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Limitation on the Use of the Horizontal

Clinostat as a Gravity Compensator

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by:

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ABSTRACT

If the horizontal clinostat effectively compensates for the influence of the gravity vector on the rotating plant, it should make the plant unresponsive to whatever chronic acceleration may be applied transverse to the axis of clinostat rotation. This was tested by centrifuging plants while they were growing on clinostats. For a number of morphological endpoints of development the results depended on the magnitude of the applied g-force. Therefore, gravity compensation by the clinostat was incomplete. This conclusion is in agreement with results of satellite experiments which are reviewed.

A clinostat, sometimes spelled klinostat, is a mechanical device used by plant physiologists to rotate a biological specimen about an axis, commonly the longitudinal axis of a higher plant. In most applications of the clinostat the axis of rotation has been held at 90° to the plumb line so that the gravitational force vector would act at all times transversely with respect to the main shoot axis. Thus, as a test plant mounted on the clinostat slowly rotates, in one revolution the gravity vector sweeps through 360° around the plant. It seems appropriate to refer to this as omnilateral stimulation by the gravity vector, if one can assume that the plant integrates the stimulus over a time at least as long as the clinostat rotation period. Rotation rates generally have been in the range from one or a few revolutions per hour to about one per min. In principle a relatively simple device, the clinostat has been in use for about a century to provide a very special kind of manipulation of the gravitational information which plants receive from their environment.

The popularity of the horizontal clinostat in certain plant physiological researches is attributable to its singular property of minimizing geotropic responses of slowly rotating plants through the substitution of a discontinuous but essentially omnilateral gravitational stimulus for a directional stimulus of the same magnitude. The rationale for this depends on a special functional property of the gravity sensors of plants whose design is different from and less well understood than those of many animals. The important operational difference is the inability of the plant to respond to gravitational stimuli of limited duration. Thus, a plant displaced from the plumb line to a horizontal position does not exhibit an obvious response (righting reaction) unless the displacement has been maintained usually for at least several minutes -- sometimes tens of minutes. This period, the minimal time of exposure to a transverse gravitational stimulus which is sufficient to elicit a geotropic response, has been called the "minimal presentation time" or simply the "presentation time". We consider the former term less cryptic and shall refer to it here as MPT. For the more georesponsive higher plants the MPT lies between about 10 and 100 sec, some 2 or more orders of magnitude longer than the comparable value for most higher animals. Mounted on a horizontal clinostat whose period of rotation is less than or at least not much greater than its MPT, the plant experiences a time averaged stimulus which remains in one plane but has no preferred direction. Since the MPT is relatively long, rotation of a small plant (a few cm in extent) can be made slow enough so that it will not produce a centrifugal acceleration of unacceptable magnitude. Of course with animals, for which a much shorter MPT is characteristic, the slowest rotation rate which can produce an effectively omnilateral stimulation by gravity still would be fast enough to impose centrifugal acceleration which would be unacceptable. Therefore, the zoologist is left without a working range in which to design a clinostat experiment for his animal material. Accordingly, the clinostat must be considered an essentially botanical device.

A plant turning on a clinostat experiences a succession of geotropic stimuli. For every small element of stimulus in one direction there is, within a time believed not resolvable by the plant, an equal and opposite element of stimulus. The condition often is referred to as "gravity compensation". The clinostat rotated plant also can be said to experience a time averaged gravitational force vector of zero and evidently for that reason the condition achieved by clinostat rotation has been called "gravity nullification" -- a term which carries some unwarranted implications.

Gravity compensation, even if completely effective, of course does not remove chronic gravitational stimulation. That can be achieved for protracted periods only in the condition of free fall as is attained by an orbiting satellite. The acceleration free state (weightlessness) is basically quite different from the chronically accelerated state of gravity compensation. The absence of convection in the former but not the latter condition is one obvious physical difference. What the clinostat achieves operationally is an alteration of a certain biological response due to its special manipulation of gravitational information input to the test subject; the physical aspects of that manipulation are in no way novel.

Several lines of reasoning suggest at least indirectly that the clinostat is an imperfect simulator for weightlessness. Long ago Newcombe (18) among others listed some limitations to its application. Experimentally the choice of rotation rate has been questioned repeatedly and found to be critical for some effects; e.g. Lyon (14). Also in some experiments of Larsen rotation rate was found to be critical only in the light, not in the dark (17). Zimmerman (22) reported a tendency for the bending of plant organs as a response to clinostating, always away from the direction of rotation (as if the plants could distinguish clockwise from counterclockwise rotation). "Curvatures of Zimmerman" as they were called evidently were rediscovered by Hoshizaki and Hamner (9). A theoretical justifica ion which could apply to such a discrimination capability may be found in an article by Freier and Anderson (6) although a more trivial explanation could be based either on irregularities in the rotation rate (backlash?) or on mechanical vibration from the clinostat drive motor as discussed by Gordon in another context (7).

The preceeding comments refer mainly to the bending responses of plant shoots or roots and not to other kinds of developmental phenomena. It often is overlooked that the observed suppression of responses in a clinostated plant applies to its geotropic reactions and to little else. Since the omnilaterally stimulated plant on the clinostat does not respond geotropically even though its axis is horizontal, it may be presumed (although it has not yet been proven) that the clinostat must produce essentially the same biological result as would occur if the plant were not stimulated at all. However, there is no reliable basis for extending that presumption to include many other facets of the plant's physiological behavior or morphological development which appear to be or are known to be affected by gravity. Even for geotropic responses the difference between omnilateral stimulation and no stimulation at all has been clearly emphasized (16).

One must keep in mind the operational distinction between <u>geotropism</u>, a term probably coined by Frank (5) for a specific type of directional response by the plant to the gravitational vector* and the broader term, <u>gravimorphism</u> (21), which refers to the ways development of form depends on the test subject's input of gravitational information (10). Gravimorphic

* Gravitropism also has been suggested as a possibly more suitable term but has not yet won popularity among understandably geocentric biologists.

effects generally cannot be simply and confidently deduced from knowledge of altered geotropic responses. Moreover such questions cannot be decided in principle; at the present stage of our knowledge of gravimorphism they are quite empirical. Speculation can be only helpful but hardly decisive in advance of direct comparisons of morphological behavior of clinostated plants and those developing under weightlessness. However, the effects of clinostating on the ontogeny of seedlings are readily determined and some of our studies on development of Arabidopsis plants bear directly on the effectiveness with which gravity compensation was achieved by clinostats.

MATERIAL AND METHODS

Our choice of test species was *Arabidopsis thaliana* (L.) Heynh. The seed stock is traceable to Prof. G.P. Redi, Univ. of Missouri; it was derived from a mutant identified as 294-187-F. Plants were cultured aseptically at 24 $\pm$ 1 C on nutrient agar in individual modules under continuous illumination. The method has been described elsewhere (2) and reported in detail (3). In all studies the growth period was 21 days from the time of planting. To provide gravity compensation the test plant modules were inserted into holders of individual clinostats ganged together in groups of 24 so they could be rotated by a single drive motor. In most experiments the rotation rate was 2 rpm. To vary the g-level in different experiments a centrifuge was employed. The clinostats were located within swinging cradles and the orientation of clinostat axes was always parallel with the longitudinal axes of the plants and at 90° to the direction of the resultant force vector. In some preliminary tests the clinostated plants were not always in swinging cradles but sometimes were mounted on the centrifuge at a fixed angle to the plumb line calculated to achieve the same effect when the centrifuge turned at the prescribed speed. Whatever g-level had been chosen, it was maintained throughout a 21-day period after which the plant modules were flooded with Karpechenko's cytological fixing solution (8). Subsequently a series of gross morphological measurements were made on each member of the population.

This procedure, repeated over a range of g-levels, provided information from which a g-functinn could be calculated for each morphological character considered. We did not make a *post facto* selection of characters; all data in the relevant categories are reported. A total of 176 plants were used.

The objective of these tests was to determine whether any of the characters studied was significantly affected by the prevailing g-level under the condition of putative gravity compensation. For each character the correlation with g-level was calculated and was tested following the method described in Ezekiel (4) to determine whether it was significantly different from zero. If so, the character was demonstrated to be g-dependent.

A series of three preliminary experiments were carried out at the NASA Ames Research Center prior to the installation of a centrifuge in our home laboratory (3). The results of those experiments did not disagree with the findings from our later studies. However, fewer plants were used in the Ames tests and, therefore, the precision of the measurements was greater in the more extensive experiments we carried out in Philadelphia. We believe the recent data are more convincing statistically and thus form a more satisfactory basis for deciding to what degree the clinostat was able to achieve gravity compensation. It would be possible, of course, to pool the data from both sets of experiments on the different centrifuges. Although this might seem advantageous (cf. Fig. 1), there were several presumably minor differences in test conditions between experiments at the NASA Center and those done several years later on the centrifuge in Philadelphia, which made it less desirable to pool data from both sources.

At least one previous research effort involved the study of gravitropism in plants which were clinostated and centrifuged in the same experiments(19). The study was designed for a purpose different from ours and its results are not applicable here.

RESULTS

Morphological endpoints of seedling development were measured and the following regression equations were determined by the method of least squares:

Total leaf length (mm), $T = 10.390 - 0.1925g$

Length of petiole (mm), $P = 5.330 - 0.1870g$

Length of leaf blade (mm), $L = 4.93 - 0.0110g$

Width of leaf blade (mm), $W = 2.924 + 0.0040g$

No. of rosette leaves, $N = 4.998 + 0.1463g$

Length of hypocotyl (mm), $H = 8.669 - 0.7087g$

Length of flowering stem (mm), $F = 44.248 - 1.627g$

Figures 1-3 are examples which illustrate some of these relationships. Fig. 1 shows for one measured character, number of rosette leaves, a comparison between data acquired at the NASA Ames Research Center and those obtained 4 years later in Philadelphia. Both positive slopes are statistically significant but are not different from one another at the 1% probability level. Fig. 2 and 3 show data from our more recent tests. Fig. 2 shows that the average length of leaves tended to shorten at higher g-levels although residual variation in results from different tests was large. Nevertheless the downward trend was statistically significant. Fig. 3 demonstrates a marked shortening of the hypocotyl as the g-level increased. We have chosen to calculate regression on the assumption of linearity although these and other test data suggest that for hypocotyl length a curvilinear relationship might better describe the g-function. For our present

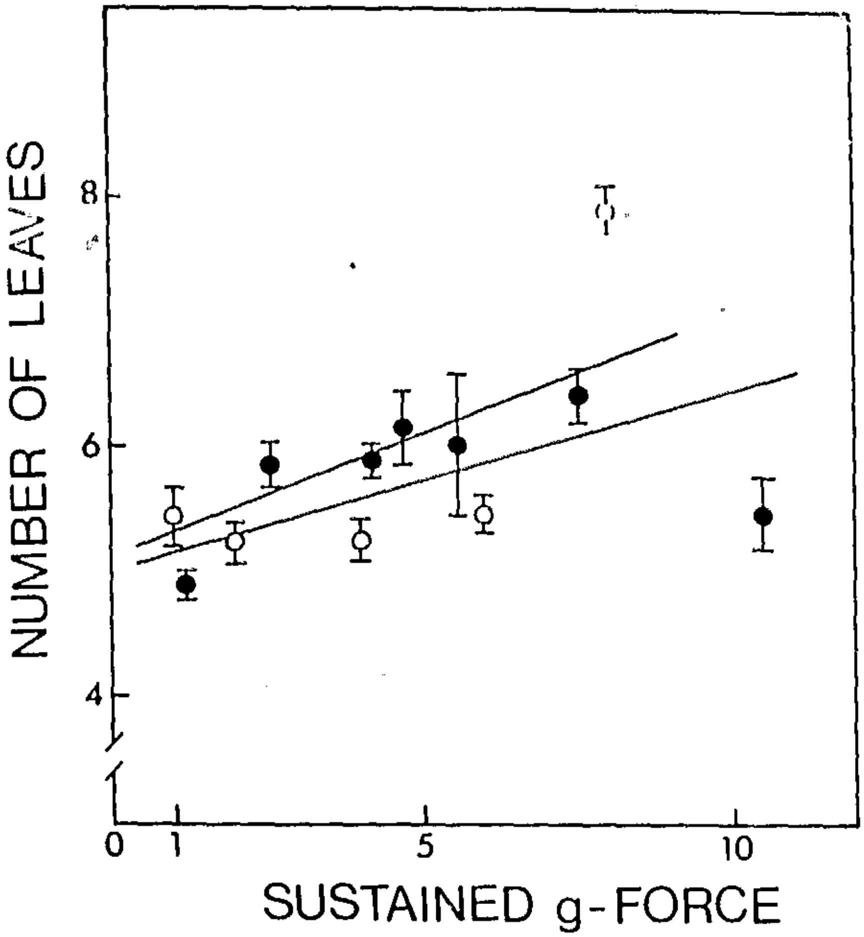

Fig. 1. Relation between mean number of rosette leaves developed and the prevailing g-level which had been maintained for 21 days of growth on clinostats mounted on a centrifuge. Open circles (and upper regression line), data from NASA Ames Research Center; solid circles (and lower regression line), data from UCSC Plant Centrifuge Laboratory.

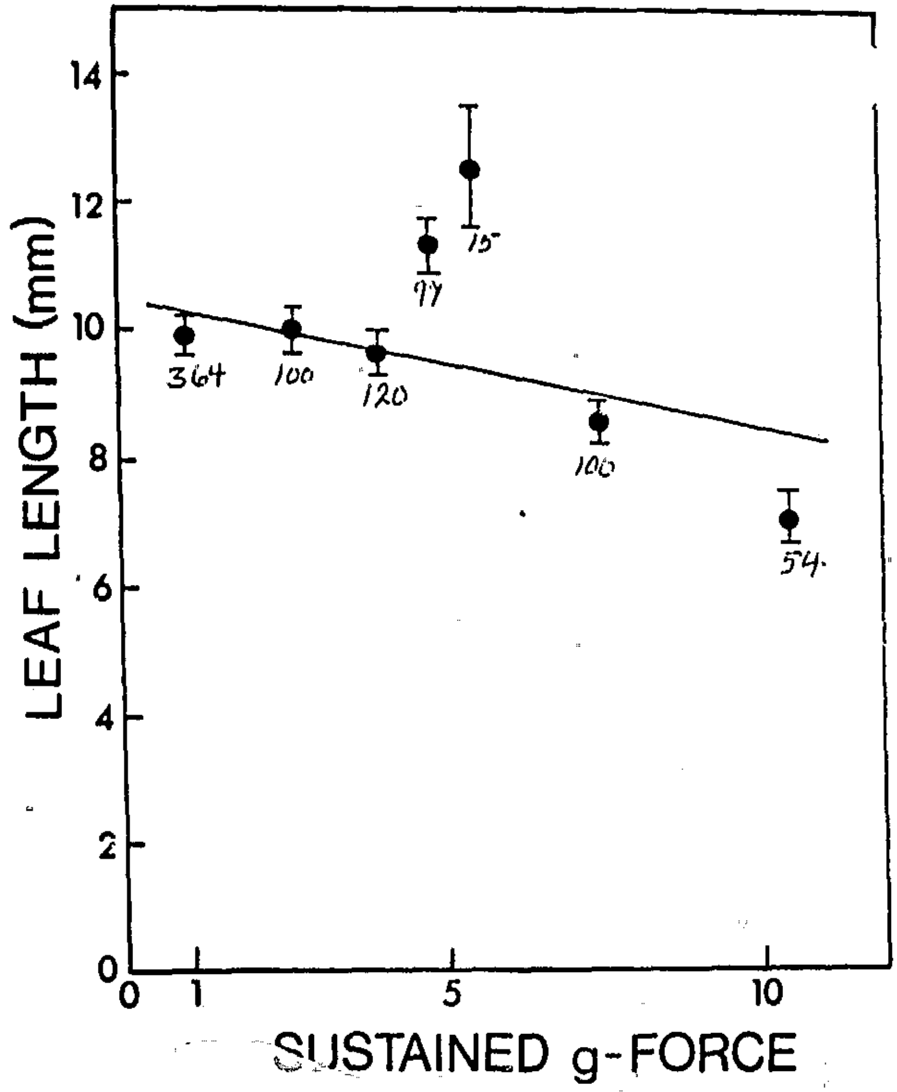

Fig. 2. Relation between mean length of rosette leaves and the g-level maintained for 21 days of growth on clinostats mounted on the centrifuge. Plotted points are averages of all measurements at the indicated g-levels. Error bars include ± 1 SE from the mean. The number below each symbol indicates how many measurements are represented.

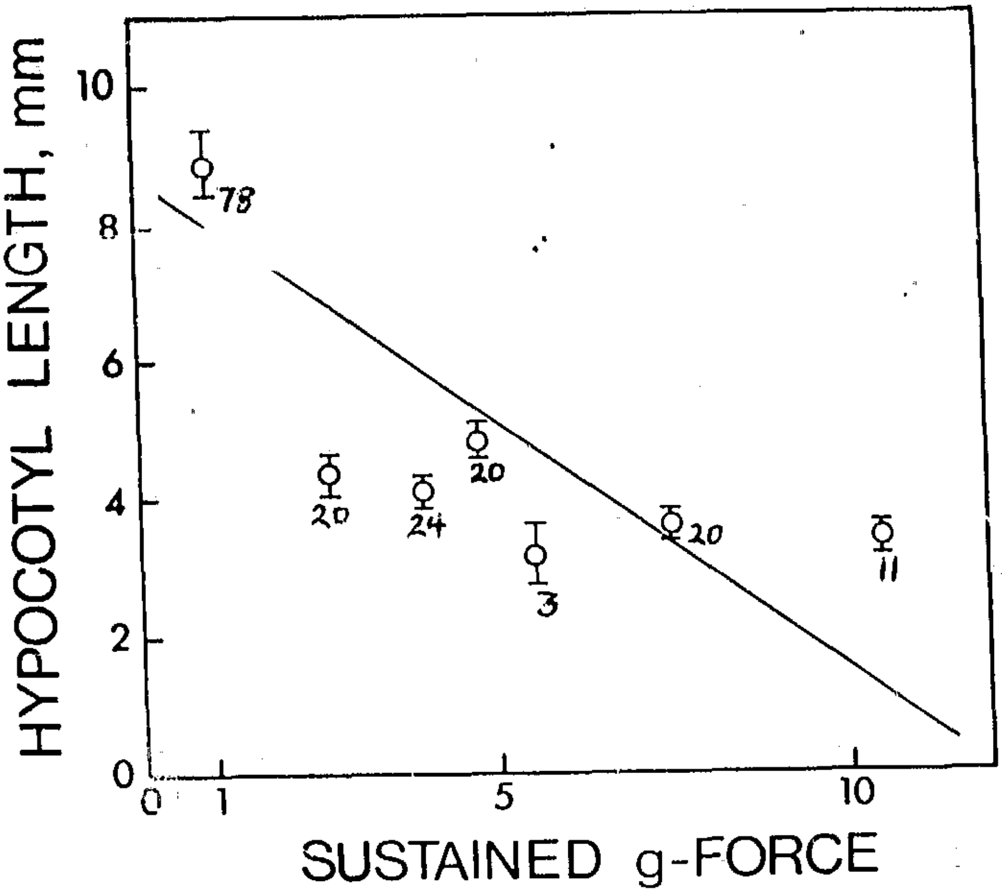

Fig. 3. Relation between mean length of hypocotyl and the g-level maintaine for 21 days of growth on clinostats mounted on the centrifuge. Plotted poin are averages of all measurements at the indicated g-levels. Error bars include $\pm$ 1 SE from the mean. The number below each symbol indicates how many measurements are represented.

purposes the distinction is not important. By statistical test the negative correlation is highly significant.

A comment is in order concerning the regression line shown in Fig 2. Inherent in the graphic method of presenting data is a shortcoming which cannot easily be overcome by conventional plotting methods. The eye tends to weight all points equally and the visual impression afforded by any plotted array of data means could be misleading, if the numbers of individual measurements contributing to the different points were grossly unequal. This was the case with the data shown in Fig. 2. Only 15 measurements contributed to the patently high value at 5.5 g. The other points were based on much larger data sets. Each represents the average of over 50 measurements (in one case, 364) and these weighed much more heavily in the least squares procedure for determining the position and downward slope of the regression line.

Table I lists all characters measured along with their correlation coefficients. The last column of the table shows the probability that the coefficients differed from zero only by chance. For over half of the characters the correlations were highly significant. (Only for two leaf shape characters was there no significant dependence on the g-level.) We conclude, therefore, that the gravity compensation achieved by our clinostats must have been incomplete.

DISCUSSION

It seems evident that one cannot discover whether a plant senses gravity unless the g-vector force is made variable in some manner. The

Table I. Statistics of the g-Functions of Morphological Endpoints of *Arabidopsis* Development on Horizontal Clinostats Mounted on a Centrifuge

Character measured	n	Regression* Coefficient ± SE	Correlation** Coefficient	Probability that Regression Coefficient Differs from Zero only by chance
Total leaf length, mm	850	-0.19 ± 0.05	-0.136	< 0.0001
Petiole length, mm	850	-0.19 ± 0.03	-0.237	< 0.0001
Blade length, mm	850	+0.01 ± 0.025	+0.015	0.67
Blade width, mm	850	+0.004 ± 0.011	+0.012	0.73
No. of Leaves	176	+0.15 ± 0.03	+0.391	< 0.0001
Hypocotyl length, mm	176	-0.71 ± 0.08	-0.546	< 0.0001
Flower stem length, mm	176	-1.63 ± 0.56	-0.214	< 0.004

* Linear regression of character value on g-level -- *i.e.*, slope of best fitted line relating the set of measurements for a given character to the g-parameter.

** Correlation of character value with g-level.

magnitude of the acceleration vector can be raised above unity by means of a centrifuge as we have done here or it can be reduced nearly to zero in satellite orbit. The first method was suggested by, among others, Larsen (16) and we can only agree with his 1953 comment that "the use of centrifugal forces . . . . . to increase the omnilateral stimulation is possible in principle, but will meet with considerable technical difficulties." The second method was employed in two satellite experiments, accomplished by NASA in 1967, in the course of which plant reactions to weightlessness were tested directly (20). Both experiments were designed to compare the epinastic responses of plants clinostated on the earth to those of plants in the satellite.

In the case of leaf epinasty of Capsicum annuum the space experiment was performed by Johnson and Tibbitts (11) although full analysis of the data was delayed because of the death of the principle investigator. Recently an analysis of the experimental data was published by Brown et al. (1) which revealed that for every manner of comparison which was attempted, in spite of qualitative similarities, the effects of clinostating were quantitatively different from the effects of weightlessness. All observed differences were statistically significant at the 1% probability level.

In the case of root epinasty in Triticum aestivum, Lyon and Yokoyama carried out clinostat tests on the ground (15) and later as "controls" for an experiment in a satellite (12,13). Root angles were measured from photographs which recorded plant profiles in "face" view and at 90° in "side" view which was contrived by the use of a mirror set at a 45° angle to the optical axis of the photographic system. Plants were photographed at the end of 2 days of growth either on horizontal clinostats in the laboratory or after recovery from the satellite. It had been part of the original design of the experiment to use the face and side views of each plant root system to construct geometrically the "true" or liminal angle between root and plant axis rather than simply to use the projected angles for the comparisons. The liminal angle, θ, for a given root could be calculated from the face view projected angle, α, and the side view projected angle, β, by the following relationship:

$$\tan \theta = \sqrt{(\tan \alpha)^2 + (\tan \beta)^2} .$$

Although Lyon did not publish the summary results of those calculations he did compute the values of θ and obtained the result shown in Table II*. It is evident that root epinasty under weightlessness was substantially greater than what was produced by the clinostat. The difference in mean liminal angles observed under the two conditions was $5.4 \pm 2.05^{\circ}$ which was significant at the 1% level ($p = 0.009$).

These results from space experiments constitute direct quantitative tests of the ability of the clinostat to simulate weightlessness for specific gravimorphic responses of two plant species. They complement the results we report for a third species using clinostats on a centrifuge. For both of these experimental approaches we now have available results which do not support the view that gravity (acceleration) compensation was achieved by rotation of test subjects on clinostats. Evidently the term, gravity compensation, may be retained in clinostat lore for geotropic reactions but it would be misleading to apply it generally to the action of a clinostat in studies of gravimorphic phenomena.

* The information in Table II was made available to us by Dr. Lyon through personal correspondence in January, 1971. Before his death we had urged Lyon to publish these results but he failed to do so.

Table II. Liminal Angles of Wheat

Roots from Biosatellite II Experiment

by C.J. Lyon*

Treatment	Lateral Roots	No. of Roots	Liminal Angle ±SE	Average of Mean Liminal Angles ± SE	% Change from Upright Plants at 1 g
Upright Plants at 1 g	Left Right	63 64	60.8±1.1 64.0±1.0	62.4 ± 0.8	0
Horizontal Clinostat	Left Right	47 50	92.1±2.3 96.2±2.0	94.2 ± 1.5	+51%
Satellite Flight	Left Right	45 51	99.5±1.6 99.7±2.3	99.6 ± 1.4	+60%

* Data and computation results from C.J. Lyon (personal communication).

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