

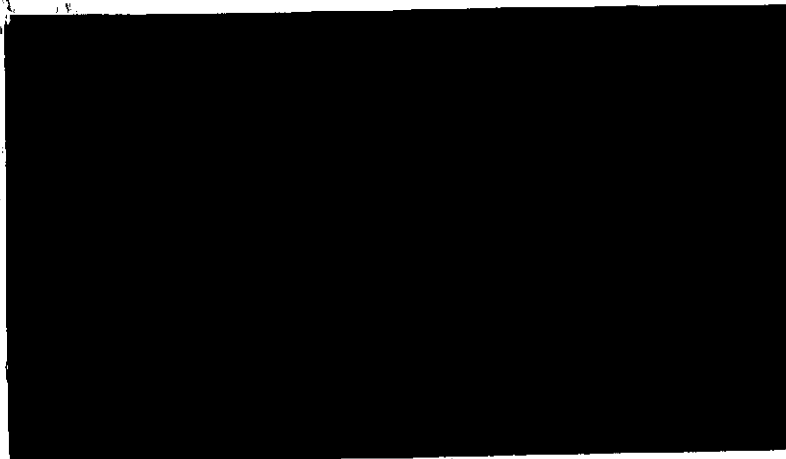
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EFFECTS OF INCREASED
G-FORCE ON THE MUTATIONS
OF SUNFLOWER SEEDLINGS

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ABSTRACT

A centrifuge was used to provide chronic acceleration in order to study the nutation of six-day old sunflower hypocotyls at 1 to 20 times normal gravity (g). At the upper end of the g-range nutational movement was impeded and at times erratic evidently because the weight of the cotyledons exceeded the supportive abilities of the hypocotyls.

Over the range from 1 to 9 g the period of nutation was independent of the resultant g-force. That finding is interpreted as evidence that the geotropic response time -- i.e., the time needed for growth hormone transport from the region of g-sensing to the region of bending response -- was not influenced significantly by substantial increments of the g-level, since geotropic response time is related to the period of nutation.

Over the same g-range the amplitude of nutation increased slightly with increasing g-level. That finding was inconsistent with a model which has been used to account for nutation as a geotropic response with overshoot (i.e., restoration of the plant's vertical alignment which continues beyond the plumb line) for it assumes the rate of hypocotyl response to displacement from the plumb line to be determined by the product of the g-force and the sine of the angle of displacement. The predicted relationship would be a strongly decreasing amplitude of nutation with increase in g. We can retain the geotropic-response-with-overshoot concept to account for the kinetics of nutation only if we employ a model for which the geotropic response is essentially independent of g in the range we tested.

A model which would be consistent with our results is one which makes geotropism a response to the direction of the g-vector but independent of its magnitude at least above some threshold value probably well below

unit g. We suggest in principle that the sedimentation of statoliths in response to a g-stimulus leads to their positioning within the statocytes which is critical but that after sedimentation has been accomplished the force on the statoliths themselves or on the cellular structures which support them is not of consequence. A model which operates on this principle can be used to derive the kinetics of nutation which would be consistent with our results.

INTRODUCTION

Various seedlings including many species of climbing vines execute growth movements which are collectively referred to as nutations. These remarkable movements, nearly always too slow to be appreciated in real time, are generally periodic, are sometimes patently adaptive -- as in the case of a tendril "seeking" a support around which to twine -- but in more numerous examples are without evident advantage to the developing plant.

By nutational movements the shoot apex describes an elliptical (often circular) path around the vertical axis of the plant. As the shoot is elongating the locus of its apex is a helix which often may be somewhat irregular. In the seedling stage the principal region of growth by extension and of nutation usually is the hypocotyl; later on movements of the epicotyl are chiefly responsible for nutation. Darwin (1) referred to these movements as circumnutation, considered their kinetics to be endogenously directed, and believed that such movements must underlie the important phenomenon -- geotropism.

Perhaps the most interesting scientific aspect of plant nutational behavior is the mechanism responsible for the movements which, although they represent more or less regular oscillations, seem to have little in common with the well known endogenous circadian movements of leaves. The period of nutation generally is about an order of magnitude less than that of circadian leaf movement and it should also be noted that temperature, which has little influence on most circadian phenomena, exerts a major effect on the period of nutation (10).

Darwin, among others, felt that the nutational pattern was a subtle

property of the plant which defies attempts to explain it in simple mechanistic terms. Heathcote is the most recent author to marshal experimental evidence in support of an inborn tendency for more or less helical motion of a portion of the shoot (3,4,5,6). Although some of his arguments are persuasive, we must acknowledge that to dismiss nutation as a chiefly endogenous phenomenon is tantamount to admitting that we are not yet wise enough to explain its mechanism.

Some physiologists and biophysicists have considered nutation from a different viewpoint and have sought to account for the characteristics of nutational motion by the assumption that it is a rather simple consequence of a continuous succession of geotropic stimulations and responses. It is well known that an appreciable time lag occurs before a geotropic response becomes manifest. In consequence, the response can be expected to overshoot to some extent. If, through nutational bending, the shoot becomes inclined away from the plumb line, geotropic response with some overshoot would tend to orient it later beyond the plumb line in the opposite direction. In the simplest case, with movement confined in one vertical plane, the oscillation could be expected to simulate that of an inverted pendulum. Of course some response amplification must occur; otherwise the oscillations would damp out.

If there is also another component of oscillation in a second plane, say at right angles to the first, then motion of the shoot tip could approximate an ellipse whose shape will depend on the relative magnitudes of the two components.

Gradmann (2.) was among the first to attribute plant nutation to such geotropic "hunting". Recently Johnsson and coworkers have provided

more extensive, carefully controlled, experimental studies and more rigorous analyses both of the problem and of their data (7,9,10). Johnsson and his colleagues strongly supported the geotropic-response-with-overshoot mechanism with which many of their results were consistent. Nevertheless it would be fair to say that the question of the basic mechanism of nutation remains moot. Relevant literature has been reviewed by Israelsson and Johnsson (7).

In recent years the geotropic hunting concept has been tested by Johnsson and others working especially with seedlings of Avena and Helianthus. They found that many of the properties of nutation (period, amplitude, temperature dependence, susceptibility to entrainment, and response to the horizontal clinostat) could be accounted for quantitatively in terms of a rather simple explicit model (7) which has been refined mathematically to improve the predictive accuracy of their model which, for convenience, we shall refer to as the "geotropic overshoot model".

On the other hand the "endogenous program model" has not been abandoned universally. Over the past decade Heathcote (3,4,5,6) working chiefly with Phaseolus, has persisted in bringing forth evidence difficult to reconcile with the geotropic overshoot model, which mostly by implication (or by default) favors an endogenous mechanism which specifies properties of nutational behavior.

A particular weakness in the quantitative argument which supports the geotropic overshoot model is that it has not been tested over a wide range of variables which possibly are relevant. The innate biological components of the mechanism (gravity sensing; stimulus transduction, auxin synthesis and transport to produce a laterally asymmetrical hormone

concentration, and the differential elongation of the hypocotyl) are difficult to manipulate experimentally. They have been taken as given. In fact the only variables tested by Johnsson and coworkers have been temperature (7) and the direction of the gravitational vector (10) -- i.e., the influence of rotation on the horizontal clinostat, and of discontinuous geostimulation (10).

A factor which could be critical in the application of the geotropic overshoot model is the magnitude of the gravitational acceleration; however no reports have appeared on the effects of making the g-force a variable as might be accomplished with a centrifuge to provide resultant g-forces in excess of 1 g or in a satellite to achieve approximately zero g or, by a combination of both methods, to explore the range between zero and unit g. The purpose of the present contribution is to examine the kinetics of sunflower nutation in centrifugal force fields over an appreciable range of g. The rationale for our experiments was based on certain quantitative predictions which devolve from the geotropic overshoot model.

Israelsson and Johnsson (7) found that they could describe the period and relative amplitude of nutation by an equation which contained only one biologically derived term, the response time for a plant's geotropic reaction after it had been displaced from the vertical position. According to the geotropic overshoot model the geotropic stimulus, S , is assumed to be approximately proportional to the plant organ's angular deviation, α , from the plumb line, at least for small angles. It often is assumed to be proportional to $\sin \alpha$ as was stated explicitly by Israelsson and Johnsson (7). It should be understood that those authors did not consider an acceleration level other than normal gravity

so that the unit value of g was implicit in the proportionality constant. Since in our studies we have made g a variable, we separate it from the constant and include it as an explicit term in the equation relating the geotropic stimulus, S , to the plant's angular displacement from the plumb line. (Vide infra, Equation f, page 20.) The bending response in turn is assumed to proceed at a rate proportional to S . However, because of a substantial time lag -- 20 min. to 1 hour depending on the temperature -- the rate of bending is largely determined by the stimulus which had been perceived at an earlier time. Formally this approximate relationship may be expressed by the equation,

$$\left(\frac{d\alpha}{dt}\right)_t = k g \sin\alpha_{t-t_\Delta} \quad (a)$$

where g is the gravitational or other chronic acceleration and t_Δ denotes the geotropic response time lag.

Israelsson and Johnsson (7) presented equation (a) as a simplification of what they considered a more exact formulation of the model. The difference for present purposes is not critical; therefore, for illustration we shall use the simpler approximation.

It is important to realize that the acceleration term, g , in equation (a) is identified as such and is not made part of the proportionality constant, k , as was done by Israelsson and Johnsson since they did not consider the consequences of conditions other than unit g . To describe the course of nutational movement they made the assumption that the oscillations must be sinusoidal in a given plane and in the 2-dimensional case must describe an ellipse for which the period of oscillation, T , should be related to the value of t_Δ . In the simplest case,

$$T = 4 t$$

(b)

As pointed out by Israelsson and Johnsson (7) the constant in Equation (b) in the simplest case should be a minimal value of 4 but in theory it could assume certain larger values. For present purposes it is only important to note that the period of nutation is a function of t_A and may be expected to be constant if t_A does not change.

It is significant for present purposes to note that t_A (and therefore T also) may be assumed to be independent of the g -level. In physiological terms this suggests that the rate of hormone transport to the region of rapid growth of the hypocotyl should not be importantly dependent on g and, if it is not, that the nutational period, T , should be approximately the same at all g -levels -- at least those substantially above zero. If T , and therefore t_A , does not vary with g , the rate of bending for any particular value of α must be essentially the same regardless of g . Therefore, from equation (a) it is evident that at all times $\sin \alpha$ must be reciprocally related to g . It follows that $\sin \alpha$ should vary with $1/g$ which means that the amplitude of nutational movement should be strongly influenced by the g -level.

In the present study we have manipulated the g -level by protracted centrifugation. We have 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 noted above, viz.

$$T \neq f(g)$$

(c)

$$g \sin \alpha = \text{constant}$$

(d)

MATERIALS AND METHODS

The chosen test species was a dwarf sunflower, Helianthus annuus L., var. "Teddy Bear". The seed was obtained from W. Atlee Burpee Co., Philadelphia. Seeds were soaked overnight and planted in peat pellets obtained from Jiffy Pot Ltd., Grorud, Norway or in a soil-type planting mixture (Burpee Planting Formula #9411-0). Seedling age was calculated in hours from the time of soaking. At the time plants were selected for photographic observation they were well watered and usually the soil or peat pellets were covered with Saran Wrap to ensure that the plant's water supply would be sufficient for the anticipated duration of the experiment.

Those tests which required that the plants be subjected to chronic acceleration were performed on the NASA-UCSC Botanical Centrifuge at the University City Science Center, Philadelphia. The centrifuge rotation rate was established in relation to the subject's location along the centrifuge radius to produce the desired g-level which was maintained well within $\pm 5\%$ at any given point within the centrifuge payload. Seedlings were located on board the centrifuge as close as 125 cm to the axis of rotation, in other cases as far as 340 cm from the axis. Plants were supported in swinging cradles so that the resultant of centrifugal and gravitational forces always was experienced by each plant parallel with its longitudinal axis. During observation on the effects of increased g-levels on nutational behavior of a set of plants, the centrifuge operated continuously at the chosen r.p.m. without any interruption.

All experiments were performed at a nominal temperature of 24° C. The test plants were enclosed in a plexiglass housing which served as a

wind screen. In many experiments temperature was monitored continuously by a thermistor probe inside the housing near the plant. Ventilation holes were provided in the plexiglass housing and temperature regulation was maintained by air conditioning of the centrifuge rotunda air space. Within any particular test the temperature varied no more than 1 degree. The extremes of temperature for all tests were 22° C to 25° C.

Test plants were illuminated continuously from above by Sylvania Wide Spectrum Gro Lux fluorescent lamps at an intensity of 175 ± 5 foot candles. The light impinging on the plants was thus directional and in choosing this method of illumination we were aware that it could affect the kinetics of nutation. Nevertheless our test plants were not etiolated. Their growth rate was reproducible but less than that of the seedlings elongating in darkness. The amplitude of their nutation was less than has been found for plants grown in white light of very low intensity or in darkness. However our principal interest was to standardize on a set of experimental conditions and to examine the effects of only the one variable, the magnitude of the g-force vector. We believe that the use of white light at constant intensity throughout seedling development and during nutational measurements had but a minor effect on the g-function of those properties of nutation in which we were interested.

Light intensities were monitored at the beginning and end of each test run using a laboratory standard G.E. No. 213 light meter.

Information on hypocotyl orientation was obtained with video cameras. The image of each seedling under observation was displayed on a TV monitor (or stored on video tape for later display) for a few seconds every 10 min. or in some tests every 15 min. The images on the monitor

were photographed with an Acme Model 6 processing camera, (Photo-sonics Inc., Burbank, Calif.) so that a permanent record was obtained on 16 mm movie film which thus became a time lapse version of nutational movements. By appropriate frame indexing the time at which each video image was recorded was easily determined and in most tests this was confirmed by including a 24-hour Accutron watch in the field of view of one of the TV cameras. The original video information was transmitted by cable from the centrifuge payload through slip rings to the tape recorder and TV monitor.

For superficial inspection of the course of nutation the film record could be projected at 12 frames per sec speed which displayed movements at from 7,200 to 10,800 times actual speed. A precise description of the kinetics of nutation was obtained by reading the film frame by frame on a Vanguard Motion Analyzer linked to an IBM card punch machine so that the coordinates of reference points on the plants and on their backgrounds were transferred to IBM cards for subsequent processing by the UCSC-IBM Computer, Model 360/75.

In preliminary tests the movements of hypocotyls was computer plotted to produce an essentially sinusoidal time course of nutation for each seedling. Relevant comparisons were made from such records in order to standardize test procedures.

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PRELIMINARY OBSERVATIONS

Once seedlings were old enough to begin nutating we found the kinetics of nutation to be influenced both by the g-level and by seedling age as shown in Fig. 1. This effect was prominent only at higher g-levels (18-20g). Only one or two plants were observed for each test condition represented in this preliminary series of experiments. Accordingly the precision of measurements of periods and amplitudes was low but the results were sufficiently consistent to indicate any gross trends in either parameter with increasing plant age. At some level in excess of about 16 g the magnitude of the force vector was found to exert a pronounced effect on nutation especially in older plants. The trends shown in Fig. 1 were dramatically evident when the time lapse records were projected for normal viewing at a frame rate of 12 sec^{-1} . It was apparent that mechanical stress on the older and larger plants was beginning to overcome the ability of the hypocotyl to raise the cotyledons against the large vector force. In consequence, the amplitude of nutation increased until the cotyledons rested on the substratum for at least part of the time which caused the period to lengthen until nutation ceased. Such changes were erratic and represented the relatively uninteresting condition of extreme mechanical stress interfering with the progress of nutation.

From these and other preliminary tests we selected 6-day old seedlings for all subsequent experiments.

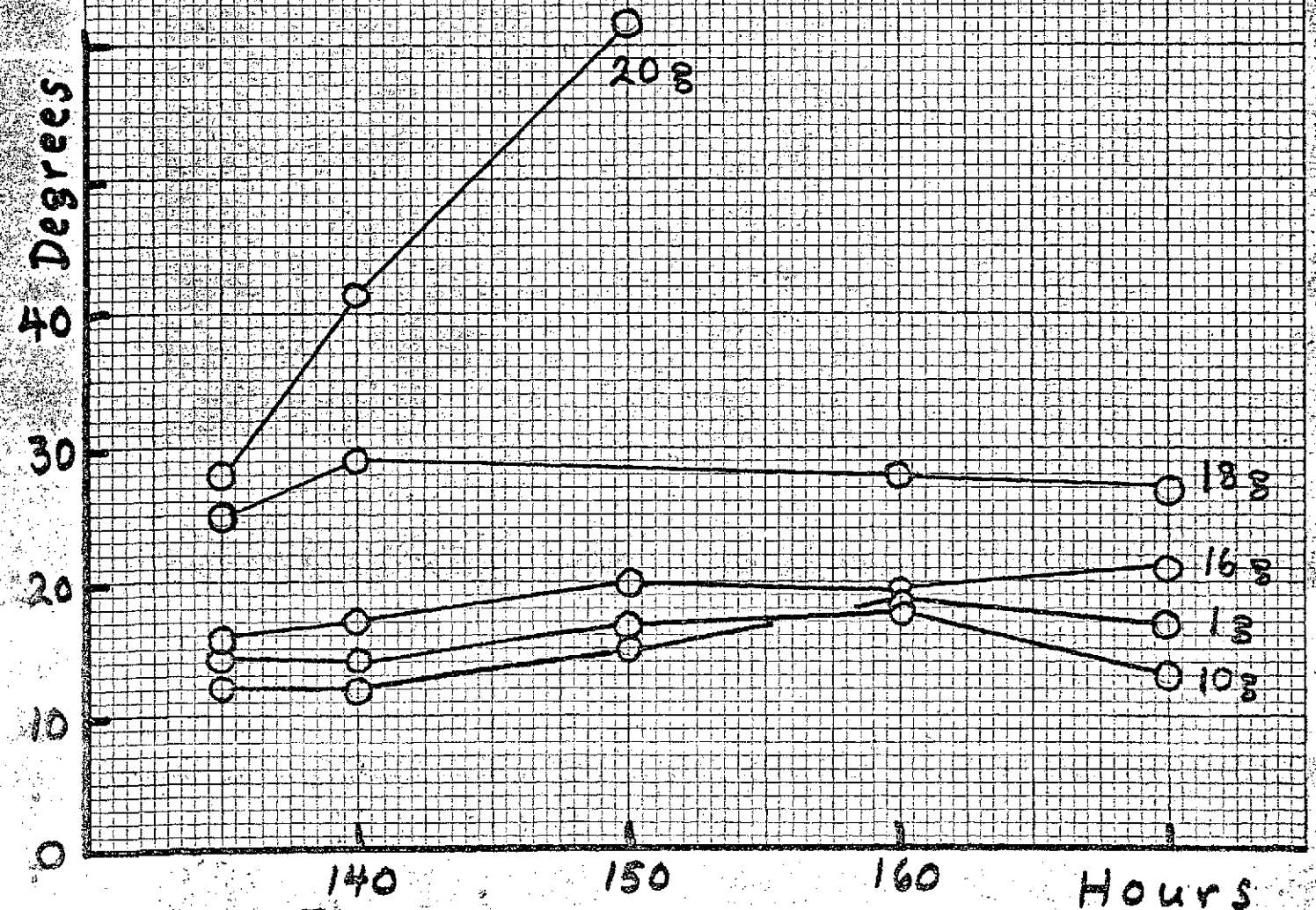
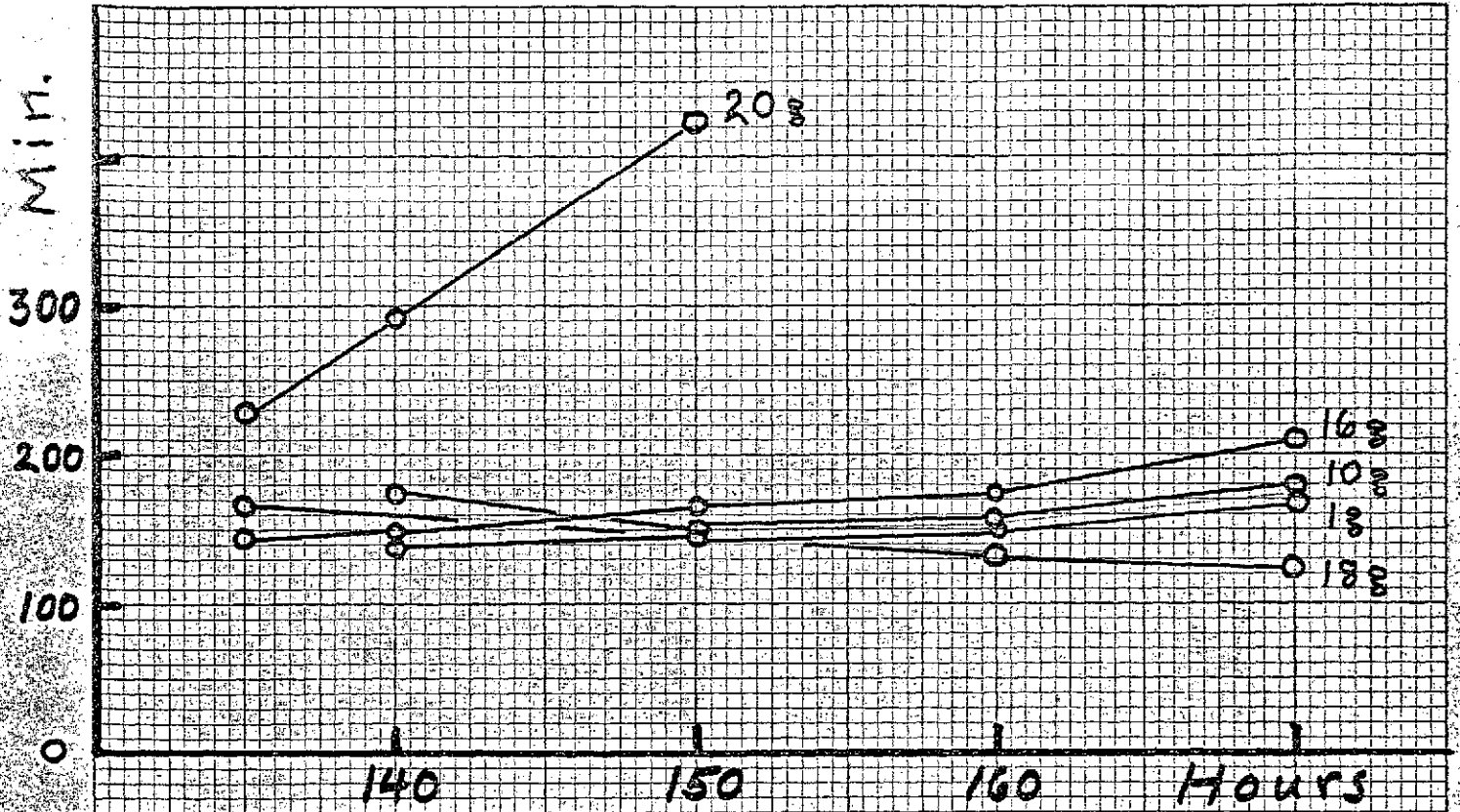
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Fig. 1 Preliminary observations on the nutational responses of plants at different ages to increased g-forces. Upper graph, relation between the period of nutation and plant age. Lower graph, relation between amplitude (extremes of nutational oscillation) and plant age.

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Figure 1



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RESULTS AND DISCUSSION

Data shown in Table 1 were collected as described on 52 six-day old seedlings at a number of g-levels from 1.0 to 20 g. For each test plant 5 nutational cycles were identified - viz., the one nearest in time to plant age, 144 hr., plus the two preceding and the two subsequent cycles. Periods and amplitudes of those 5 cycles were averaged to provide one datum point for period and one for amplitude. Similar data were obtained from other plants exposed to the same g-level in either the same or another experiment. Since, for example, 4 plants were observed at 3.7 g, 4 values were obtained for the period of nutation at that g-level and these were averaged to provide the appropriate entry in the "period" columns of Table 1 opposite 3.7 g. For the calculation of a standard error in this case n was considered to be only 4 (not $4 \times 5 = 20$); therefore the calculation was conservative.

Some of the measurements reported in Table 1 apply to only one or two test plants. We found the plant to plant variation to be relatively large; therefore we decided arbitrarily to limit the analysis of our results to those data for which at least three replicate seedlings were measured at the same g-exposure. There were 41 such test plants observed at seven different g-levels between 1.0 and 9.1 g.

Fig. 2 shows the effects of g-level variation on the period of nutation. It is evident that the period was not significantly g-dependent over a 9-fold range. If, as theory requires, the nutational period is related to the time needed for growth hormone transport to establish an asymmetric hormone concentration at the region of bending (Equation b), the result

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TABLE 1. MEASUREMENTS OF NUTATIONAL
 AMPLITUDE ⁽¹⁾ AND PERIOD ⁽²⁾ OF FIFTY TWO
 HELIANTHUS SEEDLINGS CONTINUOUSLY
 EXPOSED TO ACCELERATION

<u>g-level</u>	<u>number of seedlings</u>	<u>amplitude ⁽¹⁾ of nutation, degrees</u>	<u>period ⁽²⁾ of nutation, min.</u>
1.0	8	13.78 ± 4.24 ⁽³⁾	165.2 ± 9.7 ⁽³⁾
2.9	2	9.35 ± 1.42	160.0 ± 10.0
3.0	3	14.21 ± 1.62	185.0 ± 44.2
3.7	4	13.31 ± 2.76	153.0 ± 3.1
4.1	8	13.99 ± 2.65	159.8 ± 14.2
5.0	4	14.82 ± 2.57	165.5 ± 6.1
6.5	6	22.00 ± 4.35	205.8 ± 25.4
7.3	1	16.48	144.00
8.7	2	13.60 ± 0.37	154.0 ± 10.0
9.1	8	17.95 ± 4.46	179.3 ± 27.6
10.0	1	14.49	156.00
11.4	1	19.82	224.00
16.0	1	18.07	162.00
18.0	2	26.25 ± 0.85	145.5 ± 16.5
20.0	1	41.95	282.00

(1) Amplitude is the maximal change in angle of the hypocotyl axis from one extreme to the other in any cycle.

(2) Period is the time for a complete nutational cycle.

(3) Standard errors were calculated according to the formula,

$$\sqrt{\frac{\sum (x - \bar{x})^2}{n(n-1)}}$$

Fig. 2. Relation between the period of nutation and the g-force.

All plants were 6 days old. Plotted points are mean values.

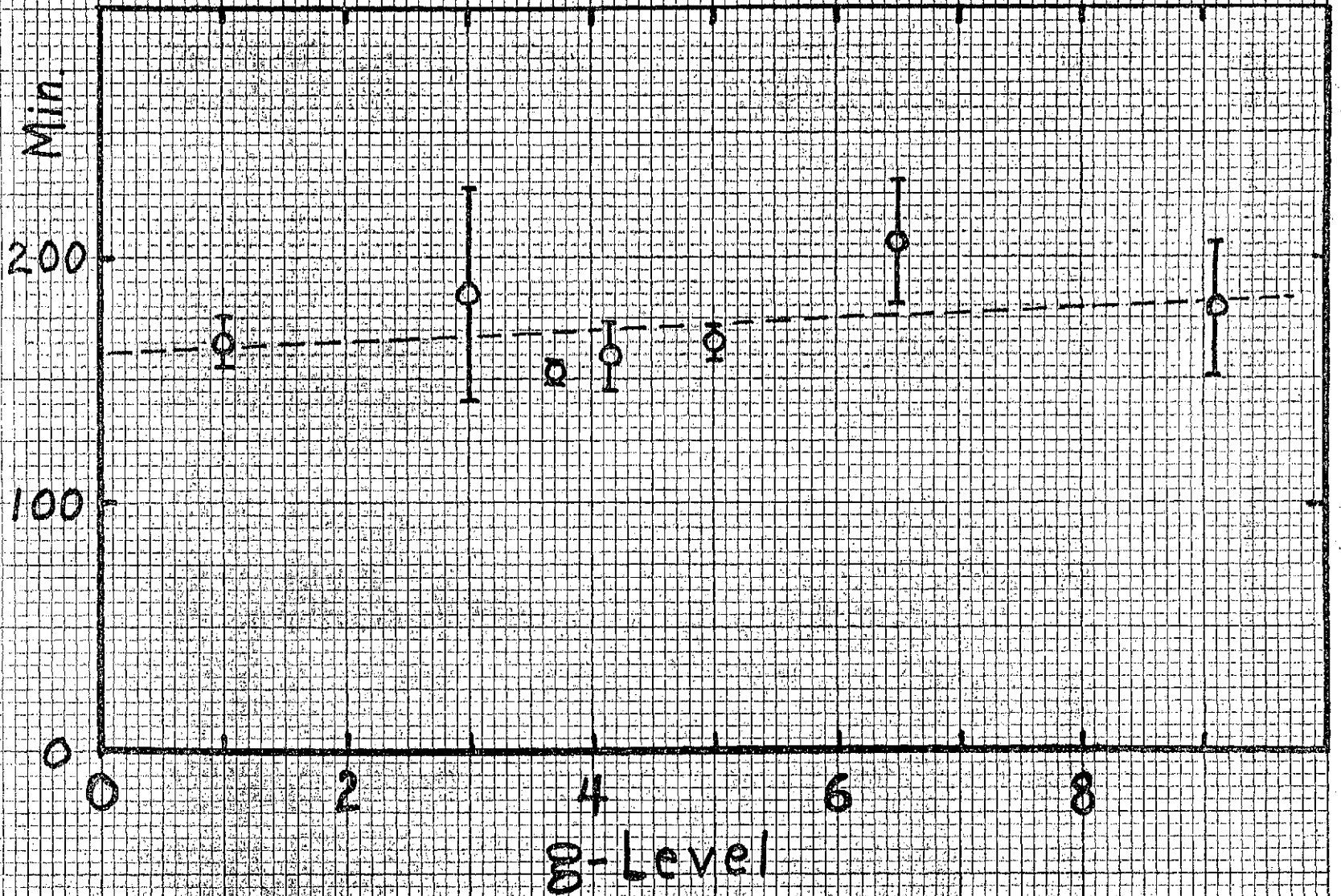
Vertical bars represent ± 1 standard error from the mean.

Dashed line is the regression line fitted by the method of least squares.

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Figure 2



seen in Fig. 2. is evidence that the transport process remains essentially unaffected by chronic acceleration at least over a 9-fold range of g .

Figure 3 shows the effects of g -level variation on the amplitude of nutation. The solid line plotted on the same coordinates is a theoretical curve derived from Equation (d) under conditions for which g is varied. The constant in Equation (d) takes the value of the sine of α_{\max} (the maximal departure from the plumb line) at unit g . For the data set reported here the amplitude of nutation as we have defined it would be twice α_{\max} . The factor .2 of course was taken into account in establishing the predicted amplitude shown by the solid line. The implied prediction was patently not fulfilled. Accordingly, the geotropic overshoot model must be incorrect or at least incomplete since it failed to predict the kinetics of sunflower nutation when the g -level was increased substantially above its normal value.

Fig. 3. Relation between the amplitude of nutation and the g-force. Circles are mean values. Vertical bars represent ± 1 standard error from the mean. Dashed line is the linear regression line fitted by the method of least squares. Solid line is a theoretical prediction as described in the text.

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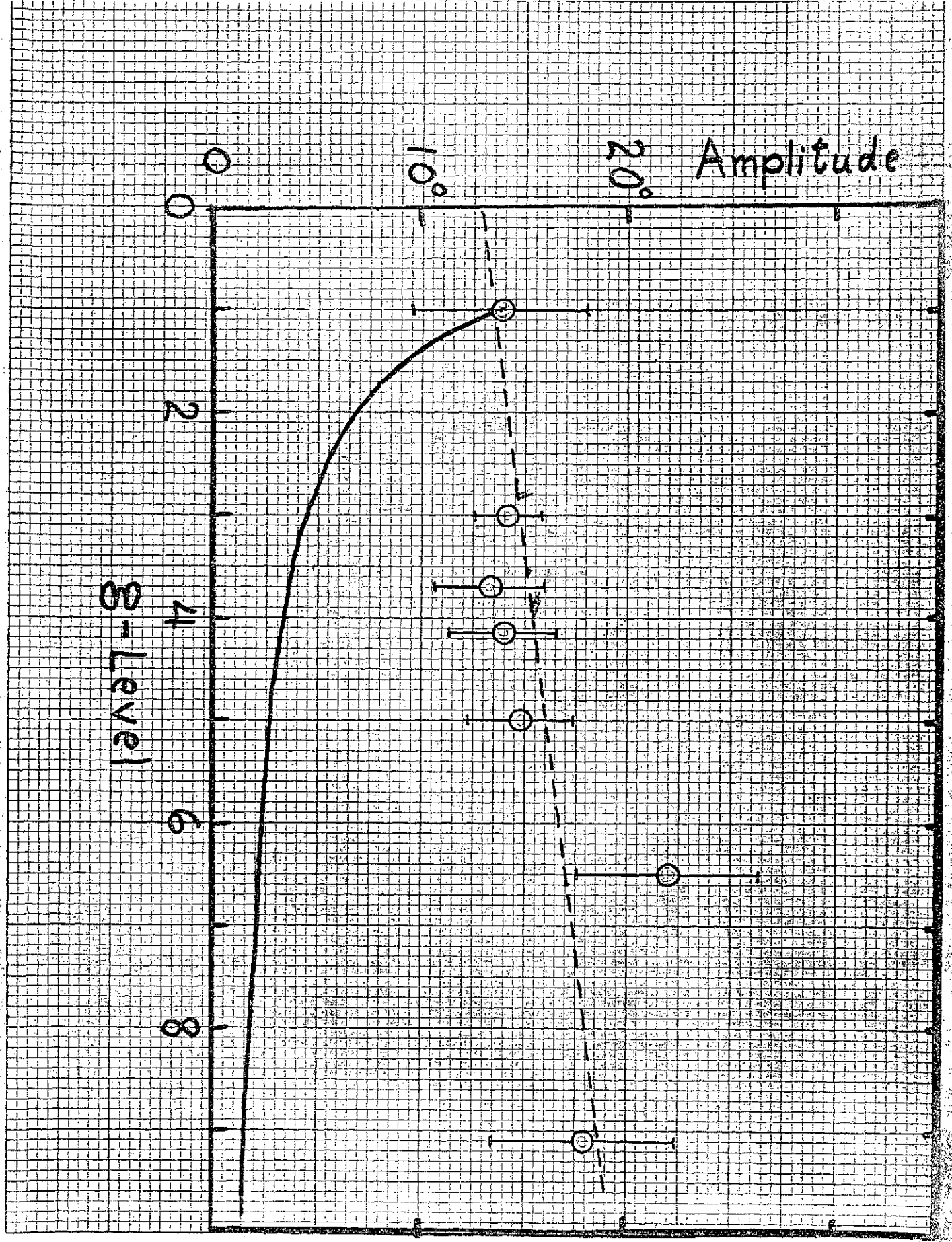


Figure 5

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INTERPRETATIONS

The basic assumptions, expressed in mathematical terms, which we identified with the geotropic overshoot model were the following

$$t_{\Delta} = k_1 T \quad (e)$$

$$S = k_2 g \sin \alpha \quad (f)$$

$$\left(\frac{d\alpha}{dt}\right)_t = k_3 S_{t-t_{\Delta}} \quad (g)$$

It was a combination of Equations (f) and (g) which led us to the simplified summary Equation (a) which is fundamental to the model. We are not inclined to distrust Equation (e) on conceptual grounds and we see no reason why t_{Δ} should be g -dependent. Moreover, since our experimental results showed T to be independent of g we have an additional reason to believe hormone transport to be not significantly affected by elevated g - viz., $k_1 = \text{constant}$.

Equation (g) simply states the concept that the rate of geotropic response should be proportional to the stimulus but it also acknowledges that a response lag or geotropic reaction time, t_{Δ} , exists.

Equation (f), however, embodies several implications which, although mathematically reasonable, may be questioned on physiological grounds.

The use of a product, $g \times \sin \alpha$, to describe the intensity of an

acceleration stimulus is gratuitous. If we consider what may be actually happening as the plant's accelerometers are stimulated by disorientation from the plumb line, it is quite reasonable to use some version of a statolith mechanism to give substance to the argument. However, the exact model of statolith action is moot. In principle there are three ways the statolith may be functioning. It could act during sedimentation, or after the end of its travel through the cytoplasm either by mechanical pressure against that part of the cytoplasm or membrane which supports it, or because of its intracellular position.

It seems unlikely although perhaps not impossible that the statolith could function during sedimentation in response to an altered g-vector. There is a measurable presentation time required for a geotropic response to be elicited, a time which closely corresponds to the period required for the sedimentation of amyloplasts in the cells of the most sensitive tissues. Therefore only after sedimentation can we expect the statoliths to be effective.

If the g-sensor is in fact a membrane pressure sensor, we should expect that its function would depend not only on the direction of the g-vector but on its magnitude as well. In that case Equation (f) might be considered a reasonable approximation. Since we found the nutational response to increased g-force was small and even of the wrong sign (cf. Fig 3), we are disinclined to credit the concept of a pressure sensor. We believe Equation (f) must be unrealistic.

Evidently we should think in terms of a mechanism which relies on a g-dependent principle to account for the reciprocity rule (8911) for minimal presentation time yet which employs a principle not dependent on

g to account for the intensity of the plant's response to a sufficient g -stimulus. A scheme which embodies both principles is a statolith model for which only the presence (i.e., differential distribution) of sedimented particles is important for generating a response to the action of the sensor. The time for achieving some critical redistribution of statoliths should be g -dependent (as has been demonstrated experimentally over a wide range of g -levels) yet the response elicited should be essentially the same at all g -levels adequate to induce sedimentation. Sensing of the g -vector direction is accomplished by the rest position of the statoliths. For a g -vector parallel with the stem axis at the base of the hypocotyl the statolith position in the epicotyl region would be predicted by a sine function of the hypocotyl's angle of displacement from the plumb line, but the final position of the statolith would not depend on the magnitude of g . Accordingly the response -- whether a simple geotropic righting reaction or a more complex nutational oscillation -- should be essentially independent of the magnitude of g (above some threshold, of course). Therefore, the g term in Equation (f) is inappropriate.

Our suggestion of a sensor mechanism which can detect the direction of a g -vector but not its magnitude is not a novel one. However, ours is perhaps the first report of a set of experimental results which serve to restrict the possible mode of action of a plant's geotropic sensor in this way. Accordingly, we subscribe to the statolith model just described in qualitative terms for it predicts a nutational behavior consistent with the geotropic overshoot model.

We note that our experimental results with respect to the period of nutation (Fig 2.) showed a regression line with a slope near zero (1.5%

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per g unit). The correlation coefficient was 0.21 and was statistically not significantly different from zero ($P > 5\%$). However, the regression line (Fig. 3) which related amplitude to g-level had a 5% slope and in that case the correlation coefficient was 0.39 which was significantly greater than zero (P near 1%). We must, therefore, accept the fact that our results imply a small but significant increase of amplitude with increasing g. This effect was minor but, nevertheless, it would not be predicted by the model we have described. We can account for the effect, if we appreciate that under increased g loading the elongating hypocotyl is more heavily stressed by the increased weight of the cotyledons at the extremes of the oscillation. The effect of this would be a small increase in angular displacement over what would occur at a lower g-level. Such an increment in amplitude should have no effect or at most only a very slight influence on the period length. There may be other reasons for the positive slope of the regression line in Fig. 3 but we believe the factor we mentioned, which has nothing directly to do with the action of the g-sensor, would be quite sufficient to explain the effect.

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PLANS FOR FURTHER EXPERIMENTS

As a consequence of our quantitative examination of sunflower nutation we believe it would be useful to test directly whether in a simple geotropic response or righting reaction the response time (adjusted for presentation time), the velocity of the bending response, and the amount of overshoot will prove to be independent of the g -level used for stimulation and that the duration of g -stimulation rather than the intensity of g will be the only effective determinant of these response modes -- all of which are predicted by the model we have favored to explain our results in the present report.

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