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Elongation and Circumnutation Oscillations of Hypocotyl of Pine Seedlings (*Pinus silvestris* L.)

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Abstract. The elongation of pine seedlings (*Pinus silvestris* L.) is associated with nutation movements. Trajectories of these growth oscillations were recorded by film technique in horizontal and vertical projection during a three day period of growth. On the basis of these data the parameters of elongation and nutation oscillations, *i.e.* rate, amplitudes and frequency of oscillations, were calculated and their changes during plant development compared. Oscillation trajectories are circular or elliptic spirals the amplitudes of which are increasing with the age of hypocotyl from 1.5mm to 7.5 mm. The frequencies of nutations are decreasing during the growth from 0.5 to 0.2 rev. h^{-1}. On the other hand, the growth rate of hypocotyl increased from values near to 10^{-3} mm h^{-1} at the beginning of the experiment to 4×10^{-1} mm h^{-1} recorded at the end of the third day. The zone of nutation curvature was slightly transferred from the middle of the hypocotyl toward the apex and its location has not been identical with that of elongation. This indicates that the system controlling nutation oscillations need not be identical with that controlling direction of elongation. At a certain stage of development behaviour of the decapitated pine hypocotyl is analogical to that of the root without the centre of georeception. A possibility of analogy of the system controlling direction of hypocotyl growth and of the system proposed for geotropical control of root growth is discussed.

The existence of nutation movements of growing pine seedlings complicates estimation of their phototropic sensitivity. Mechanisms of both these movements interfere and phototropic and nutation curvatures may be distinguished on the basis of the size of amplitudes of natural growth oscillations. For such measurements it has been suggested to estimate the basic nutation parameters of hypocotyl grown under scattered illumination for a period corresponding at least to two nutation cycles (Spurný *et al.* 1974). The size of the phototropical response is possible to determine by measurement of nutation deviations of hypocotyl before and after side-illumination.

The basic parameters of growth by elongation and nutation oscillations are affected by the age of seedlings (Baillaud 1958, Marsaud *et al.* 1965), temperature (Heathcote 1969) and by endogenous growth regulating factors. According to the recent investigations the localization of nutation curvatures of organs and position of zone of maximum elongation are closely related

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(HEATHCOTE 1969). The identity of zone of elongation and region of maximum nutation deviations was univocally recognized in roots of pea seedlings (SPURNÝ 1968). Similar data are not available for hypocotyls. It was therefore necessary to localize not only the zone of elongation but also the region of nutation curvature under conditions of scattered illumination. The knowledge of interactions of the two movement mechanisms may help to elucidate the transport and region of action of growth substances regulating elongation, *i.e.* of auxins and gibberellins (HAUPT 1972).

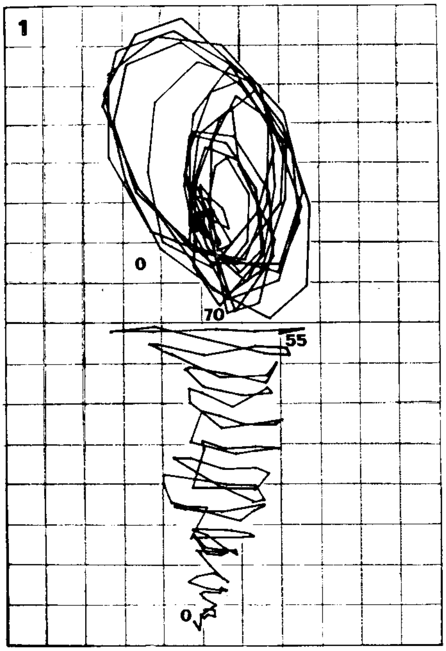
Material and Methods

The pine seedlings employed were of the same origin as described elsewhere (SPURNÝ *et al.* 1974). Ten selected individuals from pre-germinated seeds were planted into quartz sand in a growth-chamber. This chamber consisted from two parts. The first one was a tank made from "Umaplex" (150 × 40 × 40 mm) with a perforated bottom where the seedlings were planted in one line in a distance of 13 mm each from the other. The second part was a cover which maintained a high humidity inside the chamber. The growth-chamber contained the size marks and the left part of the microcultivation device was reserved for the time base. The tank and the cover were constructed in such a way which allowed simultaneous cinemathographic recording of oscillation movements of the apex in horizontal and vertical projection. This was achieved by attachment of a mirror at an angle of 45° which reflected the picture of seedlings and simultaneously reflected the light illuminating seedlings from above (SPURNÝ 1975). The recorded size of objects was reduced in ratio 1 : 12.7 and 1 : 13.5. The pictures were evaluated by a special microscope using enlargement 21 × and coordinates determining the position of the apex were plotted on a millimeter network. In this way the horizontal and vertical projections of nutation trajectories of 50 seedlings from five experimental sets (A 1—5) were obtained.

Zones of maximum elongation were localized by means of Indian ink marks. These marks were drawn on the whole hypocotyl length in a distance ca. 1 mm each from the other. Seedlings were then planted into the wet quartz sand in growth-chamber. Their growth and changes in bar distances were registered only in horizontal projection. The cultivation pots used in this experiment were smaller with attached size and time marks. The size of the object was reduced in ratio 1 : 2.34. This reduction allowed to recognize the position of marks under the microscope with sufficient accuracy. The records were evaluated on ~~a special~~ Zeiss reading apparatus at enlargement of 17.5 × which makes possible to draw the exact shape of the growing hypocotyl in each nutation phase. In this way the data were obtained about 16 seedlings from 4 experimental sets (B 1—4). All seedlings were grown under the same conditions: at 25 °C for 80 h from planting. At the end of the experiment the cotyledons started to develop and these seedlings loose their testa.

Results and Discussion

A characteristic course of apex trajectory of growing pine seedling hypocotyl in both, horizontal and vertical projection, is shown in Fig. 1. Parameters of growth oscillations, *i.e.* frequencies and amplitudes, and their changes are the same during the first three days of cultivation as in the previous experiments (SPURNÝ *et al.* 1974). Oscillation trajectories are circular or eliptic spirals with increasing amplitudes from 1.5 mm at the beginning of the experiment to values 5 times higher at the third day of cultivation. The nutation frequencies are decreasing during this period from 0.5 rev. h^{-1} to 0.2 rev. h^{-1}. The growth rate is also increasing from values near to 10^{-3} mm h^{-1} to 4×10^{-1} mm h^{-1}. This increase, however, is not high enough to compensate increasing amplitudes of oscillations and for this reason the frequency of nutations is decreased to one half. The regularity of size of oscillation spirals is increasing with the age of seedlings.



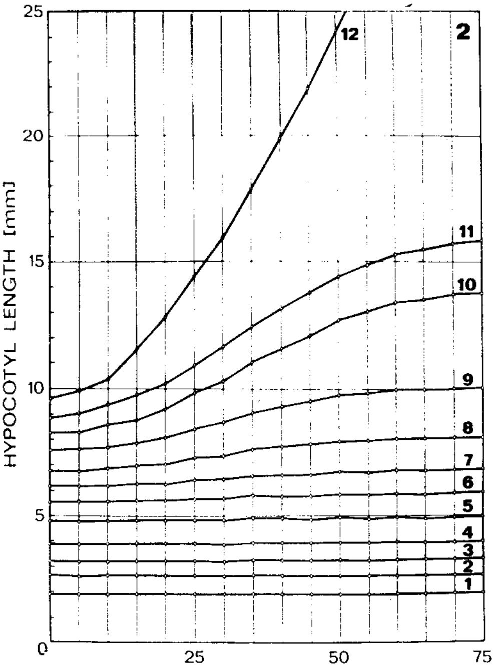
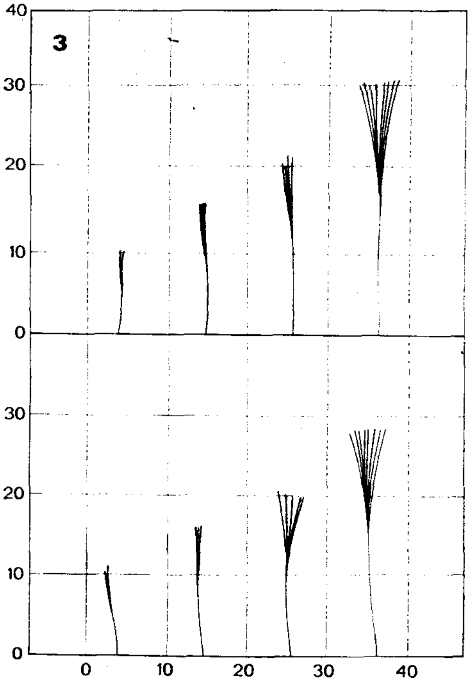


Fig. 1. Nutation oscillations of growing pine seedling hypocotyl (*Pinus silvestris* L.). Trajectories in vertical (above) and horizontal (below) projection were obtained from the cinematographic record of one plant from the experimental set A 1/2. Drawn in the 2.5 mm orthogonal network. Figures on the curve represent the beginning and the end of the experiment in h.

Fig. 2. Location of zones of maximum elongation on pine seedling hypocotyl (*Pinus silvestris* L.). Drawn according to the cinematographic record of one plant from the experimental set B 2/1. Abscissa: growth[hours], ordinate: length of hypocotyl [mm]. Figures on curves represent serial numbers of marks from the base to the apex.

Localization of hypocotyl elongation was determined on seedlings with hypocotyl 10 mm long. The results obtained from one seedling are shown in Fig. 2. At this stage of development the rate of elongation of the 6 mm long basal region of the hypocotyl is low (*ca.* 10^{-3} mm h^{-1}) and the zone of maximum elongation (*ca.* 2×10^{-2} mm h^{-1}) is located in the apex (mark No. 10, *i.e.* approximately between 7 and 8 mm of the hypocotyl). At the later stage the basal region ceases to elongate and the zone of maximum elongation is near to the apex. Starting the third day of cultivation the elongation is restricted to the zone deliminated by the last bar and the cotyledonar bud. The rate of elongation is reaching value 4×10^{-1} mm h^{-1}.

The location of elongation was analyzed in dependence on simultaneously recorded position of nutation curvature. The phases of changing shape of nutating hypocotyl are plotted at enlargement 17.5× from the cinematographic record in Fig. 3. The deviations originate in young seedlings in the middle of the hypocotyl. This region is nearer to the apex after three days of growth, *i.e.* in the oldest plants tested. The basal region of the hypocotyl does not elongate at all. At this point should be mentioned the investi-



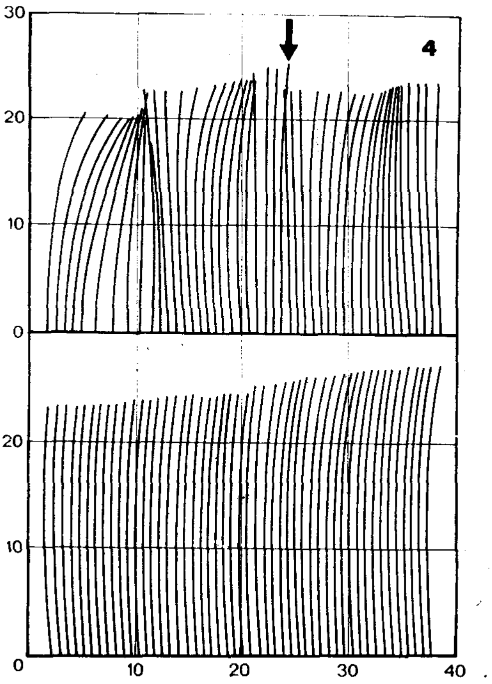


Fig. 3. Location of nutation deviations on growing pine seedling hypocotyl (*Pinus silvestris* L.). Drawn according to the cinematographic record of one plant from the experimental set B 1/3 (above) and B 2/1 (below) in the 10 mm orthogonal network. The changes in position of the centre of hypocotyl nutation curvature are recorded in 4 phases of elongation (plant from the experimental set B 1/3: 5, 15, 25 and 40 h; plant from the experimental set B 2/1: 15, 30, 40 and 65 h of growth). Nutation fan on the upper half of the hypocotyl deliminates the extent of oscillation amplitudes (= one half of nutation revolution).

Fig. 4. The effect of decapitation on elongation and nutation oscillations of pine seedling hypocotyl (*Pinus silvestris* L.). The changes in the shape of hypocotyl of one plant from the experimental set C 2/1 are drawn in the horizontal projection in 10 mm orthogonal network. Presented records in 15 min. intervals; starting from 11 h of growth these intervals were extended to 1 h. The arrow marks the time of decapitation.

gations of ARNAL (1953) and HEATHCOTE (1965) of nutation curvatures of ray coleoptiles and seedlings of *Phaseolus multiflorus*. Both authors described the same type of course of nutation oscillations which involves a characteristic wave advance from the apical to the basal region. On the basis of the velocity of transfer of curvature from the apex to the base they suggest that mechanism of nutation oscillations is closely connected with the mechanism of auxin transport. The theory of nutation oscillations as modulation of auxin production has not been, however, verified (JOERRENS 1959). The shape of nutation deviations and their course in pine seedlings is not, however, a type designated as "advance of waves from above below". It corresponds much more to circumnutations described for apical region of stems of *Dioscorea batatas*, *Quamoclit pinnata* and *Phaseolus multiflorus* (BAILLAUD 1952 and MARSAUD *et al.* 1965).

TABLE 1

THE EFFECT OF APEX DECAPITATION ON PARAMETERS OF GROWTH AND NUTATION OSCILLATIONS OF PINE SEEDLING HYPOCOTYLS (*Pinus silvestris* L.). CALCULATED FROM THE DATA REGISTERED IN THE EXPERIMENTAL SET C 2/1

Growth [h]	Hypocotyl [mm] length	Rate of elongation [mm . h^{-1}]	Frequency of oscillations time interval [h]	[rev. . h^{-1}]	Oscillation amplitudes (2a) [mm]
2	10.24	—	0 3^{30}	0.287	2.45
3	10.70	0.286	2^{15}— 5^{45}	0.287	2.56
4	10.94	—	—	—	—
5	11.35	—	—	—	—
6	12.00	0.454	4^{15}— 7^{45}	0.287	2.65
7	12.30	—	—	—	—
8	12.59	0.454	6^{15}— 9^{45}	0.287	2.96
9	13.18	—	—	—	—
10	13.67	0.430	7^{15}—10^{45}	0.287	4.09
11	14.15	0.474	9^{15}—12^{45}	0.287	4.39
12	14.15	—	—	—	—
13	15.00	0.477	11^{15}—14^{45}	0.287	4.27
14	15.28	—	—	—	—
15	15.85	0.487	13^{15}—16^{45}	0.287	4.12
16	16.10	—	—	—	—
17^{05}			Apex decapitation (1.93 mm)		
18	15.19	—	—	—	—
19	15.23	0.003	17^{05}—19^{00}	—	2.10
20	15.59	0.140	18^{45}—20^{00}	—	1.83
21	15.79	—	—	—	—
22	15.93	0.067	20^{15}—24^{00}	0.267	1.43
23	15.93	—	—	—	—
24	15.90	—	—	—	—
25	16.12	0.147	22^{15}—26^{00}	0.267	0.82
26	16.48	—	—	—	—
27	16.50	0.179	24^{15}—27^{45}	0.287	0.31
28	16.53	—	—	—	—
29	16.70	0.185	28^{15}—31^{45}	—	0.09
30	17.20	—	— does not oscillate		
35	17.54	—			
40	17.89	0.068			
45	18.20	0.051			

These diferences are not easy to explain. It seems to be neccessary to re-evaluate methods used for registration of curvature of plant organs and interpretations of results with respect to trigonometric laws. It must be stressed that optical records of curvatures of organs (*e.g.* epicotyls of *Phaseolus multiflorus* in Fig. 1 of HEATHCOTE 1965) do not show the real curvature but only its horizontal projection. Authors interpret the photographic records of organ curvatures as curvatures which proceed in a plane. But this is not the case. Only the simultaneous vertical record of trajectories of nutating organs may enable one to make a three dimensional reconstruction and to get a real picture of organ trajectory. A similar artifact may influence results of measurement of organ elongation when the organ is registered only in a horizontal projection. Distortion of real lengths of nutating organ which reached maximum in optical perihelium and ahelium may be a source of errors and incorrect interpretation of relaxation cycles.

Only the slight shift of the zone of oscillation toward the apex shows a close relationship between this process and the location of the zone of maximum elongation of the hypocotyl. But this relationship is not a correction as it was found in the case of growing roots where nutation deviations are associated with the zone of maximum elongation (Spurný 1968). Such behaviour of roots has corresponded to the proposed cybernetic model which was employed for interpretation of function of geocontrol system of growing roots. According to this model corrections of deviations of root axis from the geotropic direction induce deviations. It has been assumed that high elongation rate is favourable for occurrence of deviations and that these deviations require corrections which are signalized from the georeception centre to the elongation zone. The control of the root growth is apparently not analogical to the control of hypocotyl elongation. In the case of pine seedling hypocotyl the zone of maximum elongation is localized in a close distance under the cotyledons (3rd day of growth) but the centre of nutation deviations is below, approximately in the middle of the hypocotyl, *i.e.* at the periphery of the elongation zone. This means that the response to the hormone redistribution (its control mechanism is still irrelevant) occurs out of the zone of maximum elongation. It is possible that the control system of nutation oscillations is not, from the point of view of plant hormones, identical with a system controlling direction of hypocotyl elongation (see antagonism between auxin and gibberellin, Haupt 1972).

Some interesting information related to this problem were obtained in preliminary experiments in which the presumed control centre of hypocotyl elongation was removed. The decapitation of apex ceased hypocotyl elongation but the nutation oscillation damped out with smaller amplitudes during an interval of one oscillation period, *i.e.* 4 to 5 h (Fig. 4). The hypocotyl started to elongate after this period but oscillations ceased. In this respect the decapitated hypocotyl behaves similarly to the root without the georeception centre in the tip: the organ elongates but does not oscillate. The direction of growth is not controlled and corrected.

The problem will be investigated in the whole complexity as soon as the curvature of hypocotyl at various phases of seddling development grown under phototropic liminal- and side-illumination is known.

Acknowledgement

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M. Spurný (Brno): **Dloužení a cirkumnutační oscilace hypokotylu klíčních rostlin borovice lesní (*Pinus silvestris* L.)** — Biol. Plant. **17** : 43—49, 1975.

Hypokotyl klíčních rostlin borovice lesní (*Pinus silvestris* L.) vykonává při dlouživém růstu nutační pohyby. Dráhy těchto růstových oscilací byly filmově zaznamenávány po dobu 3 dnů v horizontálním a vertikálním průmětu. Ze získaných dat byly vyhodnoceny parametry dlouživého růstu a nutačních oscilací, tj. růstová rychlost, amplituda a frekvence oscilací a jejich změny během vývoje rostlin (obr. 1). Oscilační dráhy jsou kruhové až elipčité šroubovice s amplitudami zvětšujícími se se stářím hypokotylu; frekvence nutací se zpomaluje během růstu z počátečních hodnot 0,5 na 0,2 ot/h. Růstová rychlost hypokotylu se s vývojem rostliny zvětšuje z hodnot 1 . 10^{-3} na 4 . 10^{-1} mm/h.

Poloha zóny nutačního křivení není konstantní, ale přemísťuje se směrem k apexu; ale toto místo neodpovídá poloze zóny nejrychlejšího dloužení (obr. 2a, 3). Z toho lze soudit, že řídící systém nutačních oscilací nemusí být identický se systémem ovládajícím směr dloužení hypokotylu.

Dekapitovaný hypokotyl borovice se v určité fázi chová analogicky jako kořen zbavený centra geopercepce (obr. 4); je diskutována vhodnost analogie řídícího systému pro směr růstu hypokotylu s geokontrolním systémem v kořenech.