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Gravity Functions of Circumnutation by Hypocotyls of *Helianthus annuus* in Simulated Hypogravity[1,2]

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ABSTRACT

For more than a decade research on the botanical mechanism responsible for circumnutation has centered on whether or not these nearly ubiquitous oscillations can be attributed to a hunting process whereby the plant organ continuously responds to the gravity force and, by overshooting each stimulus, initiates a sustained oscillation or, driven by a not yet defined autogenic mechanism, performs oscillatory activities that require no external reinforcement to maintain the observed rhythms of differential growth.

We explore here the effects of altered gravity force on parameters of circumnutation. Following our earlier publication on circumnutation in hypergravity we report here an exploration of circumnutation in hypogravity.

Parameters of circumnutation are recorded as functions of the axially imposed gravity force. The same method was used (two-axes clinostat rotation) to produce sustained gravity forces referred to as hypergravity ($1 < g$), hypogravity ($0 \leq g < 1$), and negative gravity ($-1 < g < 0$). In these three regions of the *g*-parameter nutational frequency and nutational amplitude were influenced in different ways.

The results of our tests describe the gravity dependence of circumnutation over the full range of real or simulated gravity levels that are available in an earth laboratory. Our results demonstrated that nutational parameters are indeed gravity-dependent but are not inconsistent with the postulate that circumnutation can proceed in the absence of a significant gravity force.

Circumnutations of plant organs have interested botanists for more than a century. These growth oscillations usually proceed so slowly (roughly, 1–3 h per cycle) that they easily escape notice and experimenters often resort to time lapse photography to record them quantitatively. Circumnutations are scientifically interesting because they are nearly ubiquitous, often appear to be adaptive, and especially because the mechanism that must account for these cyclic movements remains moot. For an historical account of the scientific controversy over the underlying mechanism, Israelsson and Johnsson (7) and Johnsson and Heathcote (8) may be consulted. The level of our current understanding is revealed by the lack of agreement among investigators on a most fundamental issue: whether the driving force that sustains these oscillations is strictly endogenous or whether the movement is dependent on a continuing succession of geotropic responses and is, therefore, *g*-dependent.

The obvious experimental strategy for assessing the dependence of circumnutation on *g* would be to alter the *g* force in various ways, to discover any effects on the oscillations, and to reject model mechanisms found to be incompatible with the plants' observed behavior under altered *g*. Among the earliest reports of circumnutation in altered *g* environments were experiments on 90° (horizontal) clinostats. Results were conflicting. Baranetzki (1) reported cessation of nutational oscillations during rotation of plants on the horizontal clinostat, whereas Rawitscher (10) observed that circumnutation of *Calystegia sepium* continued during clinostat rotation. More recently, Israelsson and Johnsson (7) published representative data showing that nutational oscillations of *Helianthus annuus* damped out on 90° clinostats, whereas our measurements on a different cultivar of the same species confirmed persistent residual nutation both on somersaulting clinostats and on horizontal clinostats (5). Such disagreement among different observers may be attributed to differences in the behavior of different experimental material or to differences in the clinostats themselves. At best the clinostat is an imperfect gravity compensator as noted by Newcombe (9), Brown *et al.* (4), and Tibbitts and Hertzberg (11).

Less ambiguous were the results of tests in hypergravity in which the *g* force was increased by protracted centrifugation. Brown and Chapman (2) recorded both the period and the amplitude of *Helianthus* circumnutation at a series of *g* levels in the range, $1 < g < 20$. They found the oscillations to be remarkably insensitive to *g* increments over a greater than 10-fold range and concluded that, if hypocotyl circumnutation is *g*-dependent, investigation of the hypogravity region of the *g* parameter between zero and unity ought to be even more rewarding.

Except in a space experiment protracted hypogravity is an unattainable experimental condition. On earth we must resort to simulations for experiments in the range, $0 < g < 1$. This paper reports the results of a series of tests on circumnutation under simulated hypogravity.

MATERIALS AND METHODS

Biological Material. Test subjects were 4-day-old seedlings of *Helianthus annuus* L., cv. Teddy Bear. The seed was obtained from the W. Atlee Burpee Co. Seeds were planted in a loam soil-type planting mixture, Burpee Planting Formula No. 9411-0, brought to 70 ± weight % moisture. Plants were grown in darkness at 24 ± 1 C. Seedling age was counted from the time of planting.

Observations of Circumnutation. Measurements were recorded by time lapse cinematography using IR sensitive video cameras. The seedlings were photographed from above (morphologically) in order to discern the shape of the circumnutational ellipse. Plants

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were never exposed to visible light from the time of planting the seed until the conclusion of the measurements. The only illumination was from IR sources that produced a wavelength band centered on 890 nm and did not emit below 800 nm. Images were automatically recorded every 10 min and only during the brief photographic sessions were the IR sources turned on. The intensity was such that a thermal effect from absorbed radiation was negligible. Methods of data processing have been described by Brown and Chapman (2). We obtained computer printout tracings of nutational ellipses from which the period of each oscillation could be calculated and the long and short axes of the ellipse could be measured with a ruler. It was arranged for each image frame to include a scale to facilitate calibrations of linear dimensions.

We chose arbitrarily to average five consecutive, nutational cycles by a given test plant for each item of data and we attempted to center the cycles as nearly as was feasible on plant age 104 h, since we had determined that kinetic properties of hypocotyl circumnutation changed slightly but significantly with seedling age. Tests were repeated on a series of plants so that what we report here as cycle amplitude and cycle period (or frequency) represent mean values of a set of five measurements on each plant replicated on average with between seven and eight separate plants. Where results have been plotted, the length of the vertical error bars represent ± 1 SE.

Conditions of Clinostating. We employed clinostats of local design and fabrication. The rotation rate was 1 rpm. An assumption upon which clinostat simulated hypogravity depends is that the lateral component of the earth's g force is compensated (sometimes referred to as "nullified"), but that the axial component of g endures and can be calculated as $g \times \cos \theta$, where θ is the angular departure of the plant's axis from the plumb line. The frequently used horizontal clinostat represents that case for which the angle is 90° corresponding to an axial g component equal to zero.

Any particular, simulated, hypogravity condition achievable with a single axis clinostat tilted from the vertical can be duplicated in principle by an apparatus that rotates the specimen on two orthogonal axes, one of which is horizontal and provides gravity compensation whereas the other applies a centripetal acceleration in the direction coincident with the plant's longitudinal axis. Such a configuration is shown diagrammatically in Figure 1. In our case we used a centrifuge 6 m in diameter to apply whatever centripetal acceleration was required.

By a simple extension of our simulation method we were able to impose incremental g forces in the direction opposite from normal and thereby to measure effects of what are referred to here as simulated "negative" g forces.

RESULTS

Circumnutations Observed in Simulated Hypogravity. In an earlier publication, Brown and Chapman (3) reported preliminary attempts to describe the g function of circumnutation over the range, $0 < g < 1$, in which single-axis tilted clinostats were employed. For reasons that we do not yet understand, the variability of those data was appreciably larger than we later found using the identical clinostats operated in the mode of rotation on two axes. All results reported here were obtained by simulating hypogravity with clinostat rotation on two axes.

Figure 2 includes a plot of the period of nutation *versus* simulated g force. The 35% reduction in the period of oscillation (50% increase in oscillation frequency) is clearly significant. Moreover, the period extrapolates to a finite value at 0g.

Figure 2 also shows the relation between the longer axis of the nutational ellipse and the simulated g force. As expected, the amplitude of circumnutation changed markedly in the hypogravity region; at nominal 0g it was only about 20% of the value at 1g.

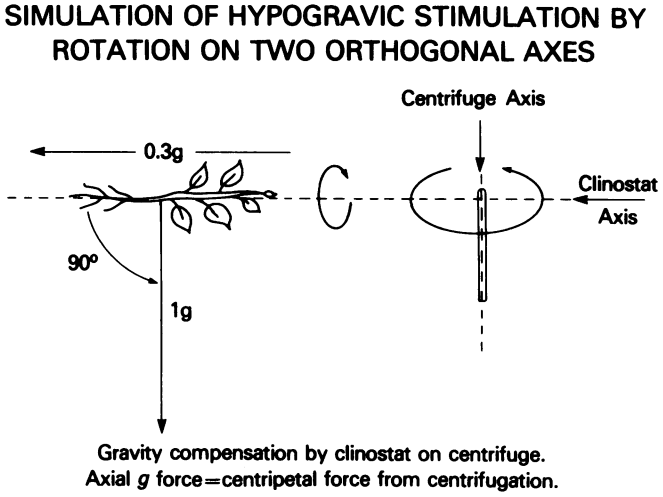

FIG. 1. Diagrammatic representation of hypogravic stimulation by rotation on two orthogonal axes. Centrifugal force is 0.3g; earth gravity acting transverse to the plant axis is compensated by clinostat rotation. Effect on test plant is that of an axially directed, sustained force of 0.3g units.

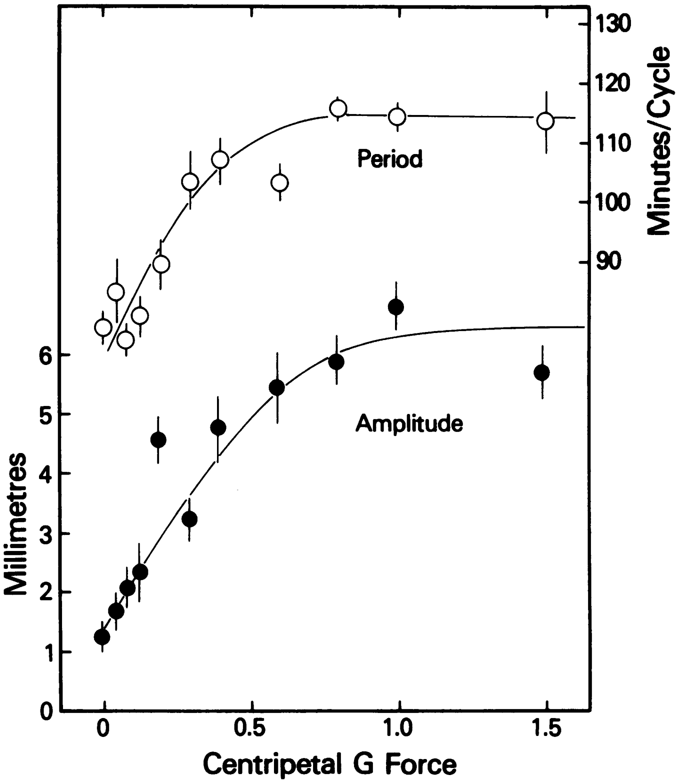

FIG. 2. Amplitude and period of circumnutation over a range of axial forces between 0 and 1.5g, achieved by rotation on two axes. Transverse component of earth gravity was compensated.

The amplitude line in Figure 2 is described by the equation

$$A = 6.2\,(1 - e^{-3.77g}) \qquad (1)$$

where A is the amplitude of oscillation in mm, e is the base of natural logarithms, and g is the centripetal acceleration in g force units. The form of equation 1 and its constants were selected to fit empirically the plotted data. They have no mechanistic implications.

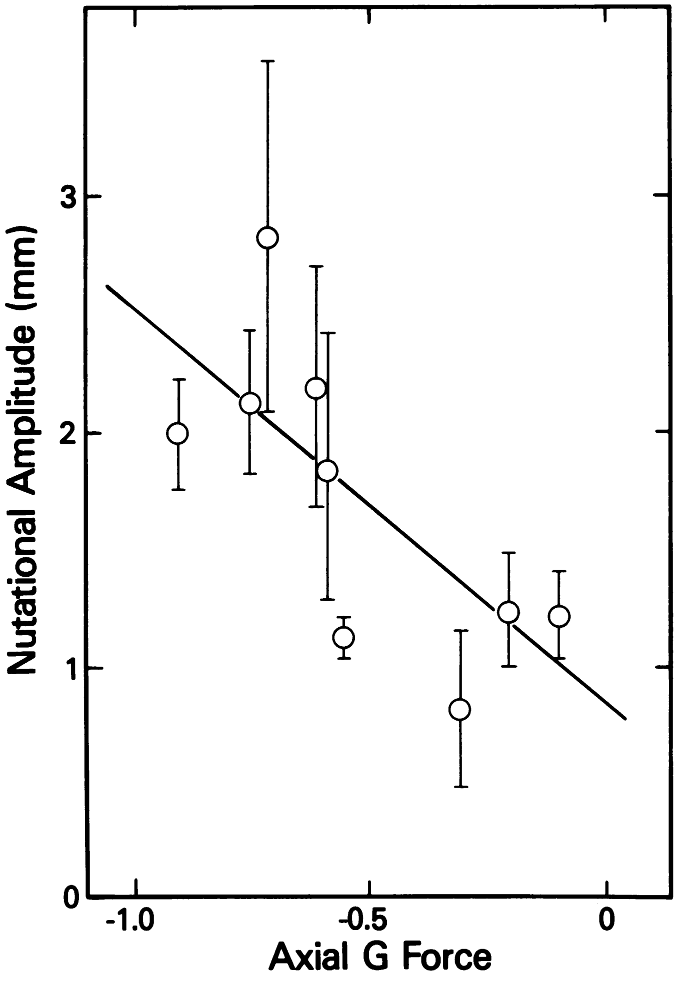

FIG. 3. Amplitude of circumnutation over a range of axial forces between -1 and $0g$, achieved by rotation on two axes. Centripetal force applied in the opposite from normal direction is defined as negative. Transverse component of earth gravity was compensated.

Circumnutations Observed When the Simulated g Force Acted in Reverse. By tilting our clinostats at angles greater than 90° from the plumb line we were able to impose g forces in the direction opposite from normal—"negative g" in that sense. We achieved the same condition by reversing the orientation of test specimens during two axes rotation so that the centripetal acceleration was imposed in the abnormal direction. With that justification for the minus sign when plotting g functions, we were able to extend our description of circumnutation to about $-1g$.

Figure 3 is a plot of nutational amplitude in the negative g region, $-1 < g < 0$. Statistical variation was larger than was the case for data taken in other g regions, but at least it seems evident that nutational amplitude increased progressively as g levels became more negative. Beyond $-1g$, the data became more difficult to obtain since the condition was one in which the seedlings were unstable and tended to revert to an "upright" orientation.

Circumnutation Observed in Hypergravity. Figure 4 is a plot of nutational amplitude in the hypergravity region. These data, taken on plants that were not exposed to visible light (to make them comparable with the rest of the data reported here) follow the same general horizontal trend as our previously reported measurements of nutation of plants undergoing centrifugation in white light (Brown and Chapman [2]; *cf.* their Fig. 4). The plants undergoing centrifugation were mounted on swinging cradles so the resultant of centripetal and gravitational forces always was normal to the base of the cradle and coincided with the plant's long axis. Above about $+15g$, the seedlings became too heavy to support themselves and failure of circumnutation to persist because of excessive mechanical stress limited the upper end of the g range in which scientifically interesting data could be acquired. Therefore, the amplitude data of Figures 2–4 taken together represent a complete g profile, insofar as that can be measured in an earth laboratory by exploiting simulations as best we can.

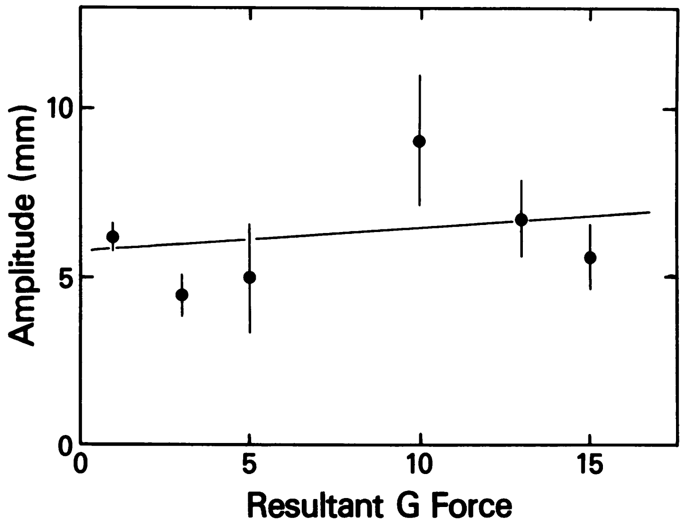

FIG. 4. Amplitude of circumnutation in hypergravity by protracted centrifugation. Ordinate: long axis of the plane projected nutational ellipse viewed from above the plant. Abscissa: vector sum of earth's gravity force and centripetal force.

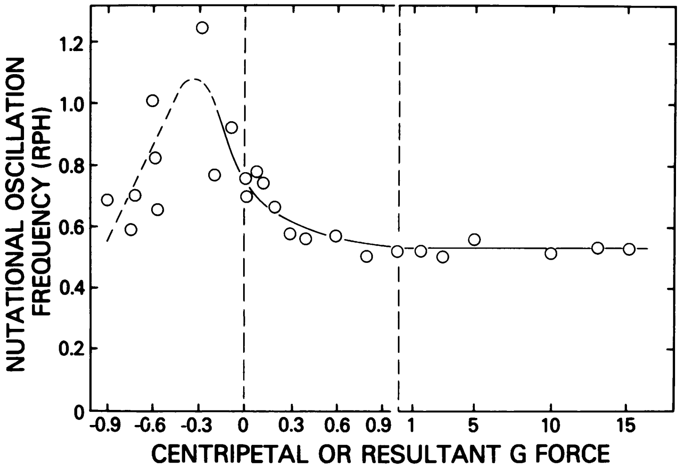

FIG. 5. Gravity profile of nutational frequency over the range of axially imposed centripetal forces between -1 and $+15g$. In all tests, transverse component of earth gravity was actually zero or was compensated.

On the same plants that provided the amplitude data shown in Figures 2–4, we obtained measurements of mean duration of the nutational cycles. Those results are shown in Figure 5 which is the g profile for nutational oscillation frequency over the range from $-0.9g$ to $+15g$. The increase of oscillation frequency in hypogravity and the evident maximum in the negative g region were unexpected. Models of circumnutation so far proposed do not predict such changes.

DISCUSSION

The data reported here strongly support the concept that circumnutation is influenced by the g force and, therefore, its mechanism cannot be fully endogenous, at least in the sunflower hypocotyl. But is circumnutation strictly dependent on exogenous *g* stimulations? Heathcote (6) presented impressive evidence that the endogenous theory may yet prove at least partly correct. Furthermore, although recognizing that all simulations have limitations, we note that our own results (5) at simulated 0*g* did not show that circumnutation damped out and, therefore, they did not support a role for gravity as the *exclusive* driving force for the oscillations.

It seems evident that a more convincing experiment can be done in an orbiting spacecraft. Such a test is under development for the first Spacelab mission which now is expected to be launched in mid-1982. The definitive test will be to observe the circumnutational behavior of sunflower seedlings in close approximation to weightlessness. If the experiment is successful, not only may we learn something about the driving mechanism for circumnutation but also we may be in a much better position to assess the validity of clinostat simulations of hypogravity conditions.

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