from the seeds. Komicheskie Issledovaniia 4: 320–23.
2. Kurtz, T. E. 1963. Basic Statistics. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.
3. Lyon, C. J. 1965a. Final report July 1, 1964 to June 30, 1965. National Aeronautics and Space Administration Contract No. NAS2-1558 for the development of Biosatellite Project Experiment P-1096. The Effect of Weightlessness on the Growth and Orientation of Roots and Shoots of Monocotyledonous Seedlings. NASA CR-75092.

4. LYON, C. J. 1965b. Action of gravity on basipetal transport of auxin. Plant Physiol. 40: 953–61.

5. LYON, C. J. 1967. Rotation axes for analysis of gravity effects on plant organs. Plant Physiol. 42: 875–80.

6. LYON, C. J. AND K. YOKOYAMA. 1966. Orientation of wheat seedling organs in relation to gravity. Plant Physiol. 41: 1065–73.

7. VELDMAN, D. J. 1967. Fortran Programming for the Behavioral Sciences. Holt, Rinehart, and Winston. New York.