Gravitational Stimulations Inhibit Oscillatory Growth Movements of Plants

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Circumnutation, Clinostat, Sunflower Seedling

Spontaneous oscillatory growth movements around (or across) the plumb line occur in young sunflower seedlings. The period is about 2.5 h. When such plants are tilted 90° and continuously rotated around their axis with a speed of 0.5 rpm, the oscillatory spontaneous movements cease. The findings are interpreted as supporting a previously published model for gravitationally induced plant movements.

Introduction

Deviations from the normal growth direction often initiate compensatory growth reactions of a plant. A young oat plant, for example, normally grows upwards but tilting it horizontally for less than 30 s is enough to induce a growth reaction (Shen-Miller [1], Johnsson [2]). The reaction manifests itself in a curvature of the plant stem some hours later. The previous "under" side of the plant elongates more than the "upper" one and this results in a detectable curvature.

The tilting of the plant with respect to the plumb line induces a gravitational stimulation of the plant and is called a geotropic stimulation or perturbation. The subsequent bending reaction is called a geotropic reaction. The geotropic reaction always counteracts the deviation from the growth direction.

The ability of the plants to compensate deviations from the plumb line, as outlined above, is an example of a physiological feedback system. In such a system oscillations can arise if certain conditions on loop gain and signal delays in the loop are fulfilled. Rhythmic passages of the plant across the plumb line (or helical movements around the plumb line) could therefore be anticipated. Interestingly enough such oscillatory growth movements are also known from plant physiology, they are often termed circumnutations (Darwin [3]). The period of these oscillations lies mostly in the range of some hours at room temperature.

However, controversies arise about the origin of the circumnutations. One group of investigators regards them as being the result of an oscillatory, geotropic feedback system as outlined above, while another group interprets them as being caused by internal timing mechanisms, not yet describable but unconnected to geotropic reactions (see review by Johnsson and Heathcote [4]). It is the purpose of this paper to contribute to a discrimination between the two approaches in the case of sunflower seedlings.

Material and Methods

a. *Material*

The experimental material was hypocotyls of sunflower, *Helianthus annuus* L. (c. v. Californicus). The seeds were soaked in water 6 – 8 days before the experiment. Grown in red light (>640 nm) and planted in soil 4 – 6 days before the experiment the plants achieved at this stage an average length of 60 mm and performed vigourous circumnutations. For details on the growing procedure, see Andersen and Johnsson [7]. The temperature was 22.5 – 24.0 °C.

b. *Definitions. Administrations of geotropic perturbations*

We will now discuss plant movements in a plane, referring to Fig. 1.

α will denote the curvature of the upper, geotropically sensitive part of the plant stem with respect to the container axis. We define α as positive when the tip is to the right of the container axis. In Figure 1 a the container axis coincides with the plumb line. Under such conditions self-sustained oscillations in α, circumnutations, will occur spontaneously. The period of these oscillations is 2 – 2.5 hours at room temperature.

α_p denotes the angle between the plumb line and the axis of the plant container as shown in Figure 1 b

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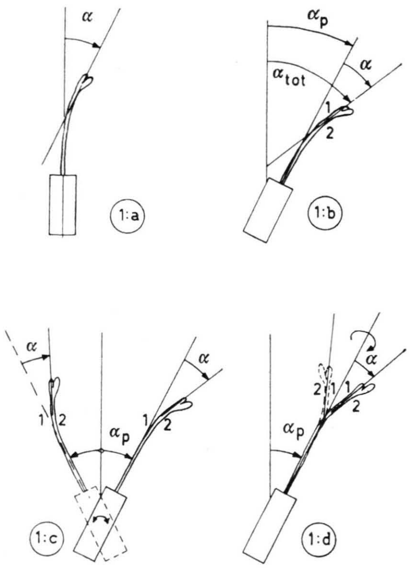

Fig. 1. a. The angle between the top of the plant stem and the container axis is called α. The container axis coincides with the plumb line. Spontaneous oscillations in α occur in sunflower seedlings.

b. A geotropic perturbation is given to the plant by tilting the container α_p degrees. The top of the stem is now tilted α_{tot} degrees with respect to the plumb line. The arrows indicate direction of positive angles. The 'under' side 2 will later elongate more than the 'upper' side 1 due to this perturbation.

c. The container is tilted to and fro around an axis perpendicular to the paper. The plant receives in this way a geotropic perturbation varying in time. In the two positions depicted, α_{tot} is $\alpha+\alpha_p$ and $\alpha-\alpha_p$ respectively.

d. The same tilting angles and thus the same perturbations as in 1 c can be administered to the plant by a repeated turning of the container axis. The position indicated by broken lines corresponds to the left position of Figure 1 c.

(index *p* stands for perturbation). We define α_p as positive when the container (and the plant) is tilted to the right and negative when it is tilted to the left.

α_{tot} finally, is the sum of α_p and α and indicates the angle between the plumb line and the upper part of the stem. It is evident that if $\alpha_{tot} > 0$, then side 2 of the plant stem will be the "under" side and will later elongate more than the "upper" side 1. If $\alpha_{tot} < 0$ side 1 will elongate more than side 2.

In the present paper geotropic perturbations α_p, varying with the time, will be added to the spontaneous oscillations in α. α_p can be varied by a suitable tilting of the container to and fro as in Fig. 1 c. The stimulation angle is $(\alpha+\alpha_p)$ in the right position of the plant in Figure 1 c $(\alpha-\alpha_p)$ in the left position.

Instead of using a device which tilts the plant according to the procedure sketched in Figure 1 c, an alternative, simpler perturbation procedure can be used and was also adopted in the present study. The plant can be rotated around the container axis, this axis being tilted α_p degrees with respect to the plumb line, Fig. 1 d. Such a rotation causes the sides 1 and 2 to receive the same stimulation as in the treatment shown in Figure 1 c (the plant position drawn with broken lines in Figure 1 d corresponds to the left position of the plant in Figure 1 c).

In the present investigation the rotation rate equalled 0.5 rpm. The main axis of the plants was parallel to the container axis which in turn was tilted 90° with respect to the plumb line in most of the experiments.

A device to give alternating geotropic stimulations according to Figure 1 d is called a clinostat in the plant physiology. As a laboratory tool it has lately received new attention since it has been claimed that certain plant reactions under "zero-g", satellite, conditions can be perfectly imitated in a clinostat experiment (*e. g.* Lyon[5], Gray and Edwards[6]).

c. *Recording of the plant movements*

Photos of circumnutating plants were taken every sixth minute The plane in which the plants performed the most vigorous oscillations was chosen to be perpendicular to the camera axis. The clinostat used in the clinostat experiments was synchronized with the flash and the camera. A red light flash (>640 nm) provided suitable background illumination.

The projected curvatures of the plants as recorded on the films were measured with the aid of a protractor. The angles could be measured by the same person with an accuracy of 1° (as checked in repeated readings of the same sequence of photos).

Experimental Results

The curvatures of plants in seven representative experiments are shown in Fig. 2. The angles, measured from the films, are given as functions of the time. Clinostat treatment started at times indicated by the vertical lines crossing every curve. It is seen that the circumnutation had a large amplitude prior to the clinostat treatment. In all the experiments of Fig. 2 the clinostat axis was horizontal, *i. e.* $\alpha_p = 90°$.

From the figure one concludes

1. that the vigorous oscillations ceased on the clinostat independent of the phase of the oscillation at which the clinostat treatment started.

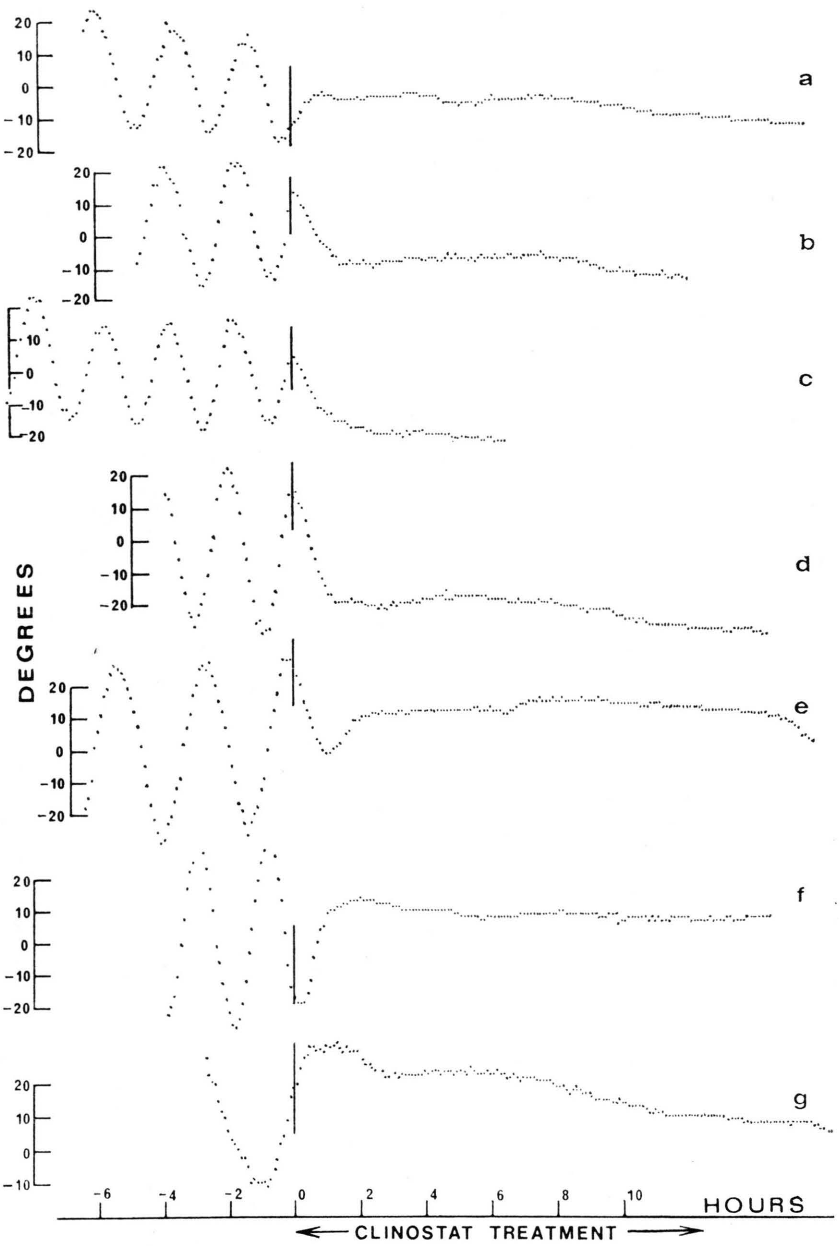

Fig. 2. Clinostat treatment inhibit oscillations. The angle α as measured from time-lapse photos is plotted as function of the time. The vertical bars indicate start of clinostat treatment (zero time of the time axis). In all plants oscillations were evident prior to the clinostat period. The oscillations died out under the following clinostat treatment.

2. that the curvatures did not stop immediately on the clinostat but went on roughly 0.5 – 1 hour after start of clinostat period (see plant *f* for an evident example).

From the literature one knows that new oscillations can be started in previously clinostated plants by giving a new geotropic perturbation (after, for example, four hours of clinostat treatment as in Andersen and Johnsson[7, 8]). These new oscillations are then phase determined by the geotropic perturbation (*cf.* Fig. 1 in Andersen and Johnsson[8]).

If α_p is less than 90°, the clinostat influence on the circumnutations will be less drastic; if $\alpha_p = 0$ the influence is, of course, absent. Results when α_p equals 45° and 10° will later be exemplified for illustrative purposes.

Theoretical Results

a. *Outline of Model*

In this section an outline of a geotropic feedback model for circumnutations will be shortly presented and used to predict the behaviour of the plants on the clinostat.

For small angles $\alpha(t)$, the circumnutations in one plane can be described by the following formula according to results published earlier (*cf.* Johnsson and Heathcote[4], and references in that paper):

$$\mathrm{d}\alpha(t)/\mathrm{d}t = -\text{constant}\int_0^\infty f(t'/t_0)\cdot\sin\alpha_{\text{tot}}\,(t-t')\,\mathrm{d}t' \,. \tag{1}$$

The sine function is introduced since that transversal component of the gravitational force vector, $g\cdot\sin\alpha_{\text{tot}}$, causes the physiological stimulation which leads to a bending reaction.

The bending rate of the plant at a certain time t, is proportional to the geotropic stimulations accumulated at t. The right hand side of Eqn (1) shows that the stimulations are weighted according to the weighting function f before they add to the accumulated stimulation.

In papers on this model f is assumed to have an exponential decay, thus the importance of old stimulations is diminished while new ones are emphasized.

Eqn (1) describe oscillatory plant movements in a plane. Such movements are also found experimentally in sunflower hypocotyls. Circumnutations *around* the plumb line, which are encountered more frequently, can, however, be regarded as composed of two perpendicular movements of the type discussed, added vectorially. Only movements in a plane will therefore be treated in the following.

Eqn (1) can be interpreted as describing a geotropic feedback system (see Johnsson and Heathcote[4]).

b. *Extension of model*

Since in the present paper we will use angles larger than 90° we now generalize Eqn (1) to

$$\begin{aligned}\frac{\mathrm{d}\alpha(t)}{\mathrm{d}t} &= -\text{const.}\int_0^\infty f(t'/t_0)\cdot r(\alpha_{\text{tot}}(t-t'))\,\mathrm{d}t' \qquad (2)\\ &= -\text{const.}\int_0^\infty f(t'/t_0)\cdot r(\alpha(t-t')+\alpha_{\text{p}}(t-t'))\,\mathrm{d}t' \,.\end{aligned}$$

r constitutes a non-linear function transforming the angular deviation of the plant into a physiological perturbation. For angles less than 90° it equals a sine function.

However, above 90° r is assumed to be different from a sine function. The reason for this is as follows: Geotropic experiments on many species have shown that a geotropic stimulation of 120° is more effectiv than one of 60°, 135° is more effective than 45° etc. (Audus[9], Larsen[10], Pickard[11]). Thus a sine function can not be appropriate for all angles up to 180°. The precise appearance of the r-function seems to be dependent on the durations of the geotropic stimulation (Larsen[10]).

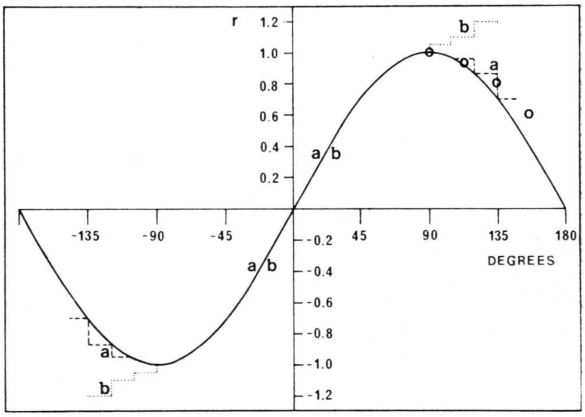

Fig. 3. Function r, used in the simulations. Two r-functions, according to curves a and b, are used in the simulation. Curve a: Equals a sine function (unbroken line) for arguments <90°. Above 90° it follows the broken lines, which in turn are approximations to experimental results shown by the circles. Curve b: Is also a sine function for arguments <90°. For arguments >90° the dotted lines are followed.

The r-function used in most simulations in the present paper is depicted in curve a, Fig. 3. Except in the sine-curve region the function was assumed to be step-wise constant. Since the normal amplitude of the oscillations was about 20° and the biggest α_{p} used was 90°, the range of the amplitudes used did not exceed about 110°. Therefore the r-function has not been specified up to 180°.

The curve a was drawn on the basis of a pre-experiment in which the reaction of plants stimulated in different angles was determined. The pre-experiment confirmed that a stimulation in position 120° is more effective than in 60° etc.; thus a sine function is not an appropriate choice above 90°.

The geotropic reactions (taken as the maximum curvatures in response to 20 min stimulation) were related as 1 : 0.92 : 0.81 : 0.60 when the stimulation angles were 90°, 120°, 135° and 150° respectively. The ratios are shown as circles in Fig. 3.

Curve b, Fig. 3, was used in some simulations and will be discussed later.

c. *Simulations of behaviour of plants on a clinostat*

In most simulations $a_p(t)$ in Eqn (2) was a square wave function with a period of 2 min and amplitude of 90°.

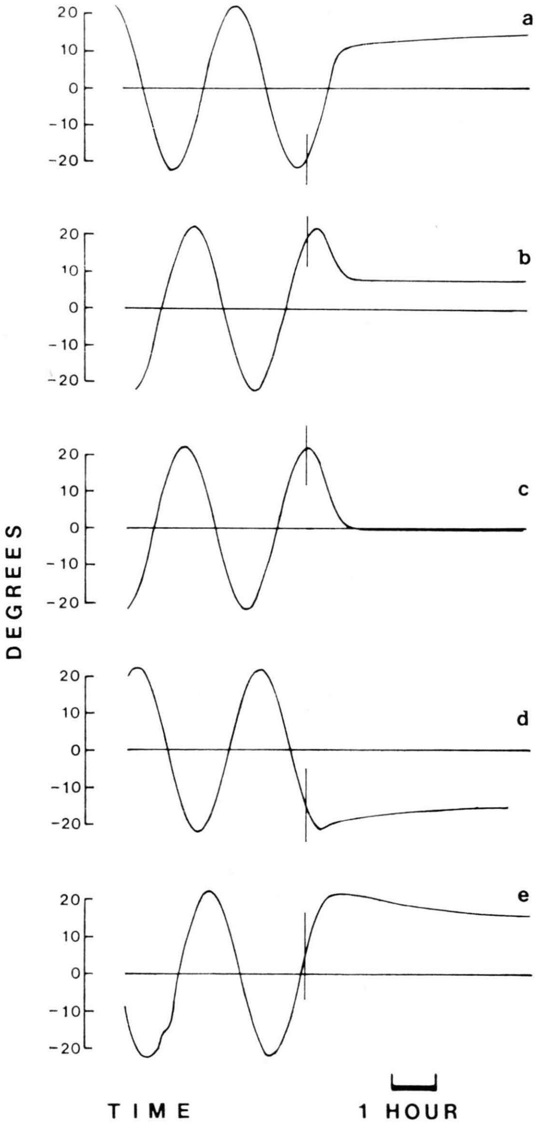

Fig. 4. Simulations of plant behaviour on a clinostat. Clinostat treatment is supposed to start at the vertical bars of every curve. The results from these simulations of clinostat treatment starting in different oscillatory phases should be compared with the experimental results in Fig. 2.

The *f*-function, as well as other parameters in Eqn (2) used in the present computations are taken from previous publications, for details see the Appendix. The *r*-function used is curve a of Fig. 3.

Results from the simulations are given in Fig. 4. It is seen that the theoretical model predicts a drastical decrease in the amplitude of the oscillations when a transient period of about one hour has elapsed. The agreement with the experimental results shown in Fig. 2 is rather good, recalling that the appearance of the *r*-function is probably only approximate. Curves which show good agreement are, for example, 2 a and 4 a, 2 b and 4 b, 2 d and 4 c, 2 g and 4 e. The most conspicuous feature of the experimental results, namely the absence of the oscillatory movements on the clinostat, is thus predicted from Eqn (2).

Variations in the *r*-function did not appreciably change the results of the simulations: The high frequency geotropic stimulations reduced the amplitude drastically. One note should be added. With an *r*-function as depicted in Fig. 3, curve b, small oscillations persisted also during the clinostat period, see Fig. 5 a.

Even if such a behaviour was not found experimentally, it is principally interesting to find that the Eqn (2) does not predict the cessation of oscillatory movements on a horizontal clinostat under all circumstances. A further comment on this point will follow in section d below.

Theoretical results from the model when the amplitude of the perturbations a_p equals 45° and 10° are given in Figs 5 b and 5 d. These simulations thus correspond to the case that the clinostat axis is tilted 45° or 10° respectively. The oscillations continue on the clinostat as they also do experimentally (Figs 5 c and 5 e). The slight influence on amplitude and period time when a_p is small has also been observed in *Cuscuta* (Baillaud[12]).

d. *The theoretical model of the circumnutations and clinostat treatment — some additional remarks*

It was mentioned that the geotropic model discussed in this paper could allow also for oscillations of plants on a horizontal clinostat (see section c). The continuation of oscillations under such clinostat treatment is therefore *not* an argument against the model or the feedback concept behind it. It could be instructive to discuss simple versions of

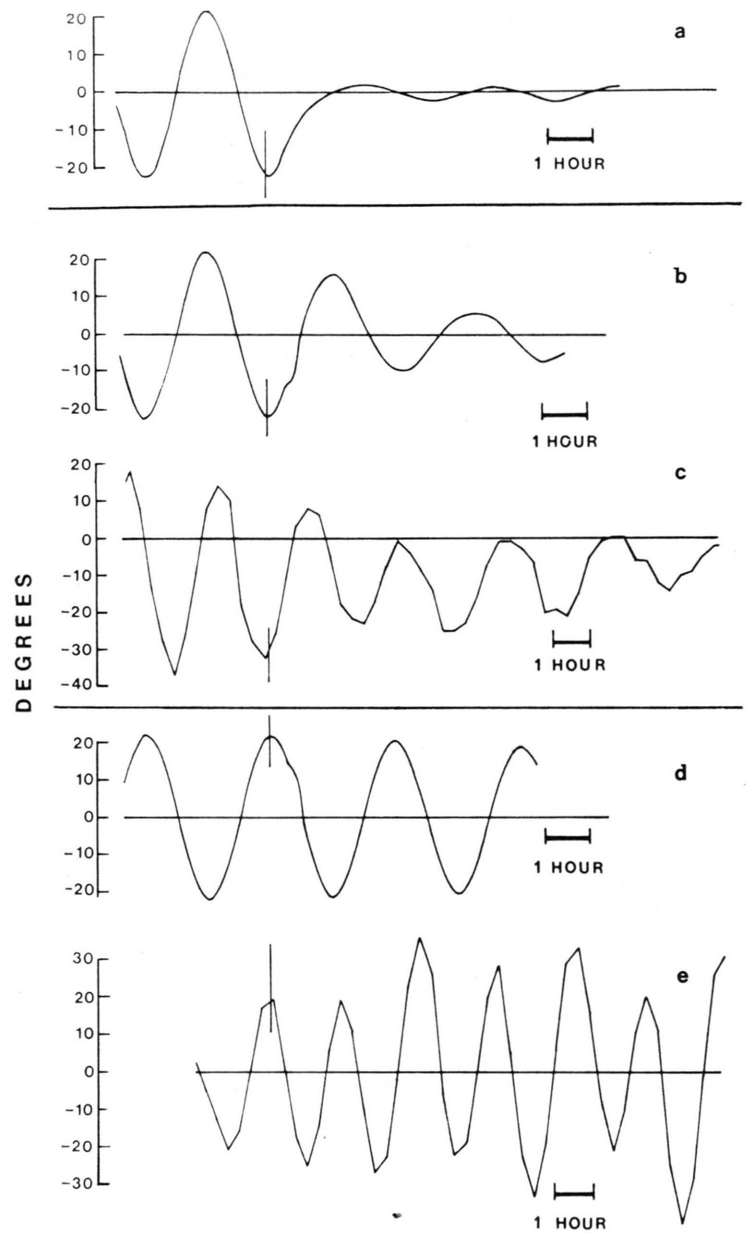

Fig. 5. Results from simulations with altered parameters.
a. Shows that small oscillations may theoretically persist during the clinostat treatment. An *r*-function according to curve b, Fig. 3, was used to achieve this result.
b. Simulation of oscillation when the clinostat axis is supposed to be tilted 45° ($\alpha_p = 45°$) *not* 90° as in Fig. 4. Oscillations are slowly damped on the clinostat (*r*-function according to curve a, Fig. 3).
c. Experimental recording of plant oscillations when clinostat axis is tilted 45°, *cf.* 5 b.
d. Simulation of oscillations when the clinostat axis is supposed to be tilted 10° from plumb line ($\alpha_p = 10°$). Oscillations persist virtually unaffected by this small tilting (*r*-function according to curve a, Fig. 3).
e. Experimental recording of plant oscillations when clinostat axis is tilted 45°, *cf.* 5 d. No damping occurs.

Eqns (1) and (2) in order to understand the importance of the *r*-function and the inclination α_p of the clinostat axis for the arousal of oscillations under clinostat conditions.

1. Sine-version of Eqn (2)

Assume that the *r*-function in Eqn (2) is a sine function, *i.e.* $r(\alpha_{tot}(t)) = \sin(\alpha_{tot}(t))$. Further on, if the weighting function f is assumed to be a delta function, *i.e.* $\delta(t' - t_0)$, then (2) changes to

$$d\alpha(t)/dt = -k \cdot \sin \alpha_{tot}(t - t_0) \quad k \text{ is a constant} > 0 .$$

Assume the plant to be repeatedly stimulated for $\tau/2$ min in position α_p and the following $\tau/2$min in position $-\alpha_p$. Since the period of the perturbations, τ, is much longer that of the oscillations, the average bending rate during τ min will be

$$\overline{d\alpha(t)/dt} = 1/\tau \int_t^{t+\tau} \frac{d\alpha(t)}{dt} \approx -\frac{k}{2}\,[\sin(\alpha(t-t_0)+\alpha_p) + \sin(\alpha(t-t_0)-\alpha_p)] \approx -k \cdot \cos\alpha_p \cdot \sin\alpha(t-t_0) .$$

If a_p is very small, *i. e.* the clinostat axis just slightly tilted (*cf.* Figs 5 d and e) then

$$\overline{\mathrm{d}a(t)/\mathrm{d}t} = -k \cdot \sin a(t-t_0)\,.$$

The small tilting of the clinostat axis does not affect the oscillations.

With a_p equal to 90°, we have a good approximation to the simulations in Fig. 4, where an *r*-function not too different from a sine function was used (curve a, Fig. 3).

In this case

$$\overline{\mathrm{d}a(t)/\mathrm{d}t} \approx 0\,.$$

Thus the oscillations are damped when the clinostat axis is horizontal.

The arguments in this version can be extended to a case with several, weighted, sine terms in the right hand side of the equation, *i. e.* to Eqn (1).

2. Non-linear version, $a_p = 90^\circ \gg a(t)$

As before we first assume that the *f*-function is a delta-function. Then Eqn (2) will give the average value

$$\overline{\mathrm{d}a(t)/\mathrm{d}t} \approx -k/2\,[r(a(t-t_0)+a_p) + r(a(t-t_0)-a_p)]\,.$$

Assume that r is not symmetrical around 90° (as was the case with the sine function) but can be written as

$$r(a_{\mathrm{tot}}) = k'\,a_{\mathrm{tot}} + l' \quad (a_{\mathrm{tot}} \approx 90^\circ)\,,$$

in a region close to $a_{\mathrm{tot}} = 90^\circ$. k' and l' are constants. Then, since r is an odd function, we have around $a_{\mathrm{tot}} = -90^\circ$.

$$r(a_{\mathrm{tot}}) = k'\,a_{\mathrm{tot}} - l' \quad (a_{\mathrm{tot}} \approx -90^\circ)\,.$$

Thus

$$\overline{\frac{\mathrm{d}a(t)}{\mathrm{d}t}} \approx -\frac{k}{2}\,[k'(a(t-t_0)+a_p) + l' + k'(a(t-t_0)-a_p) - l'] \approx -k \cdot k' \cdot a(t-t_0)\,.$$

Oscillations can arise if $k \cdot k'$ is large enough (*cf.* Johnsson and Karlsson[14]). This discussion evidently bears upon the appearance of the *r*-function close to $a_{\mathrm{tot}} = 90^\circ$. If the slope of the *r*-function is steep enough oscillations can arise on a horizontal clinostat, as was examplified by the simulation in Fig. 5 a.

The arguments in this case 2 can be extended to a case with several, weighted terms in the right hand side of the equation.

Discussion

From the experimental and theoretical results in the present paper one concludes that the model used is adequate to describe the damping and cessation of circumnutations of sunflower seedlings on a clinostat. Also the start of new oscillations after a clinostat period can be described (see *e. g.* Andersen and Johnsson[7, 8]). Whether the simple model holds also for other plant species is of course an open question.

Now, if a supposed internal oscillatory mechanism, independent of all geotropic reactions, was responsible for the circumnutations (*cf.* review by Johnsson and Heathcote[4]) the experimental results would be different. The geotropic stimulations during the clinostat treatment would lead to geotropic reactions decoupled from the circumnutations. Circumnutations would then turn up, superimposed on the geotropic reactions and, in particular, they would continue unperturbed under clinostat conditions. Since this was not the case, it seems unnecessary at the present stage to involve "internal timing mechanisms" to explain the circumnutations of sunflower seedlings.

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Appendix

In the simulations of the plant movements the following function f was used in Eqn (2).

$$\begin{cases} f(t'/t_0) = 0 & 0 \leqq t' \leqq t_0\,. \\ f(t'/t_0) = e^{-2.49(t'/t_0-1)} & t' > t_0 \end{cases} \tag{3}$$

t_0 thus accounts for a delay in the feedback system (a delay possible to interpret as the so called geotropic reaction time). t_0 equalled 30 min in the simulation. The factor 2.49 in (3) was taken from Andersen and Johnsson[7, 8].

The numerical value of the constant in Eqn (2) was taken to be 0.0035 rad/min (from Andersen and Johnsson[7]). This constant corresponds to k/t_0 in Israelsson and Johnsson[13].

Slight refinements of Eqns (2) and (3) have been published (Johnsson[2], Andersen and Johnsson[7, 8]) but for the present purposes these refinements are superfluous.

Simple Euler integration was used in the simulations.

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