

GEOTROPIC CURVATURE OF AVENA COLEOPTILES

By A. E. NAVEZ AND T. W. ROBINSON

(From the Laboratory of General Physiology, Harvard University, Cambridge)

(Accepted for publication, June 8, 1932)

I

Examination of the literature concerned with geotropic reactions in plants shows quite divergent statements about the rates of geotropic curvature exhibited by comparable organs in similar plants (*cf.*, for instance, Jost, 1923, II, p. 258). Coleoptiles of *Avena* have been used extensively for such studies. Maillefer (1910) describes the rate of curvature as gradually increasing with time, the increase being proportional to the square of the time, while Tröndle (1913) finds the rate of curvature to be constant. It is true that Tröndle's way of computing a rate of curvature smoothes out possibly existing divergences and that the procedure is highly objectionable.

On the other hand Lundegårdh (1917) finds it possible to divide the whole geotropic curvature into three phases: a "Start Phase" before the curving is actually visible with naked eye; a "eumotorische Phase" where the process goes on at constant rate, terminated abruptly by the onset of the "Gegenreaktion," the last phase.

From our own observations it is rather difficult to admit, in general, such a clean-cut subdivision of the geotropic reaction; furthermore, we are in disagreement with Lundegårdh as to the constancy of the rate of curvature, as we will later demonstrate. Most of the differences encountered probably originate in the difficulty of obtaining a record of the process free from personal or instrumental error. The apparatus developed by us for obtaining records of the process of geotropic curvature seems to be free from such defects, and it has proved serviceable in the present work. It was used to determine the steps involved in geotropic curvature in *Avena* as related to time, as well as to get more information as to the changes in the shape of the region near the tip of the coleoptile. This last point is of great interest in the determination of the "reaction time."

Technique.—For a description of the photographic recording apparatus we refer to our previous paper (1932). It will be sufficient here to say that the principle of the method used is to record automatically, on a plate sensitized to the red end of the spectrum, successive positions of the experimental plant at regularly spaced intervals; the plate may be either fixed (in which case only the moving part of the organism is recorded by images separated on the plate) or it may be moved at regular intervals of time (the records are totally separated in this case and are placed one above the other on the plate).

As we have encountered a few difficulties in obtaining straight coleoptiles of *Avena*, and as we found in some cases departures of our standard technique to be influencing to a certain extent the exact reproducibility of the results, we may describe our procedure in some detail.

The seeds used were of a pure strain of *Avena*, the so called "Cornellian oats."¹ An examination of the dry seeds showed them to be remarkably uniform in external characters; we took care, nevertheless, to eliminate the infrequent seeds which were smaller than the normal in any one lot taken at random. They were kept in an air-tight glass container which prevented water loss. These seeds, after removal of the seed coat, were planted in sterile maple sawdust (*cf.* Navez, 1929) contained in glass vials 25 mm. high by 15 mm. in diameter, the sawdust retaining 4.3 times its weight of water. The seed itself is planted dry, with the embryo side upwards, the axis of the seed being inclined about 10° from the vertical and the tip of the seed being about 2 mm. above the sawdust level (the rim of the vessel). Germination proceeds in a light-tight, ventilated germination box, flat-black inside, in an air thermostat kept at 22° or 22.5°C. The vials are fixed in a wooden base provided with holes where the vials fit loosely.

After 72 hours the seedlings are ready for use; the coleoptiles are then 25 mm. long on the average; they are at the period of maximum growth rate; *viz.*, on the almost straight portion of their growth curve. The departures from this length are small, of the order of ± 2 mm. At this moment the seedling, in its vial, is fixed in a vertical position on an adjustable mounting stand. The adjustable stand is then returned to its normal position (determined by two brass pins) in the cell kept in a water thermostat and the moment of rotation of the seedling through an arc of 90° considered as time 0. All these manipulations are done under the very dim red light provided by a Wratten safe-light lamp with a Series 2 filter and a 20 watt bulb. The lower spectral limit of such light is 635 $m\mu$ and is thus of a wave length considered as inducing no phototropic effects in the seedling. The first photographic record is taken as soon as possible; *i.e.*, within 30 seconds of the time of turning the seedling horizontally. In the experiments here reported the temperature of the thermostat where the plants were reacting was adjusted to be

¹ We are very much indebted to Dr. W. T. Craig of the Agricultural Experiment Station, Cornell University, Ithaca, N. Y., for supplying us with this strain.

equal to that of the germination thermostat (22° or 22.5°C.); this reduced the time of thermal adaptation to a minimum. Furthermore the dark room where the thermostats are kept was maintained at about the same temperature. The atmosphere of the glass cell where the geotropic reaction took place, and of the germination box, was adjusted at 75 to 80 per cent of the saturation point in water vapor. With this technique it is possible to obtain without special difficulty coleoptiles of *Avena* of uniform length and behavior; the results obtained with such material show only very small variations.

II

Examination of Fig. 2 of the preceding paper shows immediately that we may distinguish two periods in the geotropic response: the pure geotropic reaction, and the autotropic oscillations. In none of our records have we detected any mixture of these two effects occurring simultaneously, so long as we work with our pure strain of *Avena*. Our strain shows the two reactions clearly separated in time. This is not true of all strains; in a few trials with some seeds of unknown origin we have found the two manifestations conflicting one with another.

In the subsequent treatment of our data, we will consider only the true geotropic curvature.

Shape of the Curved Coleoptile.—All reacting seedlings showed a smooth, rounded curving which in the successive stages shortens its radius of curvature. In no case have we observed an almost straight tip separated from a straight base by a very short curved portion. The fact that such “curvatures” have been observed and represented by reliable workers makes it highly probable that some specific structural differences in diverse races can affect the external appearance of the bent coleoptile.

Examination of one of the pictures (Fig. 1) that we may consider as typical,—or, what is better, working on a photographic enlargement of such a picture—shows that the upper and lower profiles of the coleoptile are not one and the same curve merely shifted; this is especially so in the region of the tip. In other words, if we wish to deal with curvatures of such stems, we have to define a reference line. For theoretical convenience we have chosen for reference the axis line, the *neutral axis* of the coleoptile, with the meaning used in work on the

resistance of materials.² In the pictures this neutral axis is easily determined through the symmetrical construction of the coleoptile: the neutral axis must be equidistant at each point from both upper and lower profiles. In further discussion this line is the one used, unless specifically stated otherwise. As far as can be determined, for all the pictures of bending coleoptiles obtained, the successive neutral axes of the bending part of the stem correspond very nearly to arcs of circles rather than to portions of hyperbolas or parabolas (Fig. 1). Such arcs of circles can be defined by the positions of their centers, the radii, and the angle subtended by the arc.

The locus of the centers of curvature during the progress of bending is an arc of a curve of parabolic type; it is not a straight line perpendicular to the neutral axis at the point where the curved portion of the neutral axis becomes tangent to the horizontal straight part of the same axis, as might be expected for the simple case. The departure from simple expectation can be attributed to two causes, which may add their effects, namely (1) the stem is growing while the bending takes place; (2) the stem shows a certain amount of viscous elasticity or shearing; one might think of the first of these two causes as the main factor affecting the locus of the centers of curvature, since elongation does occur.

The radius of curvature varies also with time; Fig. 2 shows the type of relation obtained. But as in the first stages of curvature the radii are very large and very difficult to measure with accuracy, the first observation points of Fig. 2 are subject to a probable error which must be large. The angle subtended by the curving part is readily determined by measuring the angles that the tangents to the neutral axis at

² The neutral axis is a more logical reference line than one of the profiles (as used by Dolk, 1930, p. 63), for two reasons: (1) the two profiles are not the same curve merely shifted; (2) the diameter of the coleoptile varies with the distance from the tip. The same objections apply also to the method used by Dolk for determining the curvature of different regions of the stems. The graphs given in his memoir (*cf.* his Figs. 8 to 25) correspond to radii of the concave profile and are not necessarily identical in time relation with those which can be obtained for the other side, especially when using Dolk's method of measuring the radii. We have found such difficulties in the use of this method when we applied it to our seedlings as to preclude accurate measure of the curvatures of the stems.

the tip of the stem make with the horizontal. By a simple geometrical construction one can readily see that in the case of arcs of circles the angles at the center and those just defined are equal. This procedure has the advantage of relieving us from determining the centers of curvature—or the locus of these centers—and substitutes for it the

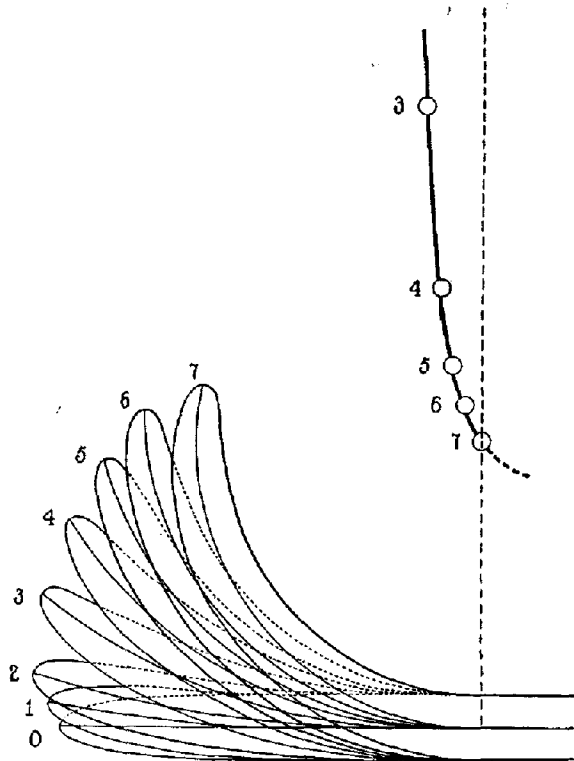


FIG. 1. Successive positions of the neutral axis of a geotropically curving coleoptile at 30 minute intervals of time, and locus of the centers of curvature of these arcs.

measurement of a tangent to an arc. As can be seen in Fig. 3, the change of this angle with time is not haphazard: it is best described by a smooth sigmoid curve having a point of inflection at about 42 per cent of total completion. This curve gives us also a way of defining a "rate of bending" which may be less arbitrary than the "rates of

curvature" as used in the literature.³ Angular displacement of the tip and linear displacement of the tip have been used to give such "rate" curves. It is obvious that even on a photograph, especially if reproduced by direct contact without enlargement, it is very difficult to measure with any precision the angle that the tip makes with the horizontal. This is particularly so when the coleoptile bends in a

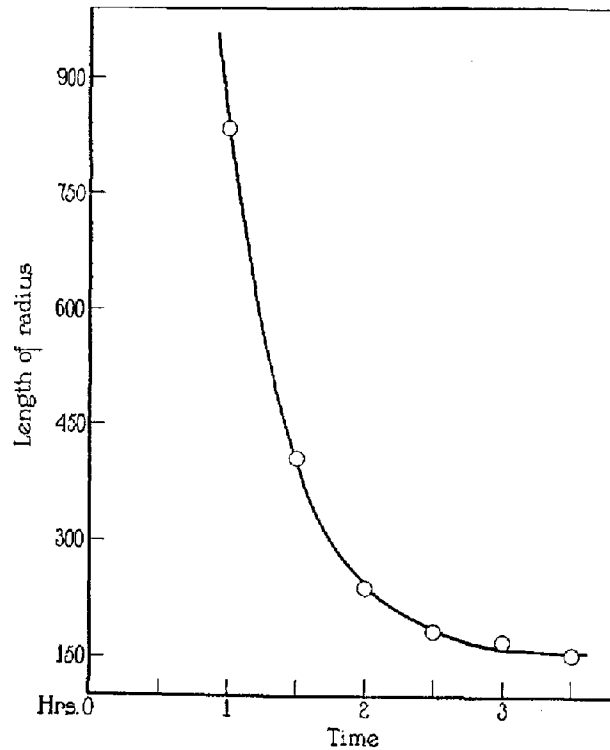


FIG. 2. The relation of radii of curvature to time

smooth, rounded curve without any straight portion near the tip. As to the estimation of this angle by visual observation, we think that the experimental error is so great as to make this measure almost

³ This curve gives us a relative measure of the amount of curving tissue, as we know for any position of the neutral axis the corresponding radius of curvature and the angle subtended by the curved part.

useless. The linear displacement of the tip (as used by Brauner, 1922, for instance), measured from the point occupied by this tip at zero time as origin, is also inadequate, because of the lack of correspondence between an arc and its chord for angles of the magnitude here observed.

The derivative of the Curve A in Fig. 3 gives us a more reasonable way of defining a "rate of curvature." It is apparent from Curves A and B that the curvature is not proceeding at a constant rate, as it has been described, and that it is difficult to divide the curve into 3 parts as Lundegårdh has done. We can only state that the rate of

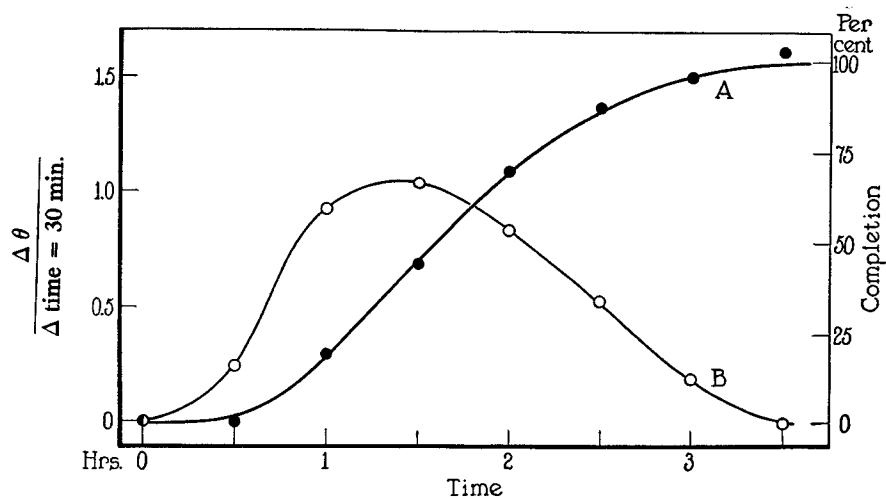


FIG. 3. Curve A, the time relation of the angle made by the tangent to the neutral axis at the tip with the horizontal.

Curve B, the derivative of Curve A, showing the variation in the "rate of curvature."

curvature at first increases with time, passes through a fairly well defined maximum at about 90 minutes after the beginning of the experiment, under these conditions, and then gradually decreases to zero.

Fig. 4 shows that for different seedlings grown under the same conditions the derivative curves are in good agreement. Similarity between curves for single seedlings shows obviously that the lack of sharply defined periods in the reaction is not a matter of smoothing out by averaging different curves, but is intrinsic.

First Steps in the Geotropic Response.—In a few pictures we observed that before any upward bending occurs, a slight drop of the tip of the coleoptile takes place. The fact that the base of the coleoptile remains exactly in its original position excludes the idea of a shift of the organ due to packing or displacement of the sawdust in which the seed grows.

As this phenomenon was not of constant occurrence we must conclude that it is probably not a fundamental step in the geotropic reaction, but that more likely we have to do with a nutational movement. Perhaps it is a passive sagging of the tip of the coleoptile, progressing

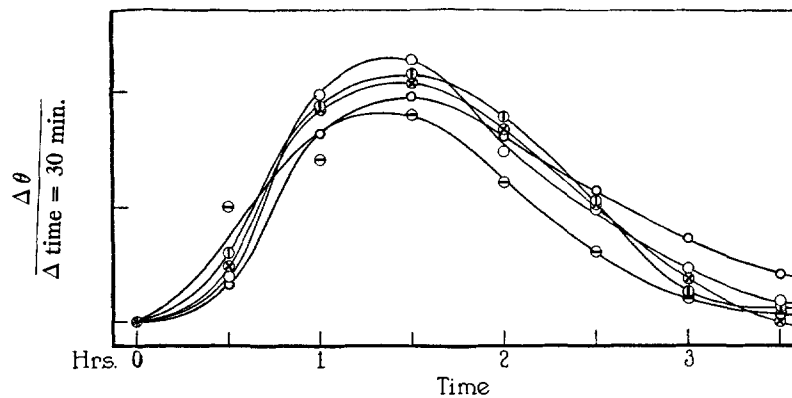


FIG. 4. A set of derivative curves (as in Fig. 3, B) showing the similarity of the course of the reaction with individual seedlings.

until the true geotropic response occurs. Bose (1906) and Maillefer (1910) are probably the only authors who have investigated this bending, but, despite the extensive tables published by Maillefer, we cannot be convinced that the sagging is the first step in the geotropic response; the phenomenon occurs in only $\frac{2}{3}$ of his observations. Even then the amplitude of the downward bending is often of the order of magnitude of the nutational movements described in the literature. In any case we would consider it as a mere mechanical sagging rather than a short phase of positive geotropic response rapidly reversed by the true geotropic bending.

III

Geogrowth Reaction

Opinions as to a "geogrowth reaction" are divided: one group of observers admits, the other rejects the idea that on geotropic stimulation the normal rate of elongation of the coleoptile is affected.

As pointed out before, the period of utilization of the coleoptiles, in our work, corresponds to the almost straight part of their growth curve.

The total duration for the geotropic reaction here studied is about 3 hours. If such a thing as geogrowth reaction exists, it will obviously be shown best by the way in which the neutral axis of the coleoptile changes with time. This change, as well as that of the upper and lower profiles, can be very easily measured on our plates. The fixity of both plate and object in respect to one another removes the difficulty of ascertaining the fixity of a reference point from which to measure elongation. This point is taken at 22 to 25 mm. from the tip, in a region where virtually no growth is found during the period of geotropic reaction. The length of each line considered is measured on the enlargement by means of a precision map measurer; the precision attained in successive readings of the same length is of the order of 1 part in 400.

As shown in Fig. 5 there is no apparent "growth reaction," either positive or negative. The Curve A, relating elongation of the neutral axis to time, is a straight line over the 3 to 4 hour period during which the geotropic reaction occurs. The fact that before and after the moment the plant is stimulated elongation proceeds at the same rate also precludes the existence of a lag period for this hypothetical geogrowth reaction, analogous to the one occurring on light stimulation (Blaauw, 1914).

Another point which also clearly comes out of the analysis of our pictures is the relative rate of elongation of the upper and lower profiles. As depicted by Curve B in Fig. 5, the upper side slows down its rate of elongation by an amount nearly equal to the one by which the lower side (Curve C) increases its rate. But in no case have we found complete inhibition of growth on the upper profile. At most we find a partial inhibition. These results depart from a good many

reported, perhaps for the reason that in our experiments no other significant stimulus was acting at the same time as the geotropic vector. All direct contacts with the plant being avoided, the method is free from interference by accidental stimulation. Very likely we are justified in attributing to such technical defects some of the results reported by previous authors.

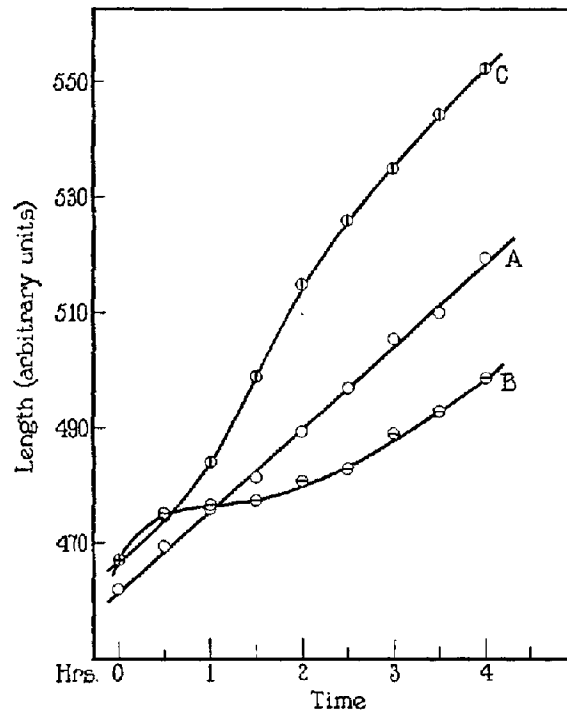


FIG. 5. Curve A, elongation of the neutral axis during geotropic response.

Curve B, elongation of the upper profile of the same coleoptile during geotropic reaction.

Curve C, elongation of the lower profile during reaction.

IV

Curvature and Distribution of Growth Substance

How do the preceding observations fit in with the hormonal theory of the geotropic curvature? In experiments performed by one of us (A. E. N.) we have tried to correlate the angle made by the tip with the

distribution of growth substance between upper and lower half of the tip. The technique used was essentially that of Went (1928) and Dolk (1930), using a 3 per cent agar-gel for extraction of growth substance; the lengths of the tips cut from the reacting plants were between 7 and 10 mm. long; the duration of contact for diffusion into the agar was always 60 minutes.

Care was taken to rub the little razor blade used for prevention of cross diffusion between both halves, before use, with a small piece of paraffin which left on it a very thin coat of this substance sufficient to suppress capillary creeping of the tissue juice. The blade protruded about 1 mm. above the small blocks of agar used to receive the diffusate. (For full details concerning this technique the reader is referred to the papers of Went and Dolk.) On the assumption that the curvature is determined by unequal growth on the upper and lower flanks of the horizontally placed stem we ought to find a definite correlation between the differences in amounts of substance in the upper and lower halves of the coleoptile, and the angle through which the stem has bent. In other words, it is when the tip of the coleoptile is placed horizontally that the difference should be maximum;⁴ as the tip is gradually brought back to verticality the difference should decrease and reach zero when the tip is again vertical. The ratio of the amounts of growth substance present in the two halves ought therefore also to vary accordingly with the angle made by the tip with the vertical.

In the set of experiments here briefly reported, we did not have a large enough number of observations to enable us to treat the data statistically; they will be amplified in a subsequent paper and are given here only as a corollary to the discussion of angular displacement with time.

In Table I the amount of substance present in symmetrical halves of tips is expressed in terms of degrees, indicating the amount of deflection observed in prepared standard coleoptiles of *Avena* when supplied with a standard block of agar containing the diffusate from the tip of the coleoptile under measurement. We know from the experiments of previous investigators, and our own, that for a tip placed vertically

⁴ Of course, this largest difference is not instantaneous. It can reach its maximum only after a lapse of time sufficient for shifting the growth substance from its state of equidistribution has been allowed. A "lag period" is thus expected.

we have an equal distribution of growth substance in what we would call "upper" and "lower" halves if the coleoptile were placed horizontally. With four tips placed on the same agar plate, this amount

TABLE I

The growth substance diffusing from tips of vertical coleoptiles is equally distributed between the two halves of the tip.

No. of tips	"Upper"	"Lower"
4	13.0°	14.0°
4	17.0°	19.0°
4	16.0°	13.0°

TABLE II

The amount of growth substance diffusing from upper and lower halves of tips of coleoptiles of *Avena* placed horizontally and subsequently reacting, varies with time. The amounts are expressed in degrees of deflection of test coleoptiles (see test) after 90 minutes.

Angle of tip to the vertical	Duration of stimulation	No. of tips tested	Average deflection of test coleoptiles	
			"Upper" half	"Lower" half
90°	<i>min.</i>			
	15	6	7°	16°
	20	4	9°	16°
	20	8	4°	13°
60°	30	4	5°	18°
		4	11°	15°
		4	12°	16°
30°	75	6	10°	15°
		4	12°	14°
		6	8°	13°
0°	110	4	11°	15°
		4	12°	14°
		3	12°	14°
0°	160	4	6°	7°
		4	12°	12°
		4	15°	16°

of substance, in three successive experiments, is represented by the figures in Table I.

For the experiments using tips of coleoptiles removed while the

stems are bending, the number of plants used for each experiment was variable and is given in the column "No. of tips tested" in Table II.

From Table II we can conclude that the distribution of growth substance is in itself directly influenced by gravity and that its distribution continues to be affected by this factor while the bending takes place. One may conceive that the apparent ratio of distribution is in part dependent on the utilization of the growth-promoting substance for further growth, and that its decrease ought to follow the progress in bending. The data thus far collected show in a qualitative way that such an assumption is valid.

SUMMARY

Applying a photographic recording method, and working on enlargements of the plates so obtained, the shape of the geotropically curving coleoptile of *Avena* was studied. This shape is expressed in terms of curvature of the neutral axis of the coleoptile; for the true geotropic response of the strain of *Avena* used, the curvature of the neutral axis is an arc of a circle. The "rate of curvature" is taken as the derivative of the curve relating time with the angle with the horizontal made by the tangent to the neutral axis at the tip. This rate increases up to a maximum and then decreases gradually. No "geogrowth" of the whole coleoptile is found. It is shown that the curvature is due to an increase in the elongation of the lower side of the horizontally placed coleoptile with a concomitant decrease of the rate of elongation of the upper side. This is correlated with a shift in distribution of "growth substance" in the tip as affected by change of position of the coleoptile.

BIBLIOGRAPHY

- Blaauw, A. H., 1914, *Z. Bot.*, **6**, 641.
 Bose, J. C., 1906, Plant response as means of physiological investigation, London, Longmans, Green and Co.
 Brauner, L., 1922, *Z. Bot.*, **14**, 497.
 Dolk, H. E., 1930, Geotropie en groeistof, Dissertation, Utrecht.
 Jost, L., 1923, Pflanzenphysiologie, Jena, G. Fischer, 1923, **2**.
 Lundegårdh, H. G., 1917, *Lunds Univ. Arsk.*, **2**, n.s., 136.
 Maillefer, A., 1910, *Bull. Soc. Vaud. Sc. Nat.*, **46**, 235.
 Navez, A. E., 1929, *J. Gen. Physiol.*, **12**, 641.
 Navez, A. E. and Robinson, T. W., 1932, *J. Gen. Physiol.*, **16**, 125.
 Tröndle, A., 1913, *Jahrb. wissenschaft. Bot.*, **52**, 186.
 Went, F. W., 1928, *Rec. trav. bot. néerl.*, **25**, 1.