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Physiology of Movements in Stems of Seedling *Pisum sativum* L. cv. Alaska[1, 2]

I. EXPERIMENTAL SEPARATION OF NUTATION FROM GRAVITROPISM

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ABSTRACT

Gravitropism and nutation in the stems of dark-grown, seedling peas (*Pisum sativum* L. cv. Alaska) were recorded on time-lapse photographs made with photomorphogenetically inactive light. Although gravitropism and nutation have been connected by several different theories in the past, our experiments indicate that the two processes are in fact dissociable. The evidence is as follows: (a) Nutational patterns are asymmetric. There is much greater amplitude of oscillation in the plane parallel (‖) to the plane of the apical hook than in the plane perpendicular (⊥), yet the average gravitropic response is equal in these two planes. (b) Brief red light irradiation given 16 to 24 hours before observation greatly increases the amplitude of nutation in the ‖-plane, but has no influence on the kinetics of gravitropic response. (c) An inhibitor of auxin transport, α-naphthylphthalamic acid, strongly inhibits nutation at 5 micromolar but affects gravitropism only at higher concentrations. (d) Nutation is also strongly inhibited by removal of the apical bud, but gravitropism is unaffected. (e) The period of nutation does not exhibit a constant relationship to the response time of gravitropism. The above evidence is inconsistent with theories that gravitropism is an asymmetrically modified nutation or, alternatively, that nutational oscillations result in a simple fashion from gravitropic overshoots. The evidence is consistent with, although not proof of, autonomous factors such as an endogenous rhythm of growth as the cause of nutation in pea stems. However, gravity and nutation do interact. Nutation in a population of seedlings can be synchronized and brought into phase by a single gravitropic induction. Furthermore, the response time and initial rate of gravitropic curvature depend to some extent on the phase of nutational curvature at which gravitropic induction is begun.

Many growing plant organs (*e.g.* tendrils, stems, roots) execute a series of oscillatory bending movements called nutations (reviewed in 24). Nutations may be confined to one plane (pendulum type) or expanded more generally into three dimensions so that, as the plant grows, the tip traces in space an irregular helix with an elliptical cross-section (circumnutation). The oscillatory movements reflect shifting states of differential cell expansion on opposite flanks. The widespread occurrence of nutation led Darwin and Darwin (11) to postulate that nutations must somehow be basic to the growth mechanism of the plant. Some recent investigators (20) have proposed that endogenous rhythms of growth underlie the often seemingly spontaneous oscillatory bendings of nutation.

Unilaterally directed stimuli such as light or gravitational attraction can also cause bending movements (phototropism and gravitropism, respectively). Most nutating organs exhibit tropistic responses which may be superimposed both temporally and spatially on the nutations. Inasmuch as these types of curvature all involve differential cell expansion, it is reasonable to expect some interaction between them. For example, the Darwins hypothesized that tropistic responses are essentially asymmetrically modified nutations.

An alternative theory is the gravitropic overshoot hypothesis (reviewed in 24) which states that nutations are not autonomous oscillations, but rather manifestations of the gravitropic response. This theory envisions brief deviations from the vertical axis as inducing a gravitropic response proportional to the sine of the angle (α), the duration of stimulation, mass acceleration (g), and the overall gravisensitivity of the plant organ (k). Because of a finite lag time between the initiation of curvature and the onset of gravistimulation (t_r), the reacting organ will overshoot the vertical only to reinitiate gravitropic response in the opposite direction. A sustained oscillation driven extrinsically by gravity is the result. Mathematical models with appropriate constants do in fact generate self-sustaining oscillations typical of nutation in two or three dimensions (Andersen, cited in 24). The relation of nutation to gravitropism is formalized in a simplified equation (22),

$$(\mathrm{d}\alpha/\mathrm{d}t)_t = -k\cdot g\cdot \sin\alpha(t - t_r) \qquad (1)$$

in which the rate of nutational curvature at time t (negative with respect to the gravity vector for a stem) is proportional to the gravitropic stimulation received t_r min before.

Three initial predictions of the gravitropic overshoot hypothesis are that the rates of nutational and gravitropic curvature should be similar, that a constant relationship is expected between the period of nutation (τ) and t_r, and that gravitropic stimulation should have phase-shifting and entrainment effects on nutational oscillations. These predictions have been confirmed for *Helianthus* (1, 2, 22, 25), but in *Phaseolus* the rate of gravitropic curvature is much slower than that of nutation and gravitropic induction failed to phase shift nutations (20).

According to the gravitropic overshoot hypothesis, compensation of the vectorial effects of gravity by rotation of plant material on clinostats should eliminate gravitropic response and, consequently, nutation. However, clinostat treatment of *Helianthus* reduced but did not eliminate nutations (10). Another prediction of the model, diminished nutational amplitude during enhanced

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gravistimulation (*i.e.* centrifugation), also could not be confirmed in *Helianthus* (9). Thus, the gravitropic overshoot hypothesis in its simplest form seems insufficient to account for all data. The data are consistent with the alternative view that some autonomous rhythm is responsible for nutations, but this hypothesis is sufficiently vague as to preclude precise predictions except that nutation, should persist in the absence of gravitropism.

More work is in order on species in which nutation and tropisms can be experimentally altered and compared. The stems of dark-grown, intact pea seedlings provide such a system. R[4] pretreatment dramatically increases the amplitude, regularity, and frequency of nutations (15). Comparable light treatments do not alter the kinetics of or magnitude of gravitropic response under continuous stimulation, although seedlings are rendered more sensitive to gravity as judged by shorter presentation times (6). These results are paradoxical relative to the above discussion. According to the gravitropic overshoot hypothesis, increased graviperception should not lead to enhanced nutational amplitude, but rather to diminished amplitude such as predicted for increased gravistimulation (9). Experiments were consequently undertaken to compare nutation in *Pisum* stems with the response time and initial rate of gravitropic curvature under stimulus-limited conditions. If nutation and gravitropism are mechanistically related, then effects on one response should logically be mirrored in the other.

MATERIALS AND METHODS

Seeds of *Pisum sativum* L. cv. Alaska (Asgrow Seed Co., Orange, CT) were soaked overnight (approximately 4 PM to 8 AM) in darkness in 2 cm standing tap water at room temperature. After washing, the seeds were planted in individual glass vials (19 mm i.d., 70 mm high) three-quarters filled with fine, washed vermiculite (Mica-Gro, type B). The seeds were then covered with vermiculite to the level of the vials, watered with 3 ml tap water, and incubated at 27 ± 1°C and 80% RH. After 4 d the plants were watered again and after 6 d the seedlings were selected for normal growth (initiation of elongation by the third internode). At this time glass rods were anchored in the vermiculite to support the seedlings at the level of the second internode (carefully avoiding disturbance to the third internode). The rods, which did not impede gravitropic response, minimized displacement of the stem during time-lapse photography and supported the weight of the stem during gravitropic induction. All plants used for experiments were 6.5 to 7 d old from the start of imbibition and had 3 to 6 cm long third internodes.

Table I. *Angular Dependence of Initial Gravitropic Kinetics*

Thirty DP plants per treatment were stimulated continuously in the ⊥-plane.

Angle	t_r[a]	Rate[a]
	min	*degrees min^{-1}*
45°	18.5 ± 0.7	1.18 ± 0.04
90°	19.4 ± 0.6	1.50 ± 0.03

[a] Values are means ± SE.

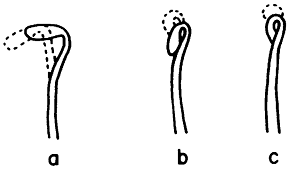

FIG. 1. Pattern of stem nutation in RP_{20} (a) and DP (b and c) in the ||-plane. Tracings from time-lapse photo frames have been superimposed to show maximum angular deflections over 90 min (a) or 210 min (b and c).

Plants were grown in the dark and exposed to green safelight (29) at planting, at watering, and during setup for experiments. R was obtained from four 15-w red fluorescent lamps (Sylvania) wrapped in two layers of red cellophane (discontinued stock, DuPont). Full irradiance at the level of the apical hooks was 3.2 w m^{-2} and standard exposure was for 4 min (760 J m^{-2} total fluence). FR light was obtained from five 300-w incandescent lamps (Photoflood, General Electric) filtered through 15 cm water and one layer of FR light-transmitting acrylic (FRF 700, Westlakes Plastics, Lenni, PA). Fluence rates were measured with a radiometer (model 65, Yellow Springs Instrument Co.). The approximate FR light fluence rate was estimated by subtracting from the total that portion transmitted by an IR light-transmitting filter (No. 7-56, Corning Glassworks). The resultant FR light fluence rate was 1.5 w m^{-2}.

Plants illuminated with R 'x' h prior to experimentation are labeled RP_x. In the absence of a subscript, RP denotes plants 16 to 24 h after R, during which time the characteristic morphological and biochemical features of the de-etiolated system were manifest (14). DP refers to plants not exposed to photomorphogenetically active light, but which have received comparable green safelight exposure.

Gravitational or nutational curvature was studied under growth room conditions and recorded on time-lapse photographs taken at 10-min intervals with the aid of an intervalometer (MC-6AH; Samenco, Decatur, IL). Images were analyzed by projection of the negatives (approximately × 1 total magnification). All plants were photographed from the side in either of two stem orientations: (a) hooks arranged at right angles to the axis of viewing (Fig. 1, for example); or (b) seedlings rotated 90° so that the plane of the hook was in line with the axis of viewing. In the first instance, movements were induced and observed in the ||-plane, defined as parallel to the plane bisecting the curvature of the apical hook. The second case is referred to as the ⊥-plane. Plants were not photographed from above because it would have been impossible to compare nutational and gravitropic curvatures directly. Up to 36 plants as well as a clock could be monitored at one time within the field of view of a 16-mm camera (Bolex, 1-inch lens at maximum aperture of *f*/1.9) located 1 m distant from the plants. Four racks of nine seedlings each were used, which were oriented either vertically or at an angle to initiate gravitropic curvature.

During photographic exposure the plants were illuminated briefly (approximately 1 ms) with green light from two Xe arc flash lamps (Mighty-Light Deluxe), each filtered through one blue light-absorbing glass filter (No. 3-68, Corning Glassworks), one R, FR, and IR light-absorbing glass filter (No. 4-96, Corning Glassworks), one interference filter (545 nm peak transmittance, 10 nm half-bandwidth, Bausch & Lomb), and one layer of green celluloid (Roscolene No. 874, Rosco Co., Port Chester, NY). This filter combination provided approximately 0.1 transmittance at the peak wavelength of 545 nm and less than 10^{-5} transmittance at wavelengths less than 520 nm and greater than 590 nm. The lamps were 0.6 m distant from the plants and approximately 30° off-axis. Film (Kodak 4×, 400 ASA) was developed 5 min in Kodak HC-110 (1:8 dilution). Under these conditions the flash lamps provided the minimum light for a usable image. The lamps did not stimulate phototropism in the test plants, nor did they cause short-term growth inhibition. Nonetheless, observation periods were kept to a maximum of 4 h (*i.e.* 25 frames).

To compare nutation with gravitropism it is important to use angles close to those actually experienced by the nutating seedling, seldom more than 30°. However, t_r and the initial rate of curvature are hard to estimate accurately for gravitropism at shallow angles, especially when complicated by overlaid nutation. As a compro-

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[4] Abbreviations: R, red light, FR, far-red light; NPA, α-naphthylphthalamic acid.

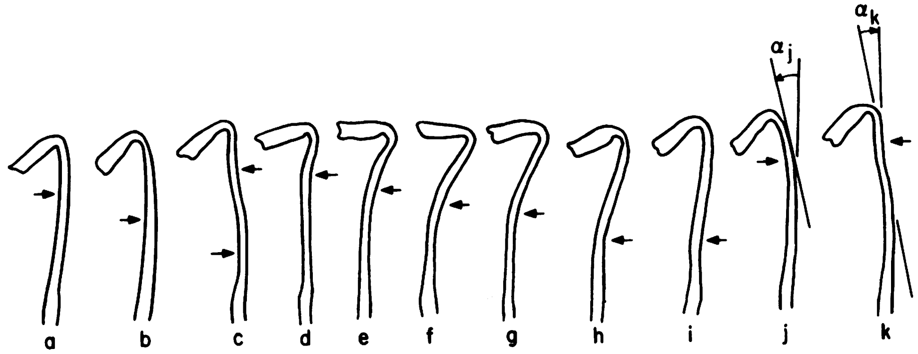

FIG. 2. Pattern and time course of stem nutation in RP$_{20}$ ||-plane. Drawings a to k are taken from projected time-lapse images at 10-min intervals. Nearly straight stem (a) first bends to left or ventral surface (b). As leftward bending proceeds basipetally down stem (small arrows), a rightward or dorsal bending intiates acropetally. Bending on the dorsal side is generally sharper than on ventral side. Measurement of angles is depicted in j and k. By definition, α_j is negative and α_k is positive.

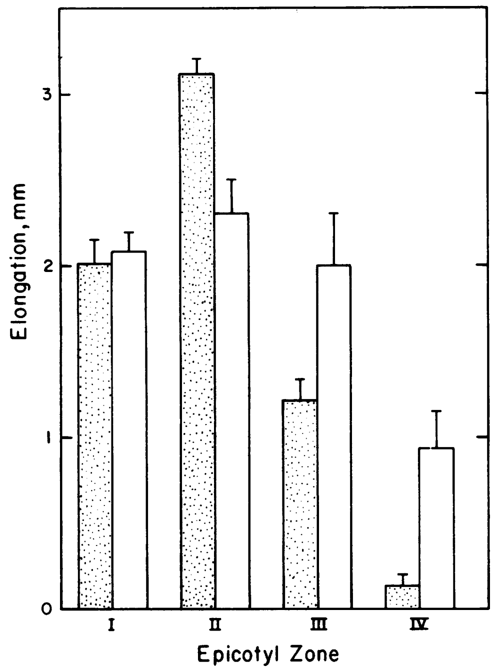

FIG. 3. Dependence of growth on stem position. Epicotyls of intact DP (stippled bars) and RP$_{23}$ (clear bars) were marked with India ink dots at 5-mm intervals starting just at base of hook and extending basipetally. Four zones were thus demarcated totalling 20 mm in length with zone I closest to hook and zone IV furthest from hook. After 4 h, growth was measured with a ruler to nearest 0.25 mm. Average values ± SE are shown. Total growth in the four zones was 7.3 ± 0.6 mm for RP and 6.5 ± 0.2 mm for DP.

mise, stimulation at 45° (⊥-plane) was found to be sufficient for good measurements and suboptimal (with respect to rate of curvature) for stimulation (Table I). Note that the results may not adhere to strict sine dependence. The change in t_r, although not significant, may be real (*e.g.* 1). In the calculations in Table I, it was assumed that gravitropic curvature begins abruptly and continues at a constant rate for 20 to 30 min (*e.g.* Fig. 8; Ref. 19). The initial rate of curvature was calculated for angular data from individual seedlings with values fitted to a linear least-squares line. The response time was estimated by extrapolation of this line back to its intercept with the axis for null curvature (0°).

The sodium salt of NPA (Alanap, Uniroyal, Naugatuck, CT) was prepared in water with 0.1% Tween-20 (Sigma) as a surfactant. Excised plant parts were daubed with anhydrous lanolin (Fisher).

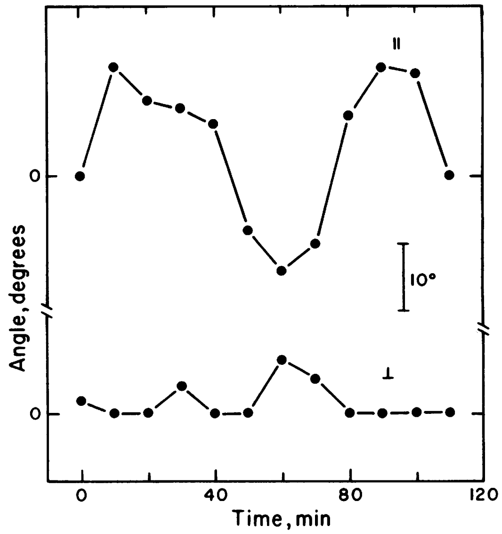

FIG. 4. Time course of nutation in the ||- and ⊥-planes of RP$_{19}$ measured on two different and representative seedlings. Angles were determined as described in Figure 2. In the ⊥-plane, dorsal side of seedling was oriented toward camera (*i.e.* looking from above, seedlings were rotated 90° clockwise relative to Figures 1 and 2).

RESULTS

Nutational curvatures in the ||-plane are depicted in Figure 1 for representative RP and DP. The stems of RP oscillate across the vertical axis (Fig. 1a), whereas the stems of DP have a more localized bending just below the apical hook and tending toward the side that forms the dorsal surface of the hook (Fig. 1b). Some DP samples actually show no detectable bending (*e.g.* Fig. 1c measured over a 3.5-h span). The time course for nutation in a typical RP is shown in Figure 2. The bending initiates somewhat below the hook region and is propagated as a wave down the stem at approximately 1.5 cm h^{-1}, a rate comparable to that of auxin transport in intact *Pisum* stems (28). Nutation in *Phaseolus* is also propagated as a wave and at similar rates (18).

Nutation in DP of *Pisum*, on the other hand, is not clearly propagated down the stem. This difference between DP and RP is probably caused at least partially by different patterns of stem growth; epicotyls of RP are elongating at rates similar to or slightly in excess of those of DP, but their growth is spread over a longer distance on the stem (Fig. 3). A similar light effect has been described for *Phaseolus* (16).

Angular measurements of nutation use the topmost discernible angle (*e.g.* Fig. 2, j and k), since it represents the most recent event and is, therefore, best suited for comparison with the kinetics of

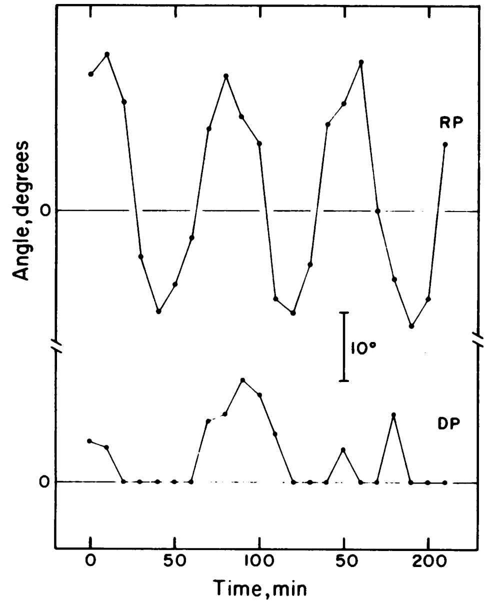

FIG. 5. Time course of nutation in ∥-plane for DP and RP_{23}. Angles were determined as described in Figure 2.

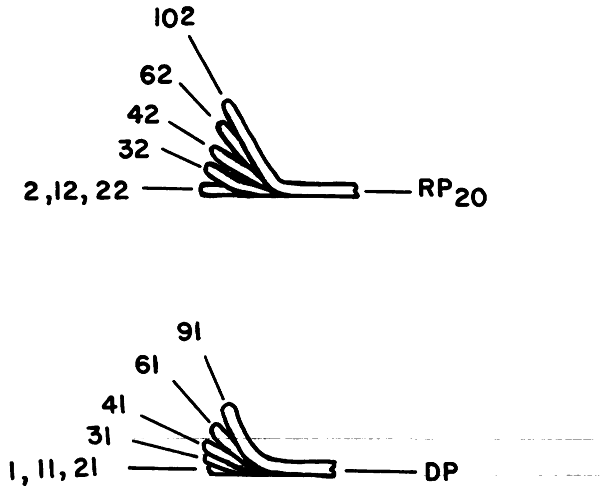

FIG. 6. Pattern and time course of gravitropic curvature in ⊥-plane for representative RP_{20} and DP seedlings. Tracing from time-lapse frames have been superimposed. Seedlings were oriented 90° from vertical at zero-time, photographed within 1 to 2 min and then at 10-min intervals. Numbered lines represent major axes of seedling and gravitropic curvature at various times (min) after start of induction. We define starting angle as 0°.

tropistic curvature, which also initiate within this region. Thus quantified, nutation in RP reveals a high degree of bilateral symmetry with clearly defined and periodic movements (τ = 70–80 min; see also Table III) observed only in the ∥-plane (*i.e.* pendulum-type; Fig. 4). The swift transition between positive and negative angles is caused by the measurement technique which reflects the initiation of bending in the opposite direction before the preceding wave has disappeared. Tendril nutation in *Pisum* also has periods on the order of 80 min (23), whereas nutation of the hypocotyl of *Helianthus* has a period approximately twice as long (1).

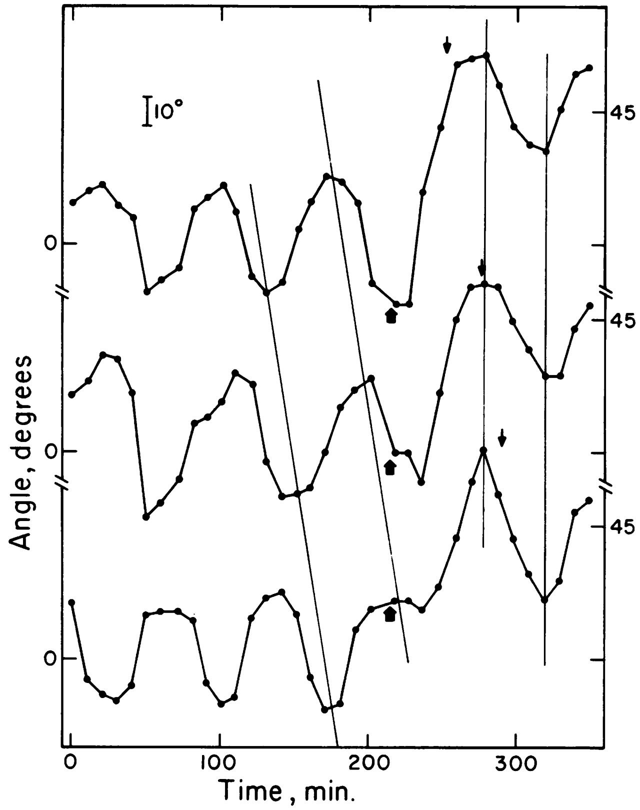

FIG. 7. Nutation and gravitropism in ∥-plane of RP_{23}. Three separate seedlings were photographed simultaneously. Slanted and vertical lines emphasize phase relationships. Heavy arrow (⬆), seedlings tipped to 45°; light arrow (↓), projected times of maxima. Angles were determined as in Figure 2, except value 45° was added to negative angles of nutation which occur after gravitropic induction so as to keep a constant baseline.

Table II. *Magnitude of Nutation*

Experiment	Plants	Observation Plane	Treatment	Nutation[a]
				degrees
1	RP_{19}	∥		37 ± 2
		⊥		8 ± 2
2	RP_{23}	∥		39 ± 1
	DP			15 ± 1
3	RP_{16}	∥	NPA, 0 μM[b]	33 ± 3
			NPA, 5 μM	20 ± 4
			NPA, 10μM	5 ± 1
4	RP_{16}	∥	Intact	26 ± 2
			Bud excised	13 ± 3

[a] Values are means ± SE.

[b] Tween-20 control.

Nutation in DP is also confined to the ∥-plane, but the angles are harder to measure because the bending is restricted to a small portion of stem just below the curved hook. A DP sample with pronounced nutation is presented in Figure 5. The period of nutation of this sample appears to be approximately 90 min, but irregularities in the pattern make it difficult to be certain. In most other DP the pattern is even less clear.

In contrast, the kinetics and magnitude of gravitropism in RP and DP are almost identical when stimulated continuously starting at 90° from the vertical and oriented in the ⊥-plane (6). Nonetheless, the pattern of gravitropic curvature differs somewhat between the two groups. For both RP and DP, initial bending (⊥-plane) is

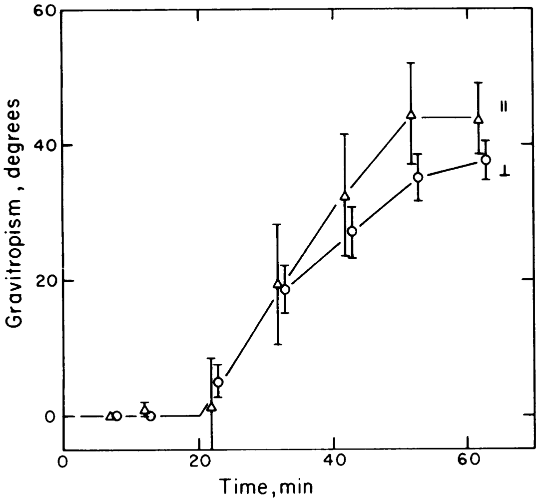

FIG. 8. Gravitropic response in ⊥ (O) and ||-planes (Δ) of RP_{19} plants tilted at 45° from vertical at zero time. Bars equal 1 × SE. Angles were determined as in Figure 2 (||-plane) or Figure 6 (⊥-plane). Starting angle was taken as zero in case of stems that were curved initially.

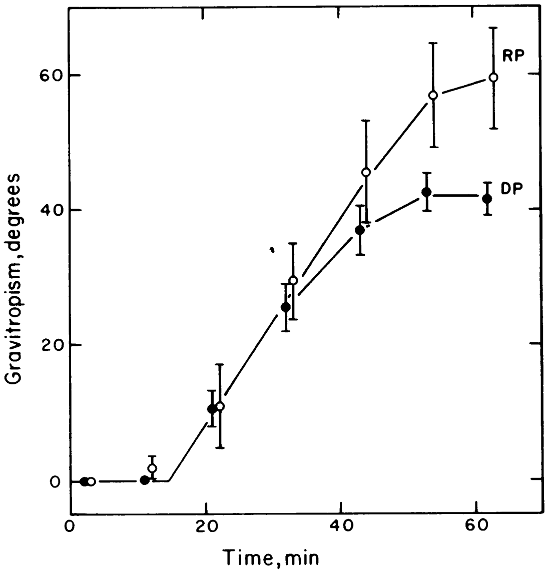

FIG. 9. Gravitropic response in ||-plane of DP (●) and RP_{23} (O). Details as in Figure 8.

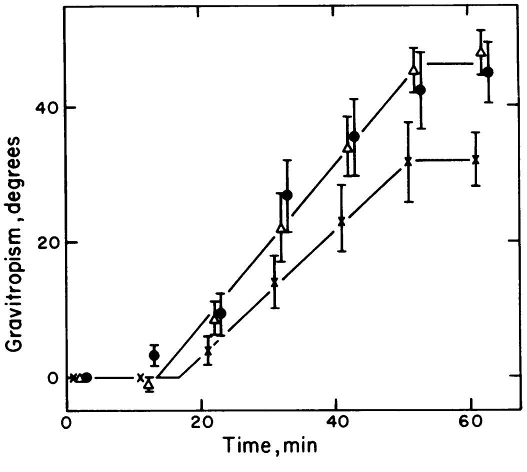

FIG. 10. Gravitropic response in ||-plane of RP_{16} treated 0 μM (●), 5 μM (Δ), or 10 μM (x) NPA. Details as in Figure 8.

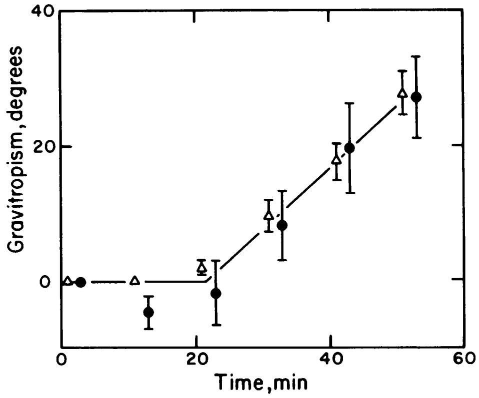

FIG. 11. Gravitropic response in ||-plane of RP_{16} either intact (●) or with apical bud excised (Δ). Details as in Figure 8.

Table III. *Relation between τ and t_r*[a]

Experiment	τ[b]	t_r[c]	τ/t_r
	min		*ratio*
1	76 ± 3	14	5.4
2	78 ± 3	21	3.7
3	71 ± 2	14	5.1
4	80 ± 3	12	6.7

[a] RP, ||-plane.

[b] Determined as time between peaks or troughs to nearest 5 min. Values are means ± SE.

[c] Determined from average gravitropic response curve.

observed near the apex (Fig. 6). Unfortunately, it is difficult to localize the reaction exactly with respect to the hook region (further complicated by different degrees of hook opening in RP and DP). However, gravitropism in DP develops in a smooth curve without evidence of migration of curvature or response down the stem (*e.g.* 12, 19, 27). In RP, however, the zone closest to the hook straightens with time and subsequent curvature is thus localized more basipetally. This difference in curvature development is presumably related to the differing patterns of growth along the stems of DP and RP (Fig. 3), although autotropic reactions (*e.g.* 13) may also be involved. The time-lapse photos do not provide resolution sufficient to analyze the curvature in more detail, so we cannot as yet decide whether basipetal migration of gravitropic response itself occurs.

Analysis of gravitropic bending patterns in the ||-plane, (Fig. 7), is more complex because of simultaneous nutational movements. Individual seedlings of a population recorded simultaneously have randomized phase angles, probably because of slight differences in τ and in the rate of development of nutation after R. All plants were given continuous gravitropic stimulation in the ||-plane with the axis of the lower epicotyl at 45° from the vertical. After tilting (Fig. 7, heavy arrow), increasing angles denote the normal upward gravitropic bending of a stem. Seedling No. 1 is stimulated near the minimum of its nutational oscillation (*i.e.* bent downward). It starts its upward gravitropic response more or less in phase with and additive to the nutational bending that would be occurring in the positive direction. The seedling thus appears to have a short gravitropic response time ($t_r \approx 13$ min) and a high rate of bending. The maximum of positive nutation, however, is delayed approximately 20 min relative to the expected time (Fig. 7, light arrow). Seedling No. 2 has increasingly negative angles at the time of tilting. It continues its natural course of nutation after tilting. Therefore, it moves further downward before bending upward, also about in phase with nutation. This seedling appears to have a long gravitropic response time ($t_r \approx 21$ min) and a slower rate of curvature. No phase shift is evident. Seedling No. 3 is near the maximum of nutation (*i.e.* directed relatively upward after tilting) and it remains more or less upward before responding gravitropically. The expected downward nutation is strongly repressed. Although this seedling also has a long response time ($t_r \approx 21$ min) and a yet slower rate of bending, t_r is shorter than expected if upward bending were in phase with nutation; that is, the rhythm of this seedling is phase advanced. This is evident from the position of the peak, which occurs approximately 10 min earlier than predicted from past history. Note that all the seedlings overshoot the vertical axis and continue to nutate, but now in phase with one another. Gravitropic induction has synchronized the oscillations. Nutation and gravitropism are, therefore, not simply overlaid, but truly interacting. Gravitropism alters the phase of nutation and nutation influences the apparent kinetics of gravitropism.

Differences in nutation with respect to stem orientation (Fig. 4) and light treatment (Fig. 5) suggest a means of comparison of nutation with gravitropic response. In a series of experiments, the nutatory behavior of seedlings was recorded for 90 to 120 min prior to gravitropic induction at 45° for 60 min. In this way, gravitropic kinetics and nutation could be compared directly in the same seedlings. As seen in Figures 5 and 7, nutations would be expected to remain fairly constant over this interval. The magnitude of nutation was taken as the maximum differential (*i.e.* peak-to-trough) angular deflection registered during the monitoring period (sufficient to encompass at least one full cycle for RP $\|$-plane oscillation). The 'magnitude' is thus approximately twice the amplitude of oscillation. In Figure 5, for example, values would be 40° (RP) and 15° (DP). No weight is given to period length or regularity. Since the nutational phases are randomized within a population of seedlings, the gravitropic response could be determined from average curvatures. The oscillations generally cancel out numerically, but the resultant averages show large standard errors which may obscure aspects of gravitropic response.

Depending on stem orientation ($\|$ or $\perp$), two groups of RP show large differences in observed magnitude of nutation (Table II, experiment 1). When the same plants were induced gravitropically, the initial kinetics (t_r and rate) are identical in both planes (Fig. 8). The slightly greater curvature at 60 min in $\|$-oriented seedlings may be caused by constructive effects of phase synchronization and overshoot (Fig. 7). *Lathyrus*, another hypogeal legume, has been reported to have highly asymmetric nutation (11) but symmetric gravitropic response (17). Considering only the $\|$-plane, RP and DP of *Pisum* show large differences in nutation (Table II, experiment 2), but little or no difference in initial gravitropism (Fig. 9). The results concur with those for gravitropic stimulation at 90° in the $\perp$-plane (6).

The magnitude of nutation does not correlate with gravitropic response, but these experiments can be criticized on the basis that DP and RP are physiologically quite different and therefore not strictly comparable. Likewise, morphological dissimilarities (bilateral symmetry) in epicotyls of *Pisum* (31) might contribute to subtle second order (albeit strictly hypothetical) differences in nutation between $\perp$ and $\|$ stem orientations. We consequently limited ourselves to a comparison of nutation and gravitropism only in RP in the $\|$-plane.

Because growth regulators are certainly important in tropisms and nutation (24, 32), we tested the effects of NPA, which at low concentrations blocks auxin transport and inhibits gravitropism (26). We dipped the apex and third internode of seedlings into NPA solutions or into Tween-20 controls for 10 s total (5 s at one orientation and 5 s at 180° orientation to nullify any gravitropic induction). The seedlings were incubated for 5.5 h and then measured for nutation followed by gravitropism. Control experiments showed that NPA effects on nutation and gravitropism had reached completion and were constant over the time span in which the responses were monitored. As is evident (Table II, experiment 3), nutation was inhibited approximately 50% by 5 μM NPA, a concentration which had no effect on initial gravitropic response (Fig. 10). NPA (10 μM) almost completely stops nutation while just beginning to inhibit gravitropism. Nutation, therefore, seems far more sensitive to NPA than is gravitropism.

The above results suggest some involvement of auxin transport in nutation. Since the apical bud is the source of auxin in etiolated peas (28), we reasoned that surgical removal of the bud should differentially affect nutation and gravitropism. Indeed excision, followed by a 90-min period to drain the stem of auxin (cut end covered by lanolin), resulted in a 50% inhibition of nutation (Table II, experiment 4) and a 63% inhibition of growth (data not shown), but in no reduction of gravitropism (Fig. 11).

Finally, several values of τ and t_r were compared, all for RP measured in the $\|$-plane (Table III). Although the period of nutation was fairly constant, the gravitropic response time was more variable. As a result, a constant relationship was not maintained between τ and t_r.

DISCUSSION

The gravitropism of *Pisum* epicotyls bears no clear relation to nutational behavior. 'Typical' gravitropic responses occur in situations in which nutation is either naturally absent or experimentally inhibited. This lack of correlation has been demonstrated in a number of ways (light treatment, stem orientation, inhibitor effects, and dismemberment) and is therefore unlikely to be simply coincidental. In particular, we feel that the persistence of an unaltered gravitropic response in debudded plants (RP, $\|$-plane), in which the high amplitude nutations are almost absent, is especially important (Fig. 11; Table II, experiment 4). It is also noteworthy that a quantitative relation between t_r and τ is lacking (Table III).

We draw two conclusions from the above evidence. (a) Nutations cannot be explained by the simple gravitropic overshoot hypothesis (*e.g.* Equation 1). (b) Conversely, gravitropism is not an asymmetrically modified nutation as originally suggested by the Darwins (11). This decision may surprise no one, but to our knowledge the matter has not been adequately resolved in the past. Jaffe (23) did conclude that tendril coiling in *Pisum* was not a modified nutation.

We have not, however, demonstrated that nutations persist in the absence of gravitropism. Thus, nutations might yet be explained by more elaborate versions of the overshoot hypothesis. For example, the effects reported in this paper might be accounted for if it were assumed that gravitropic overshoots (and hence nutations) became rapidly dampened under certain conditions (*e.g.* $\perp$-plane, debudded plants). We know of no physiological basis for this suggestion, but presumably it might involve the ability of plants to sense and to respond more rapidly to the attainment of the vertical stem position. In this regard, it may be useful to look for induced gravitropic overshoots (*e.g.* RP $\perp$-plane) and to compare them with the stable, high amplitude nutations (RP $\|$-plane) as to τ and the effect of altered gravitropic response. Even more speculative, one might propose that nutation is driven by some as yet unknown gravity-sensitive process independent of normal gravitropism and with its own unique set of properties.

The conclusions on the dissociation of nutation from gravitropism include the following assumptions in the methods of gravitropic analysis: (a) the use of gravitropic induction at 45° was based on measurements made with DP in the $\perp$-plane (Table I) which indicated the stimulus was limiting the response. Although nutation is generally in the $\|$-plane and mainly expressed in RP, it is likely that stimulation at 45° is also suboptimal in these cases, since kinetics of response do not differ between DP and RP (Fig. 9) or stem orientation (Fig. 8). (b) It is possible that differences in gravitropic response pertinent to nutatory behavior might be revealed with yet more shallow angles. But, it is unlikely that such differences would be detectable without considerably larger sample sizes to account for the 'noise' of nutation superimposed on gravitropism. (c) Even though the lower epicotyl may be accurately oriented at some angle (*e.g.* 45°), the upper (nutating) portion of the stem may have a considerably different angle of gravistimulation at the time of initial induction depending on the magnitude of the nutation and on the phase of the rhythm (*e.g.* Fig. 7). Thus, seedling No. 1, canted 20° negative at the time of tilting, experiences at 65° net angle, whereas seedling No. 3, canted 20° positive, receives only a 25° net angle. However, the average stimulation,

$$\frac{1}{N}\sum_{i=1}^{N} \sin \alpha_i,$$

may be close to the value of sin 45°. Thus, 0.5 (sin 65° + sin 25°) = 0.665, only 5% less than 0.707. (d) Averaging samples may account for some problems, but nutation and gravitropism also interact in nonadditive ways (phase-shifting). Nonetheless, the average gravitropic response of nutating samples was always about equal to the response of non-nutating samples. We can only conclude that the underlying responses of the populations were in fact the same and, on the average, not distorted by the interactions observed in individual seedlings. Clinostat treatments of plants may be a means to reduce nutation and thus explore its possible interference with gravitropism (*e.g.* 1). However, rotation on clinostats may alter gravisensitivity in ways not related to nutation (21).

We consider the data presented here to be generally consistent with autonomous factors such as endogenous rhythms of growth as the cause of nutations, although a modified gravitropic overshoot hypothesis cannot be entirely rejected. Although the ability of gravitropic induction to phase-shift nutation is consistent with the overshoot hypothesis, the data are also consistent with the involvement of a rhythm because it is reasonable to expect any process affecting growth to interact with such a rhythm. Thus, we would predict that phototropism should also phase-shift nutation if care is taken to avoid simultaneous gravistimulation. The interaction between gravity and nutation in *Pisum* (Fig. 7) but apparently not in *Phaseolus* (20) indicates that rhythms, if involved, may differ between species. However, in *Phaseolus* the kinetics of gravitropism are much slower than for nutation, so perhaps the coupling between growth regulatory processes is weaker. In *Helianthus*, in which nutations are phase-shifted by gravistimulation (25), the time courses for nutation and gravitropism are more comparable.

Future tests of the dissociability of nutation and gravitropism should attempt to determine whether nutations do indeed continue in the absence of gravitropism. An agravitropic mutant of *Pisum* may be useful in this regard (3). Another approach, surgical removal of the *Pisum* root tip, was found to inhibit both nutation and gravitropism (30). Although this evidence was taken as consistent with the gravitropic overshoot hypothesis, clearly the root cap may be both the site of graviperception and, independently, the site of cellular oscillators or the source of growth regulators important for nutation. The result is therefore not inconsistent with an endogenous rhythm hypothesis, although it does illustrate the difficulty in interpretation of a double-negative answer. Studies are planned for the space shuttle (4) where, at zero-*g* (*i.e.* outer space), the persistence of nutation could not be the result of gravitropism or other gravity-related processes. The absence of nutations under zero-*g* would unfortunately not be as definitive, since it might be argued that normal growth regulatory mechanisms, inclusive of rhythms, are disrupted under the rigors of space flight and abnormal gravity conditions. A fourth approach, simulation of zero-*g* by clinostat rotation, should be continued (10).

Finally, we consider it useful to continue comparison of environmental effects on and morphological distinctions between gravitropism and nutation. For example, stem nutation has been most widely studied in the hypocotyls of *Helianthus* in which the pattern is rather symmetrical (*i.e.* circumnutation), but in which the gravitropic response is markedly asymmetric and dependent on hypocotyl orientation (5). Asymmetries in gravitropic response and in presentation time are correlated with morphological asymmetries in the distribution of the starch sheath (*i.e.* putative statocyte tissue) and thus may be relevant to primary processes of graviperception (17). To our knowledge, this asymmetry, which ought to influence a gravitropic overshoot mechanism, has not been exploited in the study of nutation.

In *Pisum*, we need to investigate the mechanism of light promotion of nutation. If rhythms are truly involved, then are previously weak rhythms coupled in a manner more favorable for the generation of sustained oscillations, or are responding tissues made more sensitive to existing rhythmic asymmetries in growth regulator distribution? What is the basis for the bilateral symmetry in nutation? These issues, as well as implications for overall growth regulation and sensory transduction, are discussed in subsequent reports (7, 8).

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