

# Physiology of Movements in Stems of Seedling *Pisum sativum* L. cv Alaska<sup>1</sup>

## II. THE ROLE OF THE APICAL HOOK AND OF AUXIN IN NUTATION

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### ABSTRACT

The relationship between the apical hook and stem nutation in etiolated Alaska pea (*Pisum sativum* L. cv Alaska) seedlings was explored. The hook and maximum nutational displacement have the same plane of symmetry, and both are affected by light acting through phytochrome. However, the two processes do not appear to be obligatorily coupled. Light effects on nutation involve at least two components, an increase in amplitude as well as an increase in frequency. These components can be separated from one another on the basis of developmental time course or red light fluence. Excision of the plumule, leaving the hook attached to the stem, inhibits photostimulated nutation. This inhibition can be overcome by application of indole-3-acetic acid to the remaining stem. If the hook is also excised, then nutation in the stem cannot be restored by indole-3-acetic acid. It is possible, although not yet proven, that the oscillatory process regulating nutation in the stem is itself localized in the hook and that rhythms in the transport of indole-3-acetic acid are involved.

The extension growth of cylindrical plant organs is often accompanied by oscillatory bending movements (nutations) with periods on the order of 1 to several h. A complete understanding of growth regulation in plants will require the elucidation of these rhythms. In an earlier study (5), we concluded that simple gravitropic overshoots (e.g. Ref. 10) were unlikely to cause nutation in the third internode of peas. However, the nutational patterns were consistent with internal events such as endogenous rhythms of growth as the cause of oscillatory bending. Further work on the physiology of nutation should attempt to identify and to localize the rhythmic process(es), if such exist, and to explain the coupling between the primary oscillator (or clock) and subsequent growth.

Several observations seem to connect nutations in pea stems with the apical hook. In the first place, differential cell elongation on opposite sides of the stem is responsible not only for nutation, but also for the formation and maintenance of the hook. Both processes seem to be autonomous. Second, the patterns of nutation and hook curvature have a common plane of symmetry (5). Third, photomorphogenetically active light stimulates both hook opening and nutation (5, 7). Finally, formation of the hook has an irregularly rhythmic component which indicates oscillations in growth

regulation (7). This paper concentrates on the relation between the apical hook and nutation.

### MATERIALS AND METHODS

Alaska pea (*Pisum sativum* L. cv Alaska) seedlings (Asgrow Co.) were raised and handled in individual vials in the dark at 27°C and 80% RH as described in detail elsewhere (5). Plants were exposed to red light 6 d after the start of imbibition, corresponding to the early stages of elongation in the third internode. The standard red light treatment was for 4 min (760 J m<sup>-2</sup>) from four 15-w red phosphor fluorescent lamps. In the case of the fluence response curve, two lamps were used and the fluence was varied by changing irradiation time. Far red light was obtained from five 300-w incandescent flood lamps filtered through 15 cm of water and one layer of a visible light-absorbing, far red and IR light-transmitting acrylic filter (Westlake Plastics, Lenni, PA). Plants are labeled DP,<sup>3</sup> RP<sub>x</sub>, or RP (when x is between 16 and 24 h).

Nutation, total stem growth, and changes in hook angle were measured from time lapse photographs taken at 10-min intervals under growth room conditions (see Ref. 5). Gross hook angle was quantified from shadowgraphs with the aid of a protractor. As defined earlier (7), an open hook (i.e. straight stem) was defined to be 180° while a closed hook (plumule parallel to the stem) would be 0°. Negative angles indicate recurved hooks.

The pea seedling has bilateral symmetry with the plane of symmetry bisecting the apical hook (the ||-plane; see Ref. 5). Nutational curvature is most pronounced in this plane, although in some cases, the nutations are irregular and not clearly periodic. Consequently, the magnitude of nutation was chosen as the maximum peak-to-trough angular deflection in the ||-plane over the measurement period, usually 90 to 120 min.

The plumule, including the terminal apical bud and developing leaves, was removed by excision just below the petiole of the first foliage leaf. The hook was excised 1 to 2 mm below the zone where the curving stem expanded into the straight portion of the internode. The exposed tissue of the stems was coated with pastes of anhydrous lanolin (Fisher) prepared by mixing 10 g warm lanolin with 0.1 ml 95% ethanol, the latter with or without appropriate amounts of IAA (Sigma). A 90-min incubation period was always included following excision and before measurement of nutation.

### RESULTS

Nutation in RP is inhibited by excision of the plumule whereas the gravitropic response is not (5). We have since found that

<sup>3</sup> Abbreviations: DP, dark-grown plants; RP<sub>x</sub>, plants x h after treatment with standard red light; τ, period length of nutation.

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nutations is inhibited to the same extent by removal of either the plumule alone or the plumule and the hook together, provided that the seedlings are not given exogenous IAA (Table I). Note that in decapitated plants some residual movement of the stem is observed which is similar to the low amplitude, irregular nutations in the  $\perp$ -plane of DP (5). The effect of excision is unlikely to be simply the result of wounding, since nutation was not noticeably affected by a single, deep razor blade cut entirely bisecting the hook in the plane of symmetry in such a way as to leave the bud and stem connected (wounded controls, Table I). However, such cutting did inhibit further hook opening, probably through stimulation of ethylene synthesis (13).

The inhibition of nutation in RP by excision of the plumule can be at least partially reversed by addition of IAA to the stem (Table II). A wide range of concentrations was not tested, but we believe it likely that nutation would be fully restored by an appropriate, optimal level of IAA. In contrast, exogenous IAA failed to restore nutation in seedlings lacking both the plumule and the hook (Table III). In this experiment, a range of concentrations was tested and growth was restored completely at the highest concentration of IAA. The failure of the stem to nutate is thus not a consequence of inadequate growth. Moreover, the stems of decapitated seedlings are competent to execute tropistic bending movements, since RP with both plumule and hook excised will respond gravitropically, with or without added IAA, almost as well as intact plants (6).

The hook region thus appears to be necessary for the light-stimulated nutation of the stem. We therefore compared changes in nutation and hook angle after standard red light irradiation (Fig. 1). Fresh groups of seedlings were used for each assay, with each group examined for both the magnitude of nutation and the change in hook angle during the 120-min measurement period.

At the start of the experiment, the average hook angles were about 30 to 40°. Thereafter, the hooks of DP closed steadily at about 6 to 8° h<sup>-1</sup> for the next 16 h. However, the hooks of the DP began to open rapidly during the final assay period, as is typical when the growth of the internode begins to slow. The effect of red light on hook angle is clearly visible within 3 h of treatment (RP<sub>3</sub>), inasmuch as the hooks of these seedlings have already stopped

Table I. *The Effect of Wounding and Excision of Apical Parts on Nutation and Hook Opening in the Absence of Exogenous IAA*

The magnitude of nutation in RP<sub>17</sub> was monitored for 90 min starting 90 min after wounding. Hook opening was determined as the net change in hook angle over the 180-min period starting immediately after wounding.

Treatment	Nutation <sup>a</sup>	Hook Opening <sup>a</sup>
	<i>degrees</i>	
Intact controls	37 ± 3	+12 ± 3
Wounded controls	35 ± 3	+1 ± 2
Plumule excised	15 ± 3	
Plumule and hook excised	15 ± 3	

<sup>a</sup> Values are means ± SE.

Table II. *Partial Restoration of Nutation by IAA after Excision of the Plumule*

The magnitude of nutation in RP<sub>18</sub> was monitored for 120 min starting 90 min after excision.

Treatment	Nutation <sup>a</sup>
	<i>degrees</i>
Intact controls	37 ± 2
Excised, no IAA	12 ± 1
Excised, +100 µg/g IAA	28 ± 3

<sup>a</sup> Values are means ± SE.

Table III. *No Restoration of Nutation by IAA after Excision of Both the Plumule and Hook*

The magnitude of nutation in RP<sub>19</sub> was monitored for 90 min starting 90 min after excision. Growth was measured for 180 min starting immediately after excision.

Treatment	Nutation <sup>a</sup>	Growth <sup>a</sup>
	<i>degrees</i>	<i>mm</i>
Intact controls	32 ± 2	3.8 ± 0.3
Excised + 1 µg/g IAA	10 ± 1	1.3 ± 0.2
Excised + 10 µg/g IAA	12 ± 3	2.2 ± 0.1
Excised + 100 µg/g IAA	12 ± 2	3.7 ± 0.1

<sup>a</sup> Values are means ± SE.

closing. Although later samples reveal a slow (3–4° h<sup>-1</sup>) opening of the hook, the important observation is that the rate of change in hook angle in RP<sub>3</sub> is significantly different from that in DP 6 h after the start of the experiment. On the other hand, the magnitude of nutation is clearly not different in these two groups. The development of light-induced hook opening therefore precedes in time the promotion of nutation by light.

The promotion of nutation can be further resolved into two components which differ in their rate of development after illumination. The first component is the increase in magnitude of rhythmic bending (Fig. 1). The second component, not evident in Figure 1, involves changes in the period length (e.g. Ref. 5). For example, nutational patterns for the samples in Figure 1 were scrutinized for the presence of two minima or two maxima clearly resolved within either a 90-min span ( $\tau < 90$  min) or a 120-min span ( $\tau < 120$  min). The measurement period of 120 min limits the resolution. We were unable to resolve clear periods in any DP samples. Hence,  $\tau > 120$  min, if indeed the samples are periodic at all. Among RP<sub>x</sub>, we observed that only by RP<sub>12</sub> do some seedlings have periods less than 90 min, but that by RP<sub>16</sub> all seedlings have periods less than 90 min. The light-induced decrease in period thus requires markedly longer to develop than does the increase in amplitude.

Fluence response curves for the effects of red light on nutation also indicate the separation of nutation into at least two components. Low fluences (30 J m<sup>-2</sup>) cause a decrease in period length without a concomitant increase in the magnitude of nutational bending (Fig. 2). The increase in magnitude of nutation is therefore not a prerequisite for a decrease in period length. In fact, the amount of nutational curvature does not correlate with period length, since we can have plants (Fig. 1) with large amplitudes and either long periods (RP<sub>8</sub>) or short periods (RP<sub>16</sub>) as well as plants (Fig. 2) with small 'amplitudes' and either long periods (DP) or short periods (RP – 30 J m<sup>-2</sup>).

Finally, far red light reverses red light-promoted nutation, indicative of phytochrome involvement. Both effects of light on nutation, increased magnitude (Table IV), and decreased period length (data not shown) are affected, but fluence response curves were not obtained to see if the two components could be differentiated on the basis of sensitivity to far red light. Although far red light alone does cause some hook opening, it does not affect nutation significantly (data not shown). Note that far red light at the administered fluence can increase graviperception (4), thus providing further evidence separating gravitational response from nutation (5).

## DISCUSSION

Both the apical hook and auxin are clearly important for photoenhanced nutation in the stems of *Pisum*, as indicated by the effects of excision (Tables I, II, and III) and by the inhibition of nutation with  $\alpha$ -naphthylphthalamic acid (5, 6). The data are consistent with the hypothesis that nutations are caused by endog-

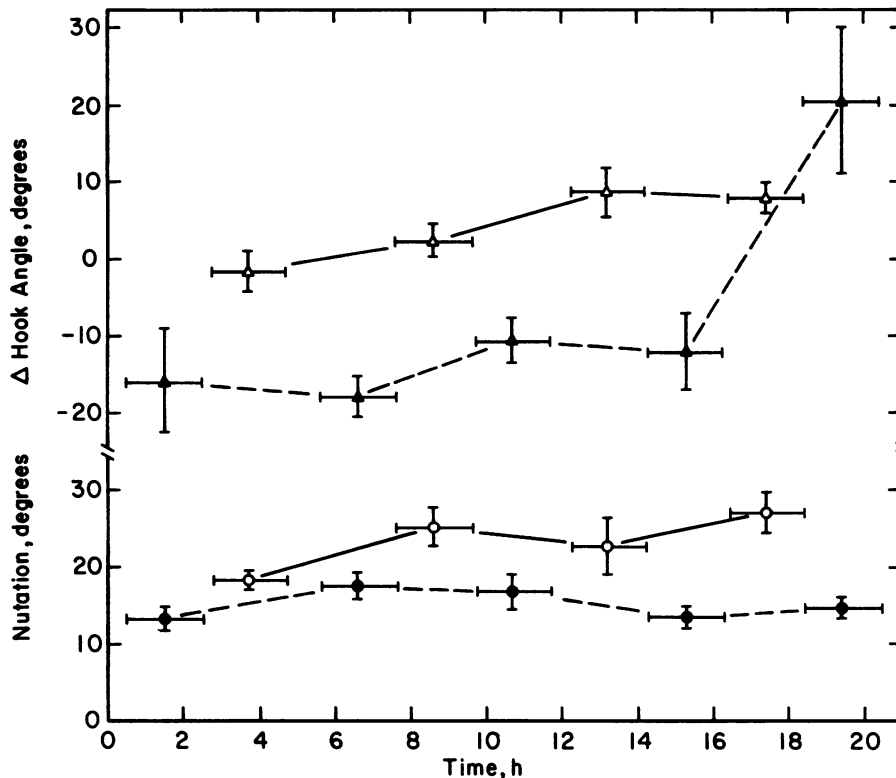


FIG. 1. Time course for changes in magnitude of nutation (○, ●) and hook angle (△, ▲) in RP<sub>x</sub> (○, △) and DP (●, ▲). Standard red light (4 min, 760 J m<sup>-2</sup>) was given at time zero. Groups of plants were selected for analysis at the indicated times. The horizontal bars represent measurement periods (about 120 min). The vertical bars represent 1 SE. Both the change in hook angle and nutation were measured on the same seedlings.

Table IV. Reversal by Far Red Light of Red Light-Promoted Hook Opening and Nutation

The magnitude of nutation in RP<sub>24</sub> was monitored for 100 min. Fluences were 380 and 180 J m<sup>-2</sup> for red light and far red light, respectively.

Treatment	Hook Angle <sup>a</sup>	Nutation <sup>a</sup>
	degrees	
2 min red	62 ± 6	37 ± 2
2 min far red	-10 ± 3	12 ± 2
2 min red + 2 min far red	-5 ± 3	10 ± 1

<sup>a</sup> Values are means ± SE.

enous rhythms, but it is still not possible to identify an oscillator or localize it unambiguously to a specific tissue. Some models of nutations, though, are rendered less probable. IAA applied to the epicotyl in the absence of the hook restores growth but not nutation (Table III), so it is unlikely that rhythmic changes in the auxin sensitivity of the responding epicotyl tissue are the cause of nutation (e.g. Ref. 9, but see Refs. 1 and 2). It also seems unlikely nutation is related to intrinsic requirements of stem growth for alternating 'expansive' and 'restive' phases (e.g. Ref. 8).

An oscillator controlling rhythmic stem nutation could plausibly be located in the hook tissue. Rhythmic auxin transport has been reported in coleoptiles (14) and lateral gradients of auxin associated with hook opening in bean hypocotyls have also been described (12). The 'bobbing' movement of *Pisum* hooks indicates that the formation of this organ can be modulated in rhythmic fashion (7). Thus, nutations could be caused by rhythmic pulses of auxin from the hook traveling out of phase down opposite flanks of the stem. However, the role of auxin in nutation may be indirect. For example, auxin in the hook may stimulate the production of secondary growth regulators which themselves may be transported rhythmically out of the hook or which may react with oscillations in epicotyl tissue sensitivity. In this latter case,

the hypothetical 'clockwork' would be localized in the responding stem tissue.

Light probably has at least two independent effects on nutations: (a) an increase in amplitude of bending; and (b) an increase in frequency (i.e. decrease in period length). The first effect might be related to growth regulator actions whereas the second is more likely related to changes in the oscillator function. Low amplitude stem nutations of uncertain periodicity have been observed in DP (5). It is not clear how these irregular bending movements are related to the high amplitude and periodic nutations of RP. For example, the effect of light may be to alter the coupling between existing clocks and growth processes, thus enabling regular oscillations to occur. In this sense, the role of light would be to enhance an on-going process. However, it remains possible that the high amplitude, regular nutations in RP represent a distinctly new phenomenon induced by light. A more detailed study of nutation in DP is therefore in order. Possible effects on nutation in DP of dim green light should also be considered.

While light-induced hook opening precedes photostimulated nutation, the lag between them is long and it is uncertain whether the stimulation of nutation is obligatorily coupled to or caused by events of hook opening. For example, wounding can inhibit hook opening without altering nutation (Table I). Light treatments may also differentiate between the two processes. A low fluence of red light (30 J m<sup>-2</sup>) significantly increased hook angle without so affecting the magnitude of nutation (Fig. 2). A somewhat higher fluence (100 J m<sup>-2</sup>) almost saturated the light effect on the period of nutation while not saturating light-induced hook opening (Fig. 2). Finally, the spontaneous and rapid hook opening observed in DP (Fig. 1) was not accompanied by changes in nutation. Of course, these observations do not rule out a connection between the hook and nutation. For example, the asymmetry of nutation may be related to the bilateral symmetry of the hook.

If hook opening and nutation are causally connected, then a

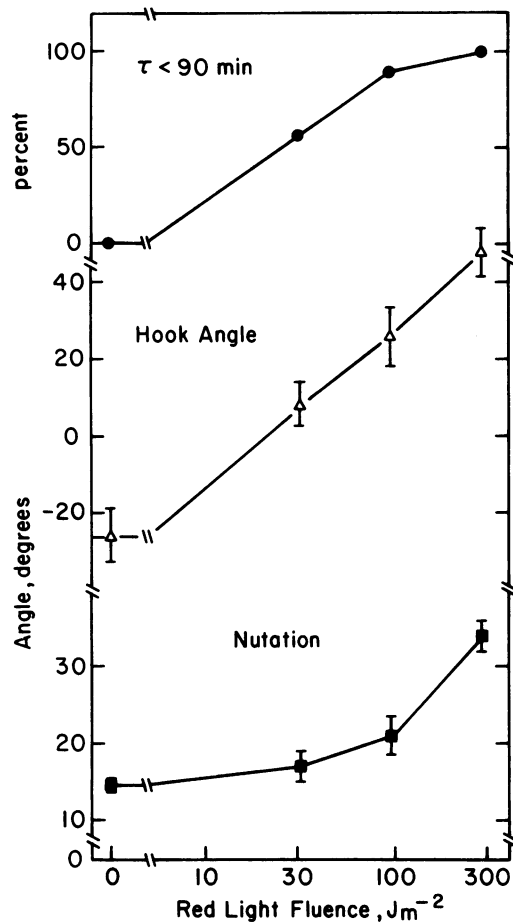


FIG. 2. Fluence response curves for red light effects on hook angle ( $\Delta$ ), magnitude of nutation ( $\blacksquare$ ), and  $\tau$ , the period of nutation ( $\bullet$ ) in RP<sub>19</sub> and DP of equal chronological age. Nutation was measured for 120 min.  $\tau$  is considered to be less than 90 min if two minima or two maxima are encompassed within a 90-min span. The vertical bars represent 1 SE. Separate groups of 20 seedlings each were used to determine absolute hook angle.

possible link between them is the growth substance ethylene, which regulates hook opening and the effects of red light thereon (11). Prolonged ethylene treatment at high concentrations leads to

horizontally directed stem growth ('diageotropism') which occurs generally in the same plane of symmetry as that for hook curvature and nutation (3). The wound-induced ethylene synthesis in *Pisum* seedlings has a rhythmic component with a period of 70 to 80 min (13), similar to that of nutation (5). Rhythms in ethylene production might be triggered by red light and affect growth either directly or indirectly (e.g. through auxin transport). However, several treatments expected to influence ethylene release or action did not affect nutation (e.g. wounding; treatment of stems with 1 mM Co(NO<sub>3</sub>) or AgNO<sub>3</sub>; Ref. 15). These treatments did, however, affect other growth processes, such as hook opening. The role of ethylene in the regulation of nutation, therefore, remains open to question.

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