Effects of Increased Gravity Force on Nutations of Sunflower Hypocotyls

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ABSTRACT

A centrifuge was used to provide sustained acceleration in order to study the hypocotyl nutation of 6-day-old Helianthus annuus L. over a range of g-forces, up to 20 times normal g. At the upper end of this g-range, nutation was impeded and at times was erratic evidently because the weight of the cotyledons exceeded the supportive abilities of the hypocotyl.

Over the range 1 to 9 g, the period of nutation was independent of the resultant force vector. Over the same g-range, the amplitude of nutation was nearly independent of the chronic g-force.

If nutation in sunflower seedlings is an oscillation caused by a succession of geotropic responses which continue to overshoot the equilibrium position (plumb line), we might expect its amplitude to be more sensitive to changes in magnitude of the sustained g-force. In order to preserve the geotropic model of nutation—viz. that it is a sustained oscillation driven by geotropic reactions, it is necessary to assume that geotropic response must increase with increasing g most rapidly in the region of the g-parameter below the terrestrial value of 1 g.

Various plants including many species of climbing vines execute growth movements which are collectively referred to as nutations. These remarkable nastic movements, usually too slow to be well appreciated in real time, are generally periodic and are sometimes patently adaptive, as in the case of a tendril "seeking" a support around which to twine, but more often they seem without evident advantage to the developing plant. By nutational movements, the shoot apex describes an elliptical (often circular) path around the plant axis. In many seedlings, the first organ to nutate prominently is the hypocotyl although in a variety of plants roots, epicotyl, and branches often nutate while they are elongating. Nutations obviously are related to growth. As the shoot axis elongates, its tip describes a helix which may be somewhat irregular. The periods of nutation usually are within the range 1.5 to 4 hr/cycle.

Darwin (1) referred to these movements as circumnutations, considered their kinetics to be endogenously directed, and believed them to be an essentially universal behavioral property of growing plants which, having the capability of modifying their nutational oscillations, thereby achieve geotropic, phototropic, and other kinds of differential growth reactions to environmental stimuli. Heathcote is the most recent author to marshal experimental evidence in support of an innate tendency for nutation of plant organs (3-6). Although some of Heathcote's arguments are persuasive, we do not find it appealing to relegate nutation to the category of endogenous mysteries especially if there is an alternative model which can be put to experimental test.

A more rigidly mechanistic view was expressed more than half a century ago by Gradmann (2) who, among others, considered nutational oscillations of the shoot to be a set of basically geotropic responses to displacement from the plumb line, each response leading to overcompensation due to the fairly long time lag between geotropic stimulation and plant reaction. The fundamental disagreement about the mechanism which accounts for nutations extends to the present day as experts in this research area continue to support either the concept of an inherent tendency for nutation (as Darwin had proposed) or the view of nutation as a geotropic "hunting" oscillation driven by the plant's gravity response mechanism (12).

Within the past decade, the mechanistic or biophysical approach has been developed to the point of an internally consistent and experimentally testable mathematical model by Anders Johnson and his colleagues (7-9, 13, 14). This model first proposed in detail by Israelsson and Johnsson (7) for nutations of Helianthus annuus, adequately accounts for the kinetics of nutation on and off horizontal clinostats (7, 11), over a range of temperatures (7), and under conditions of intermittent geotropic stimulation (11). The model describes a sustained oscillation of the hypocotyl which is unchanged either in amplitude or period. It is based on the principle that any sensible displacement of a plant organ from its equilibrium position (e.g. coincident with the plumb line) must induce a geotropic response but with a substantial time delay of some 10s of min. That time delay insures that the geotropic reaction will correct the displacement and the plant organ will necessarily overshoot the equilibrium position in the opposite direction. There will arise a series of response-with-overshoot growth movements which, if confined to a single vertical plane, would follow the pattern of an inverted pendulum (assumed, by that analogy, to be a sine function). If, however, oscillations are possible in any vertical plane, other patterns of motion can obtain. Elliptical including circular patterns are common. The Israelsson-Johnsson model was tested, elaborated, and discussed in a series of contributions involving both plant experiments and computer simulations.

For a mode of physiological behavior whose driving function is the plant's response to changing orientation with respect to the g-vector, it seemed especially desirable that nutational behavior should be examined both theoretically and experimentally under conditions of acceleration other than the normal 1 g. For example, at zero g, the nutational movement should cease because the driving force would be absent. This has not yet been demon-

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NUTATION AT INCREASED g

strated from research in a satellite although various workers found that on a horizontal clinostat (which, although it cannot provide zero g, does produce net omnilateral stimulation which sums to zero over a reasonably long interval) nutational movement was absent or at least minimal (e.g., ref. 7).

When we examine the Israelsson-Johnsson model (7) with respect to the role of the g-force, it becomes apparent that nutation should be sensitive to the magnitude of g whether that be earth’s gravity alone or the vector sum of the earth’s gravity and a sustained centrifugal force imposed experimentally.

According to the model, the geotropic stimulus is assumed to be approximately proportional to the plant’s angular deviation from the plumb line, \( \alpha \), at least for small angles. It often is assumed to be proportional to the sine of \( \alpha \) as was stated explicitly by Israelsson and Johnsson (7). The plant’s bending response is considered to be proportional to stimulus intensity. However, because of the substantial delay, attributable to the time needed to transmit a lateral imbalance of growth hormone from the sensor region (epicotyl or cotyledons) to the growth response region (hypocotyl), the rate of bending is largely determined by the stimulus which had been perceived at an earlier time, \( t - \Delta t \).

Formally, this approximate relationship can be expressed by the simplified equation,

\[
\frac{d\alpha}{dt} = \kappa g \sin (\alpha_{\text{max}})
\]

where \( \alpha \) is the angular displacement from the plumb line at time, \( t \); \( g \) is the gravitational or other accelerating force; \( \Delta t \) is the geotropic response lag; and \( \kappa \) is a constant of biophysical proportionality whose value must be determined empirically. It is important to realize that the term for the force responsible for geotropic response is potentially variable and is not made a part of the proportionality constant, \( \kappa \), as was done implicitly by Israelsson and Johnsson since they did not consider the consequences of conditions other than unit \( g \).

To describe the course of nutation, it was assumed that the oscillations are sinusoidal in a given plane and in the two-dimensional case they must describe an ellipse for which the period, \( T \), should be related to the value of \( \Delta t \). In the simplest case,

\[
T = 4\Delta t
\]

In theory, \( T/\Delta t \) could assume values larger than 4 but, for present purposes, it is important only to note that \( T \) is a function of \( \Delta t \) and it may be expected to remain constant as long as \( \Delta t \) does not change.

It was the objective of the series of experiments reported here to test the consequences of the Israelsson-Johnsson model at g-levels above unity. What would be the expected effect on nutation of sustained centrifugal acceleration a number of times greater than the normal g-force? With respect to the period of nutation, \( T \), we may explore the consequences of what is perhaps the simplest assumption, that hormone transport velocity is not g-sensitive so that \( \Delta t \) should be unaltered by an increase of \( g \) and \( T \) also should be expected to remain unchanged. If the period, \( T \), is to be independent of g-level, the time course of response to the g-stimulus cannot change either. Therefore, the term \( \frac{d\alpha}{dt} \) in equation 1 should be independent of \( g \). With higher g-stimulus, a smaller displacement from the plumb line will be sufficient to elicit the same time course of response as may be observed at 1 g. By applying a g-force up to many times normal gravity, we should expect to find a very large decrease in the amplitude of nutation because, according to our assumptions, the maximal amplitude of nutation should be reciprocally related to \( g \).

In the present study, we manipulated the g-level by chronic centrifugation and measured the period and amplitude of nutation in sunflower hypocotyls over a 20-fold range of g-levels in order to test experimentally the two predictions set forth above, viz.

\[
T = f(g)
\]

\[
g \sin \alpha_{\text{max}} = \text{constant}
\]

MATERIALS AND METHODS

Our test species was a dwarf variety of sunflower, Helianthus annuus L. cv. “Teddy Bear.” Seed was obtained from the W. Atlee Burpee Co., Warminster, Pa. Seeds were soaked overnight and planted in peat pellets obtained from Jiffy Pot Ltd., Groudn, Norway, or in a soil planting mixture (Burpee Planting Formula No. 9411-0). Seedling age was calculated from the initiation of soaking. When plants were selected for photographic observation, they were well watered and usually the soil or peat pellets were covered with 0.5 mil Saran Wrap (Dow Chemical Co.) to ensure that the plants’ water supply would be sufficient for the intended duration of the experiment.

Tests which required that plants be subjected to g-forces greater than earth’s gravity were performed on the NASA-UCSC Botanical Centrifuge at the University City Science Center, Philadelphia. The centrifuge has a diameter of 7 m. During observations of nutrition, the centrifuge operated continuously at the selected rate of rotation. The desired rotation rate was established in relation to the plant’s location along the radius of the centrifuge to produce the required g-level and this was maintained within \( \pm 3\% \). Plants were supported by the decks of cradles which were pivoted from fixed positions on the centrifuge arms. There are four such cradles which swing freely from their respective pivots. The resultant of gravitational and centrifugal forces always acts in a line perpendicular to the cradle deck and essentially coincident with the long axis of a plant mounted on the cradle. Each cradle has a deck area of 0.4 m², sufficient to accommodate several test seedlings with associated monitoring equipment. The cradle pivot points are at either of two radii such that resultant g-force to which plants can be exposed on either of the outer pair of cradles is about twice that experienced by plants on either of the inner cradles. Since the radius of rotation is relatively large (either 178 or 330 cm), and since growth extension of the 5-cm seedlings in a radial direction was less than 1 cm during our most prolonged experiments, the change of g-force at any point on the test plant due to growth displacement during an experiment could have been no more than 0.6%.

All experiments were performed at a nominal temperature of 24 C. The test seedlings were enclosed in either a Plexiglas or wooden housing which served as a wind screen. In many experiments, the temperature was monitored continuously by a thermistor probe inside the housing near the plant. Ventilation holes were provided in the housing and temperature regulation was achieved by air conditioning the centrifuge rotunda. Within any particular test, the temperature varied no more than 1 degree.

In the experiments at 25 C, all tests were 22 and 25 C.

Transfer of plants to the centrifuge involved no significant change in environmental conditions except for the influence of gentle handling. As soon as the plants were placed on the centrifuge, their nutational movements were monitored. Usually, centrifugation was started immediately, although on some occasions we observed the plants at 1 g to establish characteristics of their nutations prior to centrifugation, and in some tests...
we continued to monitor them after the centrifugation period was ended. We observed no nutational transients that could be associated with either the onset or the termination of centrifugation.

In the initial phase of this investigation, seedlings were illuminated continuously from the time of seed germination through the period of measurement of nutation. They were lighted from above by Sylvania Wide Spectrum GroLux fluorescent lamps at an intensity of 175 ± 5 ft-c. Light intensities were monitored at the beginning of each test using a laboratory standard GE No. 213 light meter.

Information on hypocotyl orientation was obtained by closed circuit TV. Video cameras were mounted on the centrifuge cradles and the raw video information was transmitted by cable from the centrifuge payload through slip rings to a TV monitor, or stored on video tape for later viewing. The image of each seedling was displayed on the monitor for a few seconds every 10 min. The image was photographed with an Acme model 6 processing camera (Photo-sonics Inc., Burbank, Calif.) so that a permanent record was obtained on 16-mm Kodak Plus-X reversal film (emulsion 7276). By appropriate frame indexing, the time at which each video image was recorded was easily determined and in most tests this was confirmed by inclusion of a 24-hr Accutron clock in the field of view of the camera.

To determine the time course of nutational movements, the film was read frame by frame on a Vanguard motion analyzer linked to an IBM card punch machine so that coordinates of reference points on the plants were transferred to IBM cards and subsequently, plotted by a computer (IBM model 360/75). Movements of sunflower hypocotyls were essentially sinusoidal as shown in Figure 1. From such data, the period and amplitude of a nutational cycle could be easily determined.

Successive cycles were somewhat variable in amplitude, less so in duration. Although the sample data (Fig. 1) suggest a rapidly declining amplitude during the short sequence plotted, over many cycles no obviously consistent trend was revealed. Variability from one cycle to the next usually was large enough to justify an averaging procedure.

Initially, we measured period and amplitude of nutation for plants of different ages and over a range of g-levels from 1 to 20 in order to establish the age span within which properties of nutation were not changing rapidly and to determine the upper limit of g-stress at which we could obtain useful data. At higher g-levels, both period and amplitude of nutation were dependent on centrifuge speed, an effect that became more pronounced as plant age increased (Fig. 2). We believe this effect was due to the fact that a plant's increased weight while being centrifuged would exceed the supportive capabilities of its hypocotyl. When the time lapse pictures were projected at 12 frames/sec (7,200 times actual speed), it was easily seen that the average plant remained more or less upright only with difficulty. At levels not much greater than 16 g, sunflower hypocotyls were not equal to the task of raising the cotyledons against such a large vector force. The amplitude of nutation became erratic and increased abruptly. The period lengthened until nutation ceased. Such changes represented the relatively uninteresting condition of extreme mechanical stress interfering with the progress of nutation. We limited further study to the acceleration range below 10 g.

At seedling age of about 100 hr, nutational behavior was fully developed and was maintained usually until the plant was more than 200 hr old. We decided to restrict our comparisons of nutational kinetics to plants which were as near as possible 6 days old. With each plant we averaged five nutational cycles—the one nearest 144 hr, the two which preceded, and the two which followed—to determine period and amplitude of nutation to be associated with whatever g-force prevailed.

At first we observed plants that were exposed continuously to white light and we were aware that the illumination could have been responsible for pulsed phototropic responses of our test plants as the angle of incidence of such directional illumination on the cotyledons altered over the course of a nutational oscillation. Reports from other laboratories support the view that differential illumination of cotyledons can influence bending in sunflower hypocotyls (15, 17). We could not be sure how important an effect this might be, although in principle, a model could
be constructed for phototropically driven oscillations based on much the same reasoning that led Israelsson and Johansson to devise their model for geotropically driven oscillations. Accordingly, we altered our methods of observation and carried out a separate series of tests in which plants were never exposed to any visible light.

For observing plants in the "dark," we employed IR-sensitive TV cameras and illuminated the plants with a low intensity tungsten source through a Wratten No. 87C filter which transmits only beyond 800 nm. Since it was not convenient to run the centrifuge in darkness, the test plants were confined to light-tight wooden boxes containing IR light sources when they were prepared for centrifugation. The IR-sensitive video cameras were mated to these "black boxes" by light-tight photographic bells. When the plant image was displayed on the video monitor, recording on film and subsequent data reduction of course were accomplished exactly as had been done in the earlier series of tests under white light.

We made one other change in our method of measuring nutation in this series of black box experiments. We arranged for the video camera to view the test plants from above, because in the earlier tests in which plants were viewed from one side, we had been able to obtain only a projected lateral image of the nutating seedling. For measurements of nutational period, that was quite satisfactory, but unless the locus of an epicotyl was exactly circular, we could not be sure whether the camera's optical axis was perpendicular to the long axis of the ellipse, or to the short axis, or somewhere in between. To avoid that ambiguity, therefore, nutation was recorded from above the plant.

The top view data required an additional step of data reduction after the coordinates of plant movement had been recorded on punch cards. Using a computer graphics program, the data were displayed on the viewing screen of a Tektronix 4010-1 terminal. This method allowed the data to be plotted point by point slowly enough for the eye to follow. Of course, if many cycles were plotted on the screen at the same time, the superimposed information would become unintelligible, but appropriately selected short segments at data (one or a few nutational oscillations) could be plotted and then converted by the computer to hard copy tracings. From these records, the shapes of the ellipses could be measured easily with an error estimated to be no more than 3%.

A third set of experiments was designed to compensate for some of the differences between plants by observing in the same set of plants responses to two different g-levels. Each seedling thus became its own "control." Plants were measured at 3 g and immediately afterward at 13 g or (with different plants) first at 13 g and then at 3 g. This was to compensate for the plant age difference of 10 hr between the first and second set of measurements. Thus, it was possible to test for an effect of g-level as well as that of plant age. We believe that the results of this third series of tests constitute our most critical test of the possible g-dependence of sunflower nutation.

RESULTS

From a series of tests on 52 seedlings illuminated by white light, plant to plant variation was relatively large. We chose to limit the analysis of our results to those tests for which three or more replicate seedlings had been measured at the same g-exposure. There were 41 such tests which had been observed at seven different g-levels between 1 and 9.1 g.

It was evident from the results (Fig. 3) that in the light, the period of nutation did not vary significantly over a 9-fold g-range. The slope of the regression line (Fig. 3) is only 1.5% per g-unit and the correlation coefficient of 0.21 was not statistically different from zero (P > 5%). If, as suggested by theory, the nutational period is related to the time needed for growth hormone transport to establish an asymmetrical hormone distribution at the region of hypocotyl bending, these results imply that the hormone transport process remained essentially unaffected by changes in the g-force at least over a 900% range.

Figure 4 shows the amplitudes of nutation for the same plants whose periods were plotted as Figure 3. Here too, the g-level did not have a prominent influence. The regression line has only a 5% slope. Nevertheless, these amplitude data showed a low but statistically significant correlation with g-level of 0.39.

A subsequent set of experiments which were carried out on seedlings which never "saw" visible light had been designed to test critically whether a 10 g increment in the g-force would produce a significant effect. Table I demonstrates that none of the properties of nutation which were measured was significantly affected by g-level. As expected, the amplitude of nutation was related to plant age.

INTERPRETATIONS

What do these results imply with regard to the underlying mechanism of nutation? They do not constitute a critical test of the Darwinian or endogenous model of nutation as defended by Heathcote (3-6) although they are not inconsistent with that model. They do test the geotropic overshoot model as elaborated by Johansson and his colleagues. We were able to use the principles of that model to predict that the period of nutation would be independent of the g-force and, should that be the case, that the maximal amplitude would be reduced in direct proportion to an increase of g. The first prediction (equation 3)
Table I. Helianthus Nutation at 3 g and 13 g

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<td>Period and g-force</td>
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<td>Amplitude (short axis) and age</td>
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was confirmed experimentally, which supports the notion that at least in Helianthus, centripetal transport of the principal growth regulator was not altered by centrifugation. The second prediction (equation 4) was not confirmed.

An important limitation of the simplest version of the Israelsson-Johnsson model of Helianthus nutation is that it assumes but does not predict that nutational oscillations are stable. If a sustained nutational oscillation is to result from a succession of geotropic reaction overshoots, this maximal angular displacement attained in the course of a particular overshoot cannot be less than the immediately preceding but opposite displacement to which it responded. (If it were less, the oscillation would damp out.) If the angle attained by the overshoot response becomes greater than that of the preceding maximal displacement in the opposite direction, and if the response is dependent on stimulus intensity without limit, each succeeding cycle would increase in amplitude until the system finally would go out of control.

Since nutational amplitude does not grow indefinitely, it appears that response to geotropic stimulation must be limited in some manner other than by stimulus intensity. Geotropic response limitation could be attributable to: (a) saturation of the sensor or of the transduction mechanism; probably the two are operationally indistinguishable; (b) saturation of the growth response mechanism.

Johnsson (10) noted that the constancy of nutational amplitude even at 1 g requires that “some type of nonlinearity must be introduced in the equations describing the oscillations.” It was suggested that the sine function, introduced because geotropic stimulation is deemed to be approximately proportional to the sine of the angle of displacement of the plant axis from the plumb line, could provide the requisite nonlinearity (7). In our experiments, the plants’ angular departures from the plumb line were less than 15°. (Extremes of total excursion less than 30°.) The sine function of small angles is nearly linear. At 15°, it departs from linearity only by 3%. Such a slight nonlinearity is patently inadequate to account for the evident saturation of the differential growth response to geotropic stimulation.

Later, Johnsson (10) invoked a logarithmic relationship between hormone concentration and growth rate presumably because that had the potential for introducing more extreme non-linearity into the model. However, even this would not account for the g-function of nutation described by the solid line in Figure 4. Therefore, the mathematical model for Helianthus nutation presented by Johnsson and his colleagues requires further modification in order to predict quantitatively nutational behavior at g-levels above unity. At least in those environments for which the g-force ranged from 1 g upward, nutational kinetic data were so nearly insensitive to g-level that we may expect to find the region of important g-dependence below 1 g.

We believe that the concept of nutation as a phenomenon of geotropic response with overshoot is most likely correct in principle. However, the kinetic properties of Helianthus nutation are not simply accounted for by the sine rule (16) suggested long ago to explain the intensities of geotropic responses in general. It now seems evident that if nutation is driven simply by a geotropic response pattern, the mechanism must be essentially “saturated” by the intensity of geotropic stimulus which occurs when the plant is at 1 g.

This conclusion probably has important implications for geotropism per se. Like its putative derivative, nutation, geotropism has never been studied at any g-force less than the earth’s unit g, notwithstanding test on clinostats which have been suggestive but not conclusive. Tests in the hypogravity environment of a satellite laboratory would be the most direct and possibly the only means of obtaining unambiguous information about the gravitational dependencies of either geotropism or nutation in what now appears to be an especially interesting range of the g-parameter, 0 < g < 1.

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