

On the artificial Production of Rhythm in Plants.

With a note on the position of maximum
heliotropic stimulation.

BY

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With four Figures in the Text.



IN the *Annals of Botany*, October, 1892 (Vol. VI, p. 245), we described a series of experiments on this subject. We remarked (p. 259) that 'Those who repeat our experiments must not expect uniform success, as there is undoubtedly a certain capriciousness in the results, which probably depends on varying degrees of vigour in the plants used.' The present research was begun in the hope of discovering a cause for this capriciousness; in this we have been disappointed, nevertheless some of our results seem worth printing.

The fundamental experiment consists in subjecting seedlings or growing shoots to a series of opposite stimuli following each other at equal intervals of time. The stimuli may be either due to gravitation or to light; in either case they tend to produce curvatures in two opposite directions. It might

¹ A note on our results was read before the Cambridge Phil. Soc. on January 22, 1900.

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be supposed that the result would be an absence of all curvature. But this is not so: what happens is that the plant curves first in one, then in the opposite direction. These to and fro movements occur with surprising but not exact regularity in a rhythm corresponding to that of the application of the stimuli. In our former experiments the reversal of the stimulus occurred at intervals of half an hour; we have now succeeded in building up a periodic movement in a 15-minute rhythm¹.

That during the continuance of the alternate stimuli a plant should nutate in a given rhythm is sufficiently remarkable, but it is far more interesting that the rhythm should continue after all stimulation has ceased, and this we again find to be the case.

METHOD.

We have again used the intermittent klinostat, employing of course a horizontal axis for geotropic experiments, and a vertical axis in the case of heliotropism.

A cord is wound round the rotating axle and supports a weight over a pulley; the axle also bears a pair of arms projecting from it at right angles and separated from each other by 180°. As long as one of these arms is fixed the weight cannot turn the axle, and the plant is geo- or heliotropically stimulated. At regular intervals a clock escapement frees the arm, and the axis rotates through 180°, when the plant is at once subjected to the opposite stimulus for another equal period of time. The act of rotation is rendered gentle by a fan-governor, so that the plant is not unduly jarred.

EXPERIMENTS.

We find that heliotropic experiments succeed with much greater regularity than those in which the stimulus is gravitational. This was to some extent evident in our 1892 results, but we can now state the case more definitely. We have made twelve heliotropic experiments² on *Phalaris canariensis*;

¹ We also tried an hourly period, but without success.

² Including one with an hourly period.

in eight of these the *stimulated rhythm* was apparent, i.e. the periodic movement continued as long as the alternate stimulation was kept going¹. In six of the eight, the *unstimulated rhythm* was observed, i.e. the periodic movement continued after the stimulus had ceased. This is the really important result, namely, that when a *stimulated rhythm* has been formed it passes on to *unstimulated rhythm* in at least three-quarters of the cases.

In the following short series we begin with the full notes of an experiment, because in our former paper such details were omitted. No. I happens to be a geotropic experiment, and we have not thought it necessary to add full details of a heliotropic example, since the principle is identical in the two classes.

HALF-HOURLY PERIOD. (GEOTROPISM.)

Exp. I (Fig. 11). Mustard Seedling. March 15, 1899.

The seedling was arranged with its hypocotyl parallel to the horizontal axis of rotation, which was perpendicular to the plane of the window to avoid alternating heliotropic effects. The geotropic curvature of the seedling was observed by means of a horizontal microscope; the readings are given in column 3 of Table I. The experiment was begun on March 14, 1899; the readings here given were made on March 15 during the 97th and following periods of revolution of the klinostat, as indicated in column 1, which is headed 'Period.'

Beginning at 10.12 a.m. it will be seen that the readings (column 3) sink in value, indicating a steady upward curvature of the hypocotyl, until 10.22; at this point the curvature is reversed, as shown by the readings suddenly increasing in value. This increase continues steadily until 10.37, and at 10.38 the klinostat rotates through 180°. At this point the horizontal microscope has to be readjusted, and the readings beginning at 10.39 (Period 98) will be seen to be falling in value instead of rising. This is the obvious result of the rotation of the horizontal axis of the klinostat; the act of

¹ Three out of the four failures were on badly grown plants.

<i>Period.</i>	<i>Time.</i>	<i>Reading.</i>	<i>Period.</i>	<i>Time.</i>	<i>Reading.</i>
97	10.12 a.m.	40	99	11.21.5 a.m.	18
	14	37		22	19
	14.5	35		23	22
	16	28		25	28
	17	23		27	40
	18	20		28	46
	20	17		29.5	52
	22	14		30	57
	23.5	24		31	62
	24	26		32	67
	25	32		32.5	73
	27	42		34	80
	28	50		37.5	83
	30	62		38.5	86
	32	72		40	93
	34	84		42	97
	35	93		44	104
	36	100		45	106
	37	103		46	107
98	10.39	44		47.5	107.5
	39.5	42		48.5	106
	40	31		50	105
	42	31		51	101
	44	24		53	90
	45	20		55	77
	46	12		56	69
	48	8		58	62
	49	6		59	55
	49.5	5		12. 1	43
	50	4		2.5	35
	51	4		3	30
	52	5		4	26
	54	11		5	20
	56	15		6.5	12
	58	19		8	4
	59	25		10	-4
	11. 2	38		12	-10
	3	43		13	-15
	5	52		14	-24
	6	56		15	-29
	7	60		16	-34
99	11. 9	47		16.5	-38
	10.5	44		17.5	-43
	13	38		18	-49
	14	32		19	-52
	16	26		19.5	-56
	17	23		20	-59
	18	21		21	-62
	19	18		22	-67
	20	17		23	-73
	21	17		24	-75
				24.5	-79
				26	-87
				30	-101

Observations ceased.

curvature remains unchanged between 10.37 and 10.39, but owing to the rotation of the axis what was a downward curvature becomes an upward curvature.

This is clearly shown in the diagram (Fig. 11), which gives

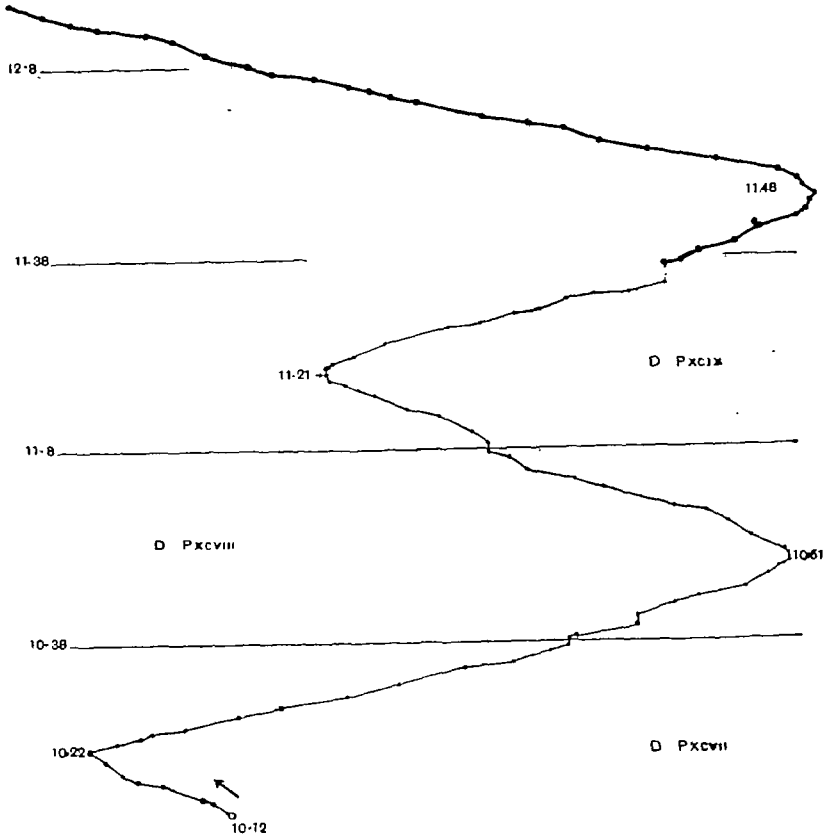


FIG. 11.

a graphic representation of Exp. I. Fig. 11 and all similar diagrams are to be read from below. The diagram is divided by a series of horizontal lines which indicate the moments at which the klinostat axis rotates; the spaces between the lines are the *periods*, beginning below with Period 97. Time

is represented along vertical lines (ordinates), and it will be seen that the times of rotation are at 10.38, 11.8, 11.38. The upward or downward curvature of the plant is represented by the line in the diagram travelling to the left or to the right. The letter *D* (for downward) being on the right in Period 97, it follows that until 10.38 movement to the *left* means an upward curvature, movement to the *right* a downward curvature. Thus from 10.12 a.m. to 10.22 the curvature was upwards; at 10.22 (as shown in Table I) the curvature is suddenly reversed, and the plant curves downwards. In Period 98, owing to the rotation of the axis at 10.38, *D* must be placed on the left; in this way it is indicated that after 10.38 the hypocotyl was bending upwards, but the continuity of the curve-line from 10.22 to 10.51 shows that, considered as a growth-curvature, it is a single act; it is in fact a single unit in what is practically half-hourly rhythm.

In Period 99 the turning-point of the curve comes at 11.21, almost exactly half an hour after 10.51, as shown in the table and in the diagram. At 11.38 the klinostat was not allowed to turn: this is indicated by a *thick* curve-line, also by the absence of a numbered period and the absence of the symbol *D* in the space 11.38–12.8. At 11.38 the plant was placed with the original plane of curvature vertical, to avoid as far as possible geotropic stimulus in that plane. In spite of the freedom from alternate stimuli, the reversal of curvature took place at 11.47½, that is, 3½ minutes before the proper moment. By referring to Table I it will be seen that after the sharp turn at 11.47½ the movement continued unchanged in direction until 12.30, when the observations were discontinued. In the diagram there is only room for the curve up to 12.10.

QUARTER-HOURLY PERIOD. (GEOTROPISM.)

Six experiments were made, on cut stalks of a Valerian, with a klinostat rotation through 180° at intervals of 15 minutes. In one experiment no curvature of any sort occurred, but in the other five cases a regular rhythm was

observed, which continued after the klinostat was stopped. In two experiments this (the 'unstimulated rhythm') showed two reversals of direction. We give a single example :—

Exp. II, Fig. 12. Valerian, $\frac{1}{4}$ -hr. period. (Geotropism.) Fig. 12 will sufficiently explain the results without giving the full notes.

There are several curious points about this experiment. The change in direction of curvature takes place not in the middle of the periods (as in Fig. 11) but at the horizontal lines, that is, the beginning of each fresh period. Thus during each period the shoot was curving downwards¹. It is impossible to say whether this is due to 'sagging,' i.e. to the weight of the shoot causing it to bend downwards as any flexible body would bend, or whether it is a true geotropic curvature which happens to coincide with the turning-point of the klinostat. The fact that it occurs in Period I makes it probable that it is a case of sagging. But the sequel of the experiment makes it clear that the alternation of stimulus was producing an effect. It is therefore probable that the physical drooping of the shoot concealed any geotropic curves as long as the klinostat was in action. The klinostat was stopped in the middle of Period IX; nevertheless, as the thick line shows,

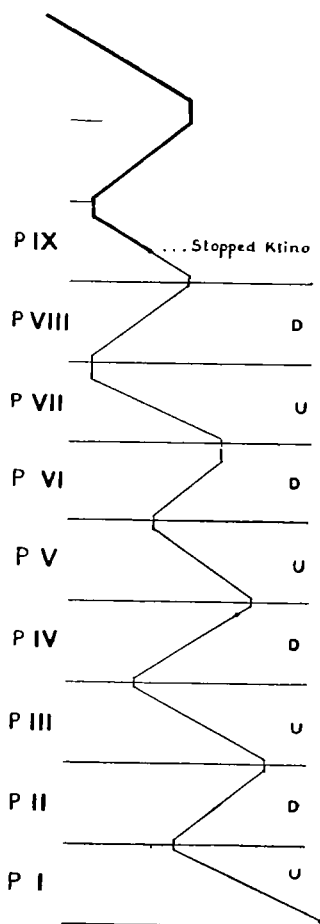


FIG. 12.

¹ It must be understood that Fig. 12 is an abstract of the observations, only the critical points of the curve being given. The same is true of Figs. 13 and 14.

the curvature of the plant was reversed at the end of the $\frac{1}{4}$ hr., and again of the next $\frac{1}{4}$ hr. These movements could not be due to physical drooping.

QUARTER-HOURLY PERIOD. (HELIOTROPISM.)

Six¹ experiments were made on the heliotropic curvature of *Phalaris canariensis* with the quarter-hourly rhythm. Four of these showed the 'stimulated rhythm,' and two

showed also 'unstimulated rhythm,' i.e. a rhythm continued after the klinostat stopped. One of these made a single turn, and the other made two such reversals of curvature.

Exp. III, Fig. 13, May 10, 1900. *Phalaris*. Quarter-hourly period. (Heliotropism.)

The experiment is remarkable as showing that the plant may acquire a rhythm in a very short time, e.g. after four periods of $\frac{1}{4}$ hr. each. It should be noted that in heliotropic diagrams the letters *D* and *L* mean *Dark* and *Light*, and that they change sides in each period precisely as the letters

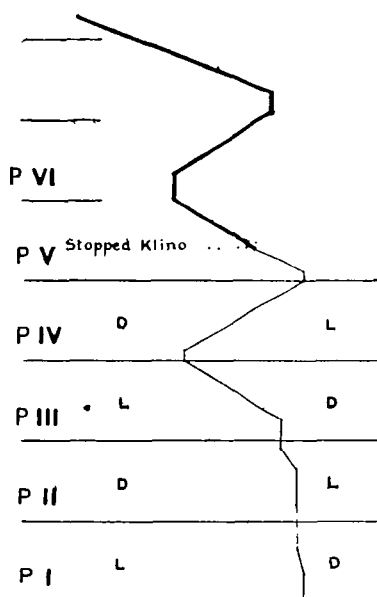


FIG. 13.

D, *U* (for *Down* and *Up*) alternate in the geotropic diagram, Fig. 12. Here again the curve changes direction synchronously with the rotation of the klinostat, but in this case there can be no question of a purely physical droop (as in Exp. II), since the axis of rotation is vertical. Neither in heliotropic nor geotropic experiments is this synchrony

¹ Several experiments were vitiated by the slow or oblique growth of the seedlings, and are therefore omitted.

universal; see for instance, *Annals of Botany*, VI, pp. 257-9, where in Exps. X and XI the reversal of the curve, in light experiments on *Phalaris*, occurs in the middle of the periods. To return to Fig. 13, the klinostat was stopped in Period 5 and the plant arranged so that no fresh heliotropic stimulation could occur in the original plane. The thick line shows two reversals of curvature occurring at approximately the right times.

Exp. IV, Fig. 14, December 1, 1899. Oat seedling. Half-hourly period. (Heliotropism.)

We give this experiment in order to make it clear that there is no necessary connexion between heliotropism and the quarter-hourly period, and that a plant can equally well acquire a half-hourly rhythm by alternate light-stimuli.

It should be noted that in Fig. 14 the curvature of the seedling, as soon as it becomes regular in Period 5, is away from the light, not towards the light as in Fig. 13; we are unable to explain the difference between the two cases. The klinostat was stopped in Period 9, and the thick line shows two reversals at approximately half an hour's interval, the first turn being somewhat belated. This is another good instance of 'unstimulated rhythm.'

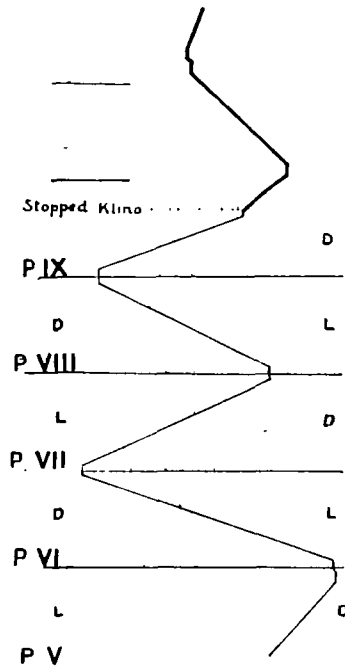


FIG. 14.

ALTERNATE UNEQUAL STIMULI.

We made a good many heliotropic experiments with alternating but *unequal* periods of illumination, in the hopes of building up an unequal rhythm. The intermittent

klinostat was so arranged that the plants were illuminated on one side for 28 minutes and then on the opposite side for 32 minutes. Twelve experiments were made, eleven with *Phalaris*, and one with an oat seedling; out of these only two failed to show stimulated rhythm, and out of these ten successful cases, three showed 'unstimulated rhythm' after the klinostat had been stopped. We hoped to find that the plants would show an inequality in the period of their rhythm corresponding to the inequality of the alternate stimuli, but the results are disappointing. If we call the number of minutes that elapse between two successive reversals of curvature, the length of the period, we can indicate the amount of regularity of the rhythm by writing in a row the lengths of successive periods. In an ideally regular half-hourly rhythm we should have—30, 30, 30, 30, 30. When we compare with this the result of an experiment made with alternate stimuli of 28 and 32 minutes, we seem to get the desired result. Thus in Exp. XXIX, the figures were—28, 30, 27, 32, 27.5. In Exp. L we got—23, 37, 22, 28, 30, 30. In Exp. LI—25, 37, 17, 40, 19. In all these there is a clear indication of the double or unequal rhythm. But unfortunately it is possible to find similar results produced by the ordinary half-hourly stimulation. Thus in Exp. II (Annals of Botany, VI, p. 250) with a regular half-hourly geotropic stimulus we got—18, 35, 14, 29. This is an unusually irregular rhythm for an experiment of this class, but it clearly renders it impossible to come to any conclusion as to the special character of the rhythm produced by unequal stimulation.

The experiments are nevertheless interesting in another way, for there was an undoubted difference in the *degree* of curvature in the two directions. That is to say that after some hours of alternate illuminations of 28 and 32 minutes the plants became perceptibly concave on the side which received the longer light-stimulus¹.

¹ The same effect was seen with the radicles of *Sinapis*, which gradually bent away from the more illuminated side.

In 'The Power of Movement in Plants' it was shown that heliotropic curvature may be brought about by modified circumnutation, i.e. by a revolving movement in which the curvature towards the light is greater than that in the opposite direction. Just in the same way, in our experiments, heliotropic curvature was produced by asymmetrical nutation.

GENERAL REMARKS.

It is often said that periodic phenomena are due to after-effect. But this, though true in a certain sense, is too vague a statement to be called an explanation. It is known that geotropic and heliotropic curvatures continue long after the stimulus has ceased to act. So that it might at first appear as if the curvatures in one direction were the after-effect of a given half-hour's stimulus, and the opposite curvatures were the effect of the next ensuing half-hour. But we are unable to construct a scheme of this sort which fits the facts. The after-effect in a curving shoot which has been stimulated for half an hour lasts a long time, and we cannot see how the series of opposite curvatures, *each lasting half an hour*, could be caused by the combination of such after-effects. After-effect in the ordinary sense is the result of the last stimulus received, and we know of nothing to make us believe that the latent after-effect of an antecedent and opposite stimulus can be held to account for the sharp reversal of curvature which we find to occur.

We believe, moreover, that an artificial rhythm may be imagined to be produced without what is ordinarily described as after-effect. Suppose an apogeotropic shoot to be placed horizontally; after some 15 minutes of stimulation it will begin to curve upwards, and will continue so to do for another 15 minutes. At this point the klinostat rotates through 180°, and the stimulus is replaced by an equal and opposite one. According to our assumption (that after-

effect is non-existent) the curvature will cease at the turn of the klinostat, and the plant will be able to receive the new stimulus, and will therefore after a period of quiescence curve in the opposite direction. We can see no reason why this imaginary state of things should not build up a rhythmic condition, so that the plant would tend to nutate at half-hourly intervals after the klinostat had been stopped. We can conceive the natural circumnutation of the plant being moulded to a half-hourly rhythm under these conditions—that is to say, without the existence of after-effect.

All we can do is to compare our results with other periodic phenomena. For instance, when the stimulus is given by the alternation of day and night, we get the diurnal periodicity of sleeping plants, which, as in our experiments, continues after the stimulus has ceased. It seems to us that such natural rhythms, as well as our artificial phenomena, are intelligible only as modifications of a fundamental rhythmic faculty in plants. Such a faculty exists as circumnutation, and we may point out that the possibility of regulating artificially the rhythmic growth of a plant is in entire agreement with the fundamental idea of 'The Power of Movement in Plants,' namely that growth-curvatures are modifications of circumnutation.

It is unfortunate that the word *after-effect* has been used in two senses:—(1) To designate the continuance of curvature after the cessation of the stimulus, which may be most conveniently classed with the phenomena of the motor mechanism, although it is also a character of the percipient element. (2) To designate such a case as the continuance of periodic movements in a sleeping-plant in continuous darkness. This should be classed with habit or memory; and is a phenomenon of the percipient or quasi-nervous element in plants. Precisely the same may be said of the artificial rhythm above described.

NOTE ON THE POSITION OF MAXIMUM
HELIOTROPIC STIMULATION.

Czapek has shown that if an apogeotropic shoot is placed at an angle of 45° , the tip being directed obliquely downwards, it receives a stronger geotropic stimulus than if it points 45° above the horizon. This may be proved to be true by the use of our intermittent klinostat. An apogeotropic organ being fixed at 45° with the horizontal axis of rotation, is subjected (by the periodic rotation of the axis through 180°) to opposite and alternate stimuli¹. If the stimulus is greater when the organ points obliquely downwards, the sum of the unequal stimuli must tend to bring the organ into line with the horizontal axis of rotation. And this was found to be the case by one of us², who made the experiment with apogeotropic grass-haulms. We have now been able to show that the same rule applies to heliotropism.

Our experiments on heliotropism were made with *Phalaris* on a klinostat rotating on a horizontal axis through 180° at intervals of half an hour. The plants were fixed³ so as to make an angle of 45° with the axis of rotation, and also, therefore, 45° with the horizontal light. It follows that the plants were alternately pointing obliquely from, and obliquely towards the light.

The following were the results :—

June 7, 1900 ($\frac{1}{2}$ -hr. klinostat). Seven *Phalaris* seedlings: after 2 hours all had become slightly more parallel to the axis of rotation.

June 11, 1900. Six seedlings: after 2 hours four were more parallel, two unchanged.

June 12, 1900. Five seedlings: after $2\frac{1}{2}$ hours all more parallel.

¹ This is the method used by Czapek; see Sitzber. K. Akad. Wien, civ, 1, p. 1216.

² D. F. M. Pertz, in the *Annals of Botany*, xiii. p. 620.

³ The plants retained a roughly horizontal position under the influence of the alternating geotropic stimuli.

June 13, 1900 ($\frac{1}{4}$ -hour klinostat). Five seedlings: after $2\frac{1}{2}$ hours all more parallel.

Thus twenty-one out of twenty-three *Phalaris* seedlings, after from 2 to $2\frac{1}{2}$ hours, approached the axis of rotation. This can only mean that they were more strongly stimulated when pointing obliquely away from the light than when pointing obliquely towards it.

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