

Botany. — *The growth of the petioles of waterplants in solutions of phytohormones.* (From the Botanical Institute, Government University, Leyden.) II. By G. L. FUNKE. (Communicated by Prof. L. G. M. BAAS BECKING.)

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V. Cell elongation—cell division.

I have measured the length of the cells of epidermis and subepidermal layer in more than 150 petioles and in each at 5 places: above, at $\frac{1}{4}$ from the top, in the middle, at $\frac{3}{4}$ from the top and below. They confirm completely the outcome of the thousands of measurements made in foregoing years, viz. that in the vast majority of cases the definite length is reached partly by cell elongation and partly by the formation of new cells. It is impossible to say more or less exactly in how far each of them accounts for the rapid increase in length of the petiole; now one may take the upperhand, now the other; sometimes elongation or division is more important in the whole extension of the petiole, sometimes only in one or some parts of it. Many factors probably influence both processes of growth, many of which, esp. the internal ones, escape thus far our control (age, respiration, assimilation, food storage, etc.). There is hardly any phenomenon in plant life so capricious as the cell dimensions. Yet there is one rule which holds true fairly generally, viz. that cell elongation is the principle means of reaching the definite length whenever a leaf is in unfavorable conditions, internal or external. Old age, sickness, poisonous solutions, etc. are invariably followed by strong cell elongation, and reversely, whenever I find abnormally long cells I feel intitled to conclude from that fact that the petiole in question has somehow suffered from injury (comp. 2). A striking example is shown by *Limnanthemum nymphaeoides* (9); concentrations 0.25 mg/L and 5 mg/L alpha-naphtalene acetic acid are resp. sub- and super-optimal for the longitudinal growth of its petioles; although the lengths reached in these concentrations are about the same, the cell lengths in 5 mg/L are abnormal for this species and ± 2 à 3 times those in 0.25; externally no difference is to be seen, but all measurements show that the growth had been achieved in different circumstances and therefore in totally different ways.

A selection of my measurements may illustrate sufficiently what has been said above.

Table 6a gives the data of a few specimens of *N. amazonum*. It happens that with the progression of the season the petioles show longer cells; therefore control specimens have been measured at different periods in summer. I would point out the following facts: No. 2 in 0.25 mg/L naphtalene acetic acid, although the longest petiole of Nos. 2—5, has by far the shortest cells; the concentrations 0.5 and 1 mg/L are obviously noxious. No. 4 has much longer cells than No. 3, although it is shorter; this is an example of the capriciousness which one meets so often and for which there is not always a ready explanation. No. 7, three times longer than No. 6, has cells of the same order of length; Nos. 8 and 9 have about twice the length of No. 7; if we only compared these three, we would be inclined to assume cell elongation as the only means of reaching the greater length; this assumption, however, has to be abandoned when we compare Nos. 8 and 9 with No. 6. No. 11 shows long cells over the whole extension, but No. 10, of the same age, is only $\pm \frac{1}{2}$ its length and this relation is nowhere to be found in the cell dimensions. No. 14 is much shorter than No. 13; it is a striking example of a petiole which has done its utmost without succeeding in reaching the surface of the water; this exertion is consequently clearly expressed by its abnormally long cells.

Table 6b, *N. Boucheana*, No. 3 was a rather old leaf which was visibly injured by the 0.5 mg/L solution; it was strongly twisted and broke off while being measured some days

TABLE 6a. Celllengths of *Nymphaea amazonum*.

No.	Particulars (conc. of horm. mg/L; depth water; date)	Length pet. in cm	Dimensions leaf blade in mm (middle vein — greatest breadth)	Average length of cells in micra									
				Epidermis					Subepidermis				
				a	$\frac{1}{4}$	m	$\frac{3}{4}$	b	a	$\frac{1}{4}$	m	$\frac{3}{4}$	b
1	Control 20 cm 4/VI	46	73— 65	100	135	105	80	60	135	175	175	130	115
2	Napht. $\frac{1}{4}$ 20 cm 23/VI	170	41— 52	80	170	165	170	220	95	225	225	335	295
3	Napht. $\frac{1}{4}$ 20 cm 4/VI	140	81— 96	185	270	220	255	320	195	290	265	360	370
4	Napht. $\frac{1}{4}$ 20 cm 4/VI	124	94—102	330	465	375 ¹⁾	320	350	250	455	505	590	485
5	Napht. 1 20 cm 19/VI	139	70— 83	335 ¹⁾	580	380	305	290	315	505	460	485	455
6	Control Vict. Basin 30/VII	51	115—158	50	80	100	95	90	75	120	180	215	150
7	Control 200 cm 1/VIII	152	104—175	40	65	110	130	115	70	140	150	190	185
8	Napht. $\frac{1}{4}$ 150 cm 2/VII	310	158—230	100	115	195	385 ¹⁾	200	145	165	285	445	305
9	Napht. $\frac{1}{8}$ 150 cm 30/VII	300	115—180	70	160	215	275	290	110	225	315	375	470
10	Control Vict. Basin 19/IX	42	48— 71	65	150	120	110	60	135	305	240	210	145
11	Napht. $\frac{1}{4}$ 40 cm 17/IX	370	50— 74	225	435	520	560	315	175	330	360	435	430
12	Contr.: seedling 5 cm 25/VIII	8	22— 29	85	90	105	90	145	85	105	135	120	140
13	Napht. $\frac{1}{4}$: seedl. 200 cm 16/IX	137	22— 28	105	270	370	365	225	110	275	310	295	340
14	Napht. $\frac{1}{4}$: seedl. 200 cm 16/IX	90	23— 29	135	385	460	460	380	190	370	355	525	350

TABLE 6b. *Nymphaea Boucheana*.

1	Control 20 cm 3/VI	29	25— 25	80	195	175	205	190	105	210	185	210	225
2	Napht. $\frac{1}{2}$ 20 cm 4/VI	140	27— 30	230	365	290	330	230	240	320	330	340	290
3	Napht. $\frac{1}{4}$ 20 cm 28/V	77	41— 48	210	590	465	460	450	240	520	500	585	370

TABLE 6c. *Nymphaea Devoniensis*.

1	Control Vict. Basin 29/VIII-41	42		65	110	90	65	100	80	170	130	100	140
2	Control Ghent 1939	50		27	110	150	135	135	92	110	210	160	195
3	Napht. 3 Ghent 1939	53		134	380	385	350	230	200	315	405	325	370
4	Napht. $\frac{1}{4}$ 40 cm 18/IX-41	213		180	170	220	270	235	175	170	210	245	265

TABLE 6d. *Nymphaea zanzibariensis rosea*.

1	Contr.: seedling 10 cm 29/VIII	19	28— 36	110	190	150	90	80	130	250	210	135	105
2	Napht. $\frac{1}{4}$: seedl. 200 cm 16/IX	226	27— 36	135	270	330	310	200	130	210	320	435	260

TABLE 6e. *Nymphaea Lotus lilacina*.

1	Contr.: seedling 5 cm 29/VIII	10	36— 38	50	55	70	60	62	65	70	95	85	105
2	Contr.: seedling 200 cm 16/IX	60	36— 39	250 ²⁾	395	720	665	365	230 ³⁾	415	565	620	490
3	Napht. $\frac{1}{4}$: seedl. 200 cm 16/IX	199	35— 40	150	430	365	460	230	170	390	390	460	310

a means: above; $\frac{1}{4}$: at $\frac{1}{4}$ from the top; m: in the middle; $\frac{3}{4}$: at $\frac{3}{4}$ from the top; b: below.
 1): many newly divided cells; 2): at $\frac{1}{8}$ from the top 435; 3): at $\frac{1}{8}$ from the top 315.

before the end of the experiment; its unfavorable condition is neatly revealed by the cell dimensions.

Table 6c, *N. Devoniensis*. In 3 mg/L naphthalene acetic acid growth was equal to that in the control, but the cell dimensions are a warning against the erroneous conclusion that this hormone would have no influence on the longitudinal growth.

Table 6d, *N. zanzibariensis rosea*. The data speak for themselves.

Table 6e, *N. Lotus lilacina*. No. 2 is one of the very rare examples in which cell elongation may be considered as the only means of reaching the definite length of the petiole; indeed it is a specimen which has been in the same unfavorable conditions as No. 14 of *amazonum* and evidently still worse; that in a normally grown petiole, cell division plays its part, is once more neatly demonstrated by No. 3.

These examples can be multiplied; my data are at the disposal of anyone interested.

Two striking facts appear from these measurements. The dimensions of the leafblades (length of middle vein and greatest breadth) indicate that no relation exists between the assimilating surface and the longitudinal growth of the petioles. The cell divisions must take place at a very rapid rate, otherwise the fact cannot be explained that so very few indications of it were visible, esp. in the longest petioles.

VI. Discussion.

Has the greatest length, which a petiole of *Nymphaea* can reach, been attained by the several treatments? This question must certainly be answered in the negative. Several species of *Nymphaea* are grown in the basin of *Victoria regia* where they get rich food and high temperature. They show a luxuriant development and notwithstanding the shallowness of the water, ± 20 cm, they form petioles of considerable length. I measured: *N. Lotus lilacina* up to 120 cm, *N. Lotus var.* up to 180 cm, *N. capensis* up to 130 cm, *N. hybrida var. hort. Oberg. Graebner* up to 230 cm. These petioles are much thicker than those grown in my experiments; they take several weeks to reach their full length and therefore, as I expected, their cell dimensions indicate a high degree of division (comp. my observations on *Victoria regia*, Nos 1, 2); the blades are large (middle vein up to 240 mm, greatest breadth up to 460 mm). If we could transport these plants in basins of, let us say, 400 or 500 cm deep, I feel convinced that they could easily outgrow them and more than that. Theoretically we could force the petioles to elongate indefinitely by growing them in shallow basins, but at the same time by preventing the blades from reaching the surface. I have tried this with *Victoria regia* (2); the petioles reached indeed a greater length, but they soon showed signs of decaying and their abnormally long cells were another indication that this treatment is injurious; yet it might be worth while to try it again with the more robust petioles of *Nymphaeaceae*.

Adding alpha-naphthalene acetic acid to the *Victoria* basin would be another way of testing the growth potentialities and a very tempting one; the quantity of hormone needed would not be enormous, 2 à 2½ gr for a concentration of ½ mg/L; it would be interesting to observe at the same time the reactions of other waterplants, of *Victoria* itself in the first place; but just because I was confident of a striking result, the consequences deterred me.

It must be emphasized, however, that my plant material in the controls, though vigorous and healthy, was not grown in such optimal conditions and therefore never reached dimensions exceeding those mentioned in the tables. The tremendous growth, as well the lengths reached as its amazingly rapid pace, were entirely due to the height of the water column and (or) to the hormones.

Of the three substances used, alpha-naphthalene acetic acid has by far the greatest activity in furthering the longitudinal growth of the petioles of *Nymphaeaceae*. This holds true for quite a number of other phenomena in plant life, e.g. the rooting response of cuttings (VAN DER LEK and KRIJTHE, 12, RAPPAPORT, 14), the parthenocarpy of fruits (HUBERT and MATON, 10, 11; see also 6), the inhibition of buds in seedlings

(MATON, 13). The sensitiveness of *Nymphaeaceae* is such that I feel intitled to propose a *Nymphaea*-test besides the *Avena*- and *Pisum*-tests. THIMANN and SCHNEIDER (15) give the concentrations in mg/L of alpha-naphtalene acetic acid giving minimal and maximal responses of straight growth of *Pisum*; when we compare these with mine for *Nymphaea* (table 7) we see that the latter is by far more sensitive; and we should not

TABLE 7. Concentrations in mg/L of alpha-naphtalene acetic acid giving minimal and maximal responses of straight growth.

	Minimum	Maximum
<i>Pisum</i> (THIMANN and SCHNEIDER)	0.05	4
<i>Nymphaea</i>	0.016	0.25

forget that the lowest concentration of 0.016 is probably still too high and that, while a maximum response in *Pisum* means an elongation of 30 à 50 %, in *Nymphaea* it may mean one of some hundreds percent. THIMANN and SCHNEIDER rightly point out the dangers of comparing testmethods as the relative activities of the auxins vary widely with the test plants. Notwithstanding this I think that these data indicate all the same that *Nymphaeaceae* may be considered as very sensitive plants worthy of further examination. On the other hand, we should never forget that they present serious practical difficulties: they can only be grown in summer; their size is such that we are restricted to a small number of specimens which are far from being uniform and whose leaves give only partly the right response; growing them under the carefully controlled conditions as are usual with *Pisum* and *Avena* is excluded; seedlings unfortunately appear to be less suitable. Considering all this I am fully aware that my proposal of establishing a *Nymphaea*-test can have hardly any practical value; but all the same circumstances may occur in which future investigators can consider its possibilities.

It is noticeable that the growth of the leafblades is not influenced at all by the hormones; in higher concentrations they show hypo- or epinastic curvature, but in the concentrations optimal for the growth of the petioles, they do not react at all. This is still more striking when we consider the flowerstalks; these do not elongate, neither as response to deepening of the waterlayer, nor to the addition of hormones; they were sometimes somewhat coiled and this was the only indication of their being "aware of" the presence of auxins.

VII. Summary.

1. The growth potentialities of the petioles of *Nymphaeaceae* and some other plants are feeble in the early seedling stage; they increase gradually and strongly and decrease only when the plants are getting old.

2. Each leaf shows individually a parallel behaviour and consequently it only gives the utmost responses to external growth-promoting factors when it is at the suitable age.

3. The two main factors inducing a sudden vigorous renewal of growth of the adult petioles are: (1) deepening of the waterlayer; (2) adding of growth substances to the water. (1) is especially efficient when the plants are young; (2) becomes gradually equally important until later on it takes the upper hand and finally can be the only means of attaining the extreme dimensions.

4. At the suitable age both factors combined or the latter alone result in causing amazing achievements of growth, up to 130 and 160 cm in 48 hours, provided the temperature is between 25° and 30°; when it is between 20° and 25° the same result can be reached but it takes more time.

5. Of the three hormones used, heteroauxin has the least influence, beta-indole butyric acid more and by far the most active is alpha-naphtalene acetic acid.

6. The optimal concentration of alpha-naphtalene acetic acid is about 0.25 mg/L

($0.25 \times 10^{-7} = \pm 7 \times 10^{-5}$ mol); in lower concentrations, even in $\frac{1}{10}$ mg/L ($3.1 \times 10^{-8} = \pm 6 \times 10^{-6}$ mol) individual petioles may give responses which are nearly as strong as the average one in 0.25 mg/L. Owing to the imposed scarcity of the plant material it can be ascertained only with some restriction that there exists a relation between the concentrations $\frac{1}{10}$, $\frac{1}{20}$, $\frac{1}{40}$, $\frac{1}{80}$ and $\frac{1}{160}$ mg/L and growth; higher concentrations are becoming gradually more noxious.

7. The sensitivity of the petioles of *Nymphaeaceae* appear to be 10 à 16 times greater than that of *Pisum*; there are, however, practical objections against establishing a *Nymphaea*-test.

8. The longitudinal growth, even the extremely rapid one, is achieved by cell elongation as well by cell division; the first one takes the upper hand whenever a leaf is in unfavorable conditions, but elongation is never the only means of reaching the definite length; this proves especially to be the case in solutions of more than 0.25 mg/L.

9. The leaf blades are not affected by the hormones in low concentrations, nor are, curiously enough, the flower stalks.

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