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

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I. *Introduction.*

My experiments on waterplants have shown that the addition of phytohormones to the water has, on the whole, little effect on the acceleration of growth and provokes abnormal phenomena as long as the temperature is below 20° (5,6). At higher temperatures, on the other hand, their influence appears to be enormous (7,8). Up to now I have mainly used a concentration of 3 mg/L, because this was one of the optimal concentrations found by VEGIS (16) in his experiments with heteroauxin on the premature sprouting of the turiones of *Stratiotes aloides*; no other authors, so far as I know, have ever applied growth substances on waterplants. This year I studied the effect of lower concentrations of different kinds of phytohormones on the petioles of several species of *Nymphaeaceae* and other plants.

Part of the plant material was provided by the courtesy of the Zoological Gardens at The Hague and the Botanical Gardens of the University of Amsterdam. Experiments were made with seedlings and with specimens grown from tubers. Four basins were used in the Victoria hothouse of the Botanical Gardens at Leyden, especially built for the observation of growth in deeper waterlayers; two of them have a depth of 150 cm, two of 200 cm; during hot summerdays the temperature of the water varied between 26° and 31° at the surface, between 24° and 28° at the bottom; during the rainy period in August it sank gradually to 23°—20°. Other experiments were made in small aquariums of 20 cm deep; these were placed on radiators in a tropical hothouse; their temperature varied between 24° and 29°. Towards the end of the summer I could make some experiments in an aquarium of 40 cm deep which was electrically heated at a constant temperature of 28°. Only three kinds of hormones have been applied, viz. heteroauxin, beta-indolebutyric acid and alpha-naphtalene acetic acid, beta-indole propionic acid and beta-naphtalene acetic acid having been shown to have only a slight influence or no influence at all (6).

II. *The influence of heteroauxin, beta-indole butyric acid and alpha-naphtalene acetic acid.*

Four equally developed young specimens of *Nymphaea amazonum*, grown from tubers, were planted in aquariums of 20 cm deep. Part of the leaves were full grown, which means that their blades floated on the surface and that their petioles elongated no more. A few days later the hormones were added in a dose of 0.5 mg/L; one aquarium served as control. The petioles were measured every day; they showed facts which have appeared invariably in all my former experiments. The older leaves reacted very feebly or not at all; the very young ones needed some days before responding to the addition of the hormones; the fresh adult ones, ranging between, did not react equally well, apparently owing to a difference of their respective ages, although their appearance was very much alike. Table 1, therefore, gives only a selection of the measurements which illustrate best the action of the hormones.

Part of the control leaves showed a renewal of growth towards the end of the experiment, but their elongation, nevertheless, stayed very much behind that in the solutions of growth substances. As the depth of the aquaria is only 20 cm, the result was that the greater part of the petioles floated on the surface in large coils which intertwined.

We see that alpha-naphtalene acetic acid is by far the most active hormone and heteroauxin the least. In naphtalene acetic acid the older leaves were strongly twisted and fragile and often broke in 2 or 3 pieces when they were measured; this seems to indicate that the concentration 0.5 mg/L is more or less noxious; we will see further on that the cell measurements confirm this view, cell elongation taking the upperhand of cell division.

TABLE 1. Petioles of Nymphaea amazonum in solutions of different hormones (petioles in cm; depth of watercolumn 20 cm).

Hormones	May							June		
	24	26	27	28	29	30	31	2	3	4
Control	23	24	24	24	25	25	25	25	25	25
	13	20	22	23	25	30	35	42	45	46
Heteroauxin	25	66	70	71	76	78	81	82	84	85
0.5 mg/L	18	47	50	54	56	60	63	66	67	67
β-ind. butyr. acid	24	63	77	84	89	92	93	100	101	101
0.5 mg/L	20	58	77	85	91	99	103	105	110	110
α-napht. acet. acid	21	73	100	114	121	129	132	134	137	138
0.5 mg/L	8	29	41	59	85	108	117	136	142	149

This experiment has been repeated with identical results; it has been made also with the species *N. Boucheana* and *N. jubilé lilacina.* In every case we see that the action of alpha-naphtalene acetic acid is most efficient and also slightly noxious, witness the twisting of the petioles, that of heteroauxin least, while that of beta-indole butyric acid is intermediate. The definite lengths were always reached in 10 à 14 days.

Experiments with seedlings of *N. Obergaertner, N. Devoniensis, N. zanzibariensis rosea, N. jubilé lilacina* and *N. stellata* were made under the same conditions. As the plants were very young the concentration used was only 0.25 mg/L and even this dose proved to be rather too much; nevertheless, all these species clearly demonstrated the same succession in effect of the hormones in furthering growth. The same holds true for *Victoria regia* (first floating leaves) and petioles of *Ranunculus sceleratus.* Only one species showed about the same rate of growth in everyone of the three hormones, viz. *Limnanthemum nymphaeoides,* although also here it appeared that the higher concentrations of alpha-naphtalene acetic acid were more noxious than those of heteroauxin and beta-indole butyric acid. A detailed account of the experiments with the latter species has been given elsewhere (9). Full data of the measurements of all species investigated are at the disposal of everyone interested; as they only confirm those given in table 1, it is needless to give them here.

III. *The influence of different concentrations of alpha-naphtalene acetic acid.*

As it has been stated, that of the three hormones used, alpha-naphtalene acetic acid has by far the strongest furthering effect on the growth of the petioles of many waterplants, this substance was consequently applied in various concentrations.

Four young specimens of *Nymphaea amazonum* were planted in the same way as described above and the following doses were added: 0, $\frac{1}{8}$, $\frac{1}{4}$ and 1 mg/L. Table 2 gives an idea of the rates of growth.

We see that the utmost lengths reached in the solutions $\frac{1}{4}$, $\frac{1}{2}$ (table 1) and 1 mg/L do not vary noticeably; yet it may not be said that the plant is insensitive to the difference in concentrations. As has been said above, 0.5 mg/L is already slightly noxious which is indicated by the fragility and the twisting of the petioles and their very long cells; these phenomena all occurred in the 1 mg/L solution also and to a higher degree; the greater part of the petioles did not reach the ultimate length because they broke long before the end of the experiment while being measured. Therefore I consider the concentration

TABLE 2. Petioles of Nymphaea amazonum in different concentrations of α-naphtalene acetic acid (petioles in cm; depth of watercolumn 20 cm).

Concentrations	June										
	4	5	6	7	9	10	11	12	16	19	23
Control	16	20	22	24	25	27	28	28	40	44	44
	2	4	6	9	17	20	21	21	25	25	36
$\frac{1}{8}$ mg/L	22	34	53	71	86	97	100	103	104	109	109
	8	18	29	41	71	83	94	97	104	111	111
$\frac{1}{4}$ mg/L	20	36	60	79	112	113	120	127	134	134	134
	1	1	5	9	20	46	63	76	146	163	170
1 mg/L	19	32	58	94	116	130	133	134	136	139	139
	13	21	43	68	124	138	150	159	165	166	166

0.25 mg/L as the optimal one. Experiments with higher concentrations could not be made in a sufficient number for two reasons: (1) the plant material was restricted; this holds true for the specimens grown from tubers, (which will be the difficulty in every botanic garden); seedlings were available in a larger number but we will see further on that they are less suitable for this sort of experiment; (2) in the course of the summer the plants became too tall to be planted in small aquaria of 20 cm depth; the experiments had to be continued in the large basins in the Victoria hothouse, but as these have a capacity of 1500 L each, it will be easily understood that concentrations of more than 1 mg/L could hardly be applied.

The few experiments, however, which I could make, and those of former years, all confirm the impression that concentrations higher than 1 mg/L of all three growth-substances are still more noxious and check the longitudinal growth. One of these may be briefly described. *N. jubilé lilacina* is a species whose responses to phytohormones are in all respects very similar to those of *N. amazonum*; four young specimens were planted in aquariums of 20 cm deep and 3 mg/L of the three hormones were added. Growth in heteroauxin and beta-indole butyric acid was only a little more rapid than in the control, but in alpha-naphtalene acetic acid it was nearly equal to that. All sorts of abnormal phenomena, observed during my earlier investigations with this concentration, occurred again and most distinctly in alpha-naphtalene acetic acid: strong twisting of the petioles, stiff hyponastic curling of the blades which, moreover, remained very small. After 7 days the plants were washed and removed into pure water; they recovered within 48 hours, and most of them even within 24 h.; the twisting of the petioles, the curling-up of the leafblades disappeared and the blades grew out to normal dimensions; the petioles did not elongate any more (except of course the very young ones). Seven days later the hormones were again added, but this time only in a dose of 0.5 mg/L. The same leaves, which had reacted so unfavourably to the concentrations 3 mg/L, started growth anew (except in the control aquarium) and at the same pace as has been observed in all similar experiments. After 10 days they had reached lengths of 28 à 32 cm in control, 65 à 70 cm in heteroauxin, 70 à 80 cm in beta-indole butyric acid and 130 à 146 cm in alpha-naphtalene acetic acid. Therefore we may conclude that the hormones are distinctly noxious in a concentration of 3 mg/L and especially so alpha-naphtalene acetic acid which has obviously a lower optimal concentration than the other two. My experiments of 1938 (6) and 1939 (7) confirm this view (with the restriction of *N. Boucheana*, which form is apparently less sensitive to higher concentrations).

The experiments in the deep basins took place as follows: young plants, which had developed in a shallow layer of water, were planted in pots and these were hung in the basins at a depth somewhat greater than the length of the petioles; next day the leafblades had reached the surface and the pots were lowered 20 à 30 cm; this was repeated every day and each time part of the leafblades (not the oldest ones) appeared at the surface well within 24 hours, so that at the end of 4 to 6 days the pots had reached the bottom of the basins and even then growth did not stop but continued for at least some decimeters. The leaves made a very healthy impression and nothing indicated that they were at the end of their achievement; (the pots having a height of 20 cm, the real waterlayers were resp. 130 and 180 cm). The very youngest leaves, which only begin to develop after the immersion is completed, do not, as a rule, reach the surface but if they do, with "visible exertion" and after a considerable time.

Adult petioles of ± 30 cm thus elongate up to ± 200 cm in about one week. This is in itself a remarkable performance which, however, I rather expected after what I have observed in former years. When nothing further is done to them the leaves do not elongate any more, their "excess growth" (by which I mean the part of the petiole above the surface) is restricted to 30 à 40 cm. When, however, 0.25 mg/L alpha-naphtalene acetic acid is added, growth starts anew and during 8 à 12 days elongation continues at a very rapid pace. The petioles reach lengths of more than 300 cm, which means that their greater parts are meandering on the surface, intertwining with each other. A few examples out of many others may give an impression of the rate of growth in the deep basins after the surface has been reached.

TABLE 3. Excess growth of petioles of Nymphaea amazonum in watercolumns of 150 and 200 cm (petioles in cm).

	No.	June											
		16	17	18	19	20	21	23	24	25	26	27	28
Control													
Basin 150 cm	1	142	143	150	156	156	157	160	162	163	163	164	164
Basin 200 cm	2	192	193	200	206	206	207	210	212	213	213	213	213
α-napht. ac. acid													
0.25 mg/L	1	140	167	175	195	207	217	231	236	236	236		
Basin 150 cm	2	132	150	185	203	230	238	257	262	270		270	
	3				100	160	218	270	280	288	290	296	296
	4					58	94	224	263	290	310	310	310
	5										108	160	202

Leaves No. 1 and 2 in the solution of naphtalene acetic acid are obviously rather old ones which are no more capable of extreme responses to the hormone, although an elongation from 30 to resp. 140 and 132 cm as a result of the deepening of the watercolumn, followed by the growth since June 16th as indicated in the table, remains remarkable all the same. Nos. 3, 4 and 5, however, were younger ones which were observed just at the moment of their utmost capacity of reaction. The strongest elongation in 24 h equals and even surpasses the record of growth rate which I observed in 1940 (7) in a 3 mg/L solution of beta-indole butyric acid with *N. Boucheana* and other species. No. 4 has grown 130 cm from Saturday 21/6 till Monday 23/6; I estimate that in 24 h the increment has been resp. ± 70 and ± 60 cm; we will see farther on that even this record can be beaten.

This experiment was repeated several times with *N. amazonum* and once with *N. jubilé lilacina, Boucheana* and *Lotus rosea*; it always yielded identical results. During August and September the weather was unfavorable and the temperature of the water sank to 23° and even to 20°; this retarded the growth, but all the same the ultimate lengths reached were about 300 cm. Daily growth of 60 cm and more did not occur very often, but 40—50 cm per 24 h were quite usual.

I applied several concentrations of alpha-naphtalene acetic acid in these basins also, viz. $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$ mg/L. There being only four deep basins, the experiment had to be made in two successive parts. The pots were not lowered deeper than 150 cm. Table 4 gives the data of the full grown petioles; those which could not reach the surface were neglected.

TABLE 4. Ultimate lengths of petioles of Nymphaea amazonum in a watercolumn of 150 cm and in different concentrations of α-naphtalene acetic acid.

	Length of petioles in cm							Average	Average of excess growth
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7		
Control	202	183	178	152	152	140		168	**38**
α-napht. ac. acid $\frac{1}{64}$ mg/L	186	179	179	178	166	149		173	**43**
α-napht. ac. acid $\frac{1}{32}$ mg/L	255	240	196	184	162	146		197	**67**
α-napht. ac. acid $\frac{1}{16}$ mg/L	233	225	162	159	153			186	**56**
α-napht. ac. acid $\frac{1}{8}$ mg/L	300	235	219	170	152	151		205	**75**
α-napht. ac. acid $\frac{1}{4}$ mg/L	310	295	283	275	267	250	230	273	**143**

The specimen in the $\frac{1}{16}$ concentration was a less prosperous one than the others, hence its rather poor development. The number of the petioles is by far too small to take an average from them; it will e.g. be seen that in the controlbasin one grew to a length which is not reached in the $\frac{1}{64}$ solution. Given due consideration to all this it is surprising that the averages nevertheless indicate to some degree a relation between growth and concentration of the hormone. These results seem to confirm my opinion that 0.25 mg/L alpha-naphtalene acetic acid (2.5×10^{-7} or $\pm\ 7 \times 10^{-5}$ mol) is optimal for exerting the utmost of growth capacity in the petioles of *N. amazonum* and probably of many more species of *Nymphaeaceae*. It should be remembered, however, that in $\frac{1}{8}$ and even in $\frac{1}{32}$ mg/L (3.1×10^{-8} or $\pm\ 6 \times 10^{-6}$ mol) individual petioles can show the same rate of elongation.

A similar experiment has been made with *N. Boucheana*; as, however, my material of this species was scanty, I had to be content to test it in three concentrations only, viz. $\frac{1}{32}$, $\frac{1}{16}$ and $\frac{1}{8}$ mg/L. The average excess growth in the control was 36 cm, in $\frac{1}{32}$ mg/L 57 cm, in $\frac{1}{16}$ mg/L 70 cm and in $\frac{1}{8}$ mg/L 109 cm. Also in this case there seems to exist a relation between growth and concentration of naphtalene acetic acid. These results, added to those obtained in the small aquaria indicate that for this species too the optimal concentration must be about 0.25 mg/L.

N. amazonum and *N. Lotus rosea* have been tested in the deep basins in two concentrations of beta-indole butyric acid, viz. $\frac{1}{32}$ and $\frac{1}{4}$ mg/L; their excess growth was equal to that in the control. This confirms my former experiments (and those of 1940); for optimal growth achievements in this substance higher concentrations are needed.

IV. *The two main factors inducing rapid elongation.*

There are two methods to induce a sudden vigorous renewal of growth of adult petioles of waterplants: (1) transplanting in a deep waterlayer and (2) adding of phytohormones.

In the last described experiments these methods have been applied in succession and each of them has shown its action very neatly; (immersion in a deep waterlayer in which growth substance had already been dissolved gives the same results; adding of growth substance first and deepening of the waterlayer afterwards has not yet been tried.)

The question which of these methods has the strongest influence is not simple to answer, because it depends on many factors of which the most important is the condition of the plant material. Seedlings seemed to be preferable to specimens grown from tubers because they present a more homogeneous material. It appeared, however, that in their very juvenile stage they are not suitable for experiments; they hardly responded to a deepening of the waterlayer and even very low concentrations of phytohormones were apt to injure them. Only towards the end of July (they were sown in April) they were sufficiently advanced to give reliable results; in most cases this stage is not characterised by morphological changes but must depend on metabolical processes. The plants remained throughout the summer in shallow dishes in a few cm of water and developed 5 or 6 leaves per specimen with petioles of not much more than 10 cm. As soon as they appeared sufficiently matured a number of experiments were made with them; they were planted in an aquarium at depths of 8, 18, 28 and 38 cm, at several levels in the deep basins, in pure water, and in various concentrations of growth substances. They showed that, although elongation was sometimes considerable, yet their growth capacity had by far not reached the amazing potentialities which are to be seen in older material. In the deep basins only those specimens were able to reach the surface which had already formed small tubers. This was the case with *N. zanzibariensis rosea*; the petioles elongated in 18 days from about 20 cm to more than 200; this is quite an achievement for these slender organs and I think it most remarkable that after this the small tubers not only were still present, but that their volume had even slightly increased; one would expect quite the contrary; this opens possibilities for further research. Subsequent addition of growth substances could not induce any more growth, nor could it stimulate those petioles which had stayed behind (compare (9), *Limnanthemum nymphaeoides*).

It seems that the height of the watercolumn is the most efficient factor for inducing rapid longitudinal growth in very young plants; as they grow older hormones gradually become equally important, till later on they take the upperhand and finally can be the chief means of attaining extraordinary dimensions. This is further demonstrated by an experiment which I made towards the end of the summer in an aquarium of 40 cm deep; it lasted from August 27th until September 18th and was made with material grown from tubers which had developed throughout the summer in shallow water. It was therefore some three months older than that which I used in the aquaria of 20 cm deep; all traces of

TABLE 5. Petioles of Nymphaeaceae in 0.25 mg/L alpha-naphtalene acetic acid (petioles in cm; depth of watercolumn 40 cm).

Species	No.	September								
		3	4	5	6	8	12	16	17	18
N. amazonum	1	41	64	90	108	115	115			
	2	40	91	136	183	220	220	222	228	236
	3		19	44	92	252	350	367	370	
	4						35	212	222	222
N. stellata	1	50	56	60	77	89	95	101	105	
	2	48	73	97	105	135	142	145	145	
	3	32	61	108	125	141	172	172	178	
	4			4	9	37	130	210	210	
N. Devoniensis	1	47	58	68	100	110	116			
	2	43	68	102	118	130	151	158	160	
	3	40	89	134	165	195	203	213	213	

primary leaves had long since disappeared, the leaves were more robust with large blades and thick petioles. Three species were used, viz. *N. amazonum, N. stellata* and *N. Devoniensis.* Their longest petioles measured up to 40 à 45 cm; during one week they did not change and then, on September 3d, 0.25 mg/L alpha-naphtalene acetic acid was added. Once more I had the occasion to observe the almost explosive renewal of growth. Some data will illustrate this in table 5.

No. 1 of all three species are typical samples of leaves which, although looking quite fresh, have just passed the age on which the "top response" is possible. No. 3 of *amazonum* and No. 4 of *stellata,* on the other hand, are very young leaves which show clearly that growth is never extraordinarily rapid in the beginning, but that at a certain stage it can take an enormous jump. This is especially the case in No. 3 of *amazonum* which has beaten two records: (1) 160 cm growth in 48 hours (probably resp. ± 90 and ± 70 per day) and (2) an ultimate length of 370 cm which has not even been reached in the deep basins.

It is perhaps not superfluous to emphasize that in an experiment like this one it is indispensable to distinguish the leaves by individual characteristics in order to identify them from the nearly undisentangible mass of intertwined petioles and that utmost care is needed to undo them from it without breaking them.

(Continued.)