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

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I. *Alpha-naphtalene acetic acid in the basin of Victoria regia.*

In last year's report on experiments with waterplants (3 i) I considered the possibility of adding alpha-naphtalene acetic acid to the *Victoria* basin as a means of testing the growth potentialities of the species growing in it, of *Victoria regia* itself in the first place. During the summer of 1942 I had the opportunity of executing this experiment. The development of the different species in the basin had reached its height in the middle of August and can be briefly described as follows:

Victoria regia: the plant possessed 8 leaves, numbered 1—8 in order of decreasing stage of maturity; Nos. 1 and 2 were old and approaching their end, No. 8 was not yet fully unfolded; (Nos. 9—11 appeared during the experiment). There was one flower on the point of opening (No. 1), some buds (Nos. 2—3) and a number of maturing fruits; compare table 1.

Nymphaea capensis, N. Lotus, N. Obergaertner Graebner, N. jubilé lilacina: their development was luxuriant; a great number of leaves were joined in neatly fitting mosaics; each species had some flowers and flowerbuds; the longest petioles did not exceed 130 cm, the flowerstalks measured between 30 and 55 cm.

The hormone was added to the basin on August 17th. The measurements taken on this day must needs be accepted as control; esp. in the case of *Victoria* we may safely assume that without artificial interference the mature leaves would not have developed beyond what they show on this date. Observations of former years and those made in other botanical gardens will without doubt support this opinion.

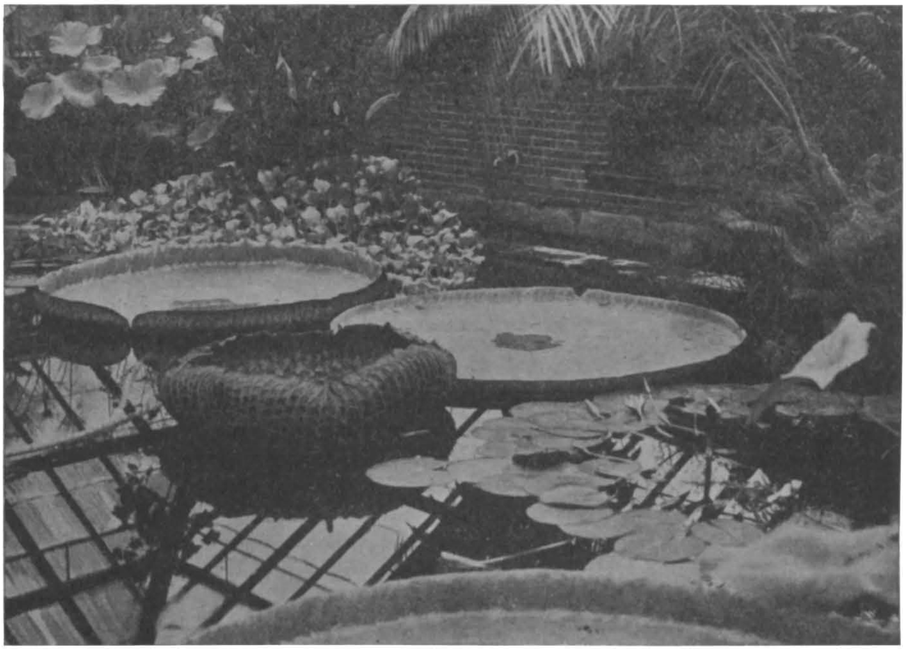
The petioles of the different species of *Nymphaea* can reach a greater length than those mentioned above (compare 3 i), when conditions are extremely favorable, but in this case too we are justified in taking for granted that a further spontaneous vigorous growth in the latter half of August is hardly to be expected.

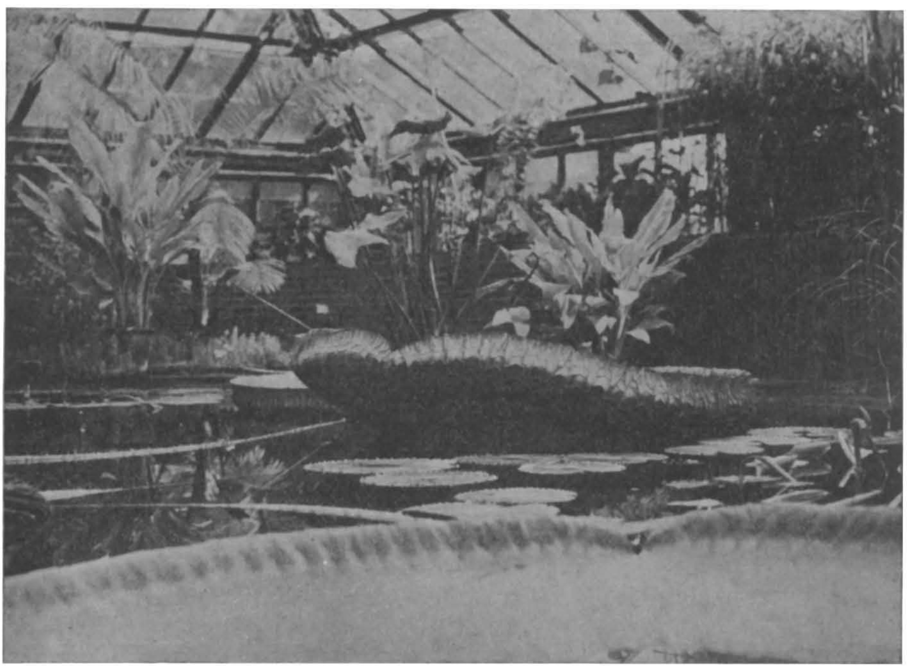
Therefore, any sudden change in the development of the plants after the addition of the hormone may be safely ascribed to the influence exerted by this substance.

The capacity of the *Victoria* basin is approximately 17.6 m^3. Last year we saw that a concentration of 0.25 mg/L of alpha-naphtalene acetic acid has the strongest influence thus far known on the growth of several species of waterplants. Therefore I added 4.4 gr of this auxin to the basin, giving due attention to a very thorough mixing. The temperature of the water was about 24° C throughout the experiment; this is not optimal, but quite sufficient for a ready response of the plants.

Reaction of *Victoria regia*: this species appeared to be extremely sensitive; 24 hours after the addition of the hormone its habitus was already abnormal, not so much because the petioles had elongated, but because they had bended towards the surface, with their upper halves emerged, thus being very conspicuous. The younger leafblades had grown hyponastically and consequently their upstanding borders were curved inwards and their horizontal parts were more or less lifted out of the water, showing the brilliant violet of the lower surface with the network of protruding veins; see figure 2. They further showed all sorts of irregular undulations, with in the centre a sunken part on which water gathered later on; the latter phenomenon occurred also in the older leaves; see figure 1.

Everyone of these peculiarities became more accentuated during the following days.





Victoria regia. Five days after the addition of 0.25 mg/L alpha-naphtalene acetic acid; 22 August 1942.

Fig. 1. Leaf No. 5, adult; left background;
leaf No. 1, old; right background;
leaf No. 8, young; foreground;
on the right: Nymphaea Lotus.

Fig. 2. Leaf No. 6, young; in the foreground part of leaf No. 2.

The photographs show some of the leaves 5 days after the addition of the hormone, viz the young ones Nos. 6 and 8, together with two older ones, Nos. 1 and 5, whose blades hardly responded to it. Table 1 gives an impression of the elongation of the petioles. A length of 290 cm may be considered as the extreme which can be reached in ordinary circumstances; the excess growth caused by the auxin, therefore, amounts to between 100 and 200 cm. The leafblades were pushed against the border of the basin, but the petioles, nevertheless, had not enough space to remain straight and formed large meanders, all the more conspicuous because they floated on the surface.

TABLE 1. Growth of Victoria regia in 0.25 mg/L α-naphtalene acetic acid.

Petioles in order of their age	August							September	
	17	18	20	22	25	28	31	10	16
1	290	290	340	355	370				
2	285	300	365	390	420	420	430		
3	260	262	340	350	410	415	425	435	
4	245	260	350	355	415	435	460	465	
5	205	230	310	345	390	400	420	430	
6	185	200	285	330	375	440	470	480	482
7	125	180	275	330	355	370	400	400	420
8	55	105	190	250	295	320	385	390	390
9		50	135	180	255	295	295	340	
10			50	90	152	260	340	380	380
11						60	125	300	
Flower stalks									
1	40	80	130	160	165	165	172	180	190
2		30	105	150	172	174	190	200	215
3			30	85	105	170	170	170	

The development of *Victoria* reaches its height during August, but new leaves and flowers are continually formed till the month of October and even November, though their dimensions become gradually smaller. The auxin shortened considerably this vegetation period; it practically came to a stop towards the end of September with everything characteristic of the approaching death: tiny flowerbuds and small leafblades which unfold only partially.

The phenomena which have been recorded here, parrallel those which have so often been observed in various species of *Nymphaea*: excessive and rapid elongation of the petioles, hyponastic curving of the blades, shortening of the vegetation period. As *Victoria* is a very large plant, these responses were especially striking, although they are really astonishing enough in the genus *Nymphaea* itself, and in some respects even more so. So far, however, there is nothing fundamentally new, but for the noteworthy fact that the concentration 0.25 mg/L is sufficient to produce these reactions so distinctly in the bulky organs of *Victoria*. I am even inclined to consider this dose as too high, witness the hyponasty of the leaves which in *Nymphaea* hardly ever occurs in the concentrations optimal for the growth, but regularly in the higher ones.

In former years I have stated that flowerstalks of *Nymphaea* do not elongate, neither as response to deepening of the waterlayer, nor to the addition of hormones. It was therefore a surprise to see how rapidly and easily the flowerstalks of *Victoria* reacted to the alpha-naphtalene acetic acid in this experiment. Table 1 gives an impression of the elongation of three of them; these were the youngest ones; those with ripening seeds stretched to between 130 and 150 cm; without interference they would not have surpassed ± 50 cm; therefore, their response to the auxin is relatively much stronger than that of the petioles. It will be understood that these long stalks were not rigid as normal ones, but that they floated on the surface of the water.

Reaction of *Nymphaea*: the four species growing in the *Victoria* basin responded much in the same way to the auxin and gave similar surprises. With a view to my former experiments I had expected that the petioles would elongate considerably; this was far from being the case. The longest ones, those whose blades are at the periphery of the leaf mosaics, practically did not grow at all; only the inner petioles stretched to lengths between 110 and 130 cm, that is to the same as the longest ones measured from the beginning, with the consequence that the mosaics "opened" to a broad circle; an example of this is to be seen on the right in figure 1.

The average length of the petioles, therefore, became much greater owing to the auxin, but the amazingly rapid growth which I observed in 1941 in a solution of the same concentration did not occur at all. This inconsistency of results may most probably be ascribed to the difference of age of my plant material. Last year I worked with young specimens, this summer with fully grown ones which either have lost their reaction capacity for the greater part or (and) are only sensitive to higher doses of the hormone.

Another surprise, this time a very positive one, was presented by the flowerstalks. Similar to those of *Victoria*, they responded to the addition of the auxin by an immediate renewal of growth and within a few days reached lengths of 100 to 125 cm, whereas the normal ones are about 50 cm and seldom as long as 60. These stalks were also too flaccid to keep themselves upright and floated on the surface of the water amidst the numerous petioles, but easily discernible by their greater thickness. One flower and one bud are visible in figure 1. Cell measurements and determinations of the dry weight gave the impression that in these organs the stretching is due to cell elongation only, and that cell division, which is so important in the petioles, hardly occurs here, if at all. I had not the opportunity, however, to measure a sufficient number of stalks and therefore cannot draw any definite conclusion. Flowerstalks which developed 10 days and more after the addition of the auxin showed the normal dimensions and remained erect; apparently at that time the alpha-naphtalene acetic acid had lost its efficacy.

It remains a remarkable fact that in young plants of *Nymhaea* the petioles, on one side, respond so much more strongly to the hormone, and the flowerstalks, at the other, hardly if at all, when compared with what we see in adult plants which are at the height of their development. The only means of elucidating this problem would be a series of experiments with *Nymphaea* and *Victoria* throughout their different stages of development and with several concentrations of auxin. My former researches demonstrated that the sensitiveness to hormones of the petioles of *Nymphaea* is feeble during the seedling stage and increases with age; the same apparently holds true for the flowerstalks, although for these organs other concentrations seem to be needed in order to show their ready response. When, however, the plants become too voluminous to be manipulated in aquaria or small basins, and the *Victoria* basin is the only place where their full expansion can be studied, there arise practical difficulties for the continuation of the experiment and hardly any botanical garden will be sufficiently equipped to overcome them.

Among the other species grown in the *Victoria* basin there is only one which showed a slight response to the alpha-naphtalene acetic acid, viz. *Eichhornia crassipes*; its flowerstalks reached lengths about twice that of the normal ones, viz. 50—60 cm in stead of 30—35 cm; they remained, nevertheless, upright above the water. The leaves of *Eichhornia* showed no reaction; neither did the floating stems of *Hippuris*, or any of the other species, such as papyrus and canna, which are rooted in the *Victoria* basin; this negative result is quite what one would expect, at least as far as the latter are concerned.

II. *The influence of alpha-naphtalene acetic acid on cut petioles of waterplants.*

In the course of my experiments with waterplants I have observed several times that when a petiole somehow becomes detached from the plant, it can continue its growth in a solution of auxin to a considerable degree. During last summer I began to study this phenomenon more methodically.

Young specimens of *Nymphaea coerulea* were grown in a shallow aquarium in a solution of 0.25 mg/L alpha-naphtalene acetic acid. Twelve leaves were cut from the plants at their bases one day before the beginning of the experiment and divided into two groups, 6 mature and 6 young ones; they were kept in separate basins in the same concentration of auxin. The growth is recorded in table 2 a. In table 2 b the same is done for 6 petioles of *Limnanthemum nymphaeoides*, which were treated in exactly the same way, with the exception that the concentration of the hormone was 2 mg/L (comp. 3 h).

The rather old petioles of *N. coerulea* elongated less than the other groups, which fact agrees with former results. Apart from that it may be said that on the whole the petioles do not diverge in their growth from those which remained on the plants. The longest specimens were always found on the plants, but as the individual growth of petioles can vary considerably, I cannot say whether this is accidental or not.

Both in *N. coerulea* and *Limnanthemum* the dry weights of the petioles per cm are the same in every lot. The celllengths were found to be a little shorter in the cut petioles than in the others; this is the opposite of what one might expect, because the former ones were in less favorable conditions; (comp. 3 b and 3 i).

TABLE 2. Length of petioles, on the plant and petioles separate.

	August							Control in H_2O
A. Nymphaea coerulea; 0.25 mg/L α-naphtalene acetic acid.								
	3	4	5	6	7	8	10	10
On the plant aver. of 12 pet.	34.6	37.0	45.5	48.8	59.2	?[1])	60.8	36.2
Longest petiole	48	48	54	71	91		91	
Cut pet. av. of 6 mature sp.	31.7	35.8	40.3	43.0	44.9	45.5	46.7	34.1
Longest petiole	41	44	47	50	53	54	56	
Cut pet. av. of 6 young sp.	31.7	37.8	46.0	52.8	58.7	61.2	63.0	35.8
Longest petiole	35	44	57	68	77	79	81	
B. Limnanthemum nymphaeoides; 2 mg/L α-naphtalene acetic acid.								
On the plant aver of 10 pet.	27.0	43.2	58.0	60.4	61.5	62.1		29.5
Longest petiole	33	54	70	79	86	89		
Cut. pet. av. of 6	27.3	37.8	49.7	54.6	57.0	58.5		30.7
Longest petiole	30	41	52	60	66	68		

[1]) Inextricable.

On the last day of the experiment most blades of the cut leaves had a more or less yellowish colour, whilst those on the plants were still a healthy green. This indication of the nearing end is not to be wondered at, but the experiment shows none the less that the response to alpha-naphtalene acetic acid is equally strong in the two examined species, whether the leaves remain attached to the plant or not. A small number of petioles of *Nymphaea hybrida* yielded identical results. Therefore it seems that during the process of rapid elongation no food or other substances are needed from the plant itself. When we consider that cell division is one of the ways by which the rapid growth is achieved, we are inclined to come to the conclusion that the petioles themselves must contain the reserve materials for the formation of new cells.

III. *The influence of different compounds on the growth of waterplants.*

My former experiments have proved that water intake, cell elongation and consequently dry matter per unit of petiole length, are by no means so closely connected as one might be inclined to expect. When plants are taken from their natural habitat and placed in aquariums, the dry weight of the petioles sinks within a few days to half or even less than what it was before. Therefore it may be assumed that respiration plays a part here, although my experiments with aërated and non-aërated water are far from giving conclusive data in this direction (3 d). Assimilation, thickening of the cell walls, storage of food and other life processes are likely to prove to be of influence here. Nevertheless, I thought it worth while to investigate whether stimulation of the respiration might make the plants more sensitive to growth hormones. As aëration yielded no results, I tried another method, viz. of adding amino acids to the water because these substances are known to have a marked influence on the catabolic processes (2,6).

I applied the amino acids alone and together with optimal concentrations of alpha-naphtalene acetic acid; basins with this hormone only and with pure water served as controls. It was my idea that the hormone should indicate whether the growth capacity of the petioles was as it should be, and further that in a solution which activates their growth so strongly they might be more sensitive to the amino acids, in case these failed to exert any action when applied alone. Among the compounds at my disposal I chose asparagin, cystin and tyrosin in doses of 1 and 10 mg/L. Test plants were *Nymphaea coerulea* and *Limnanthemum nymphaeoides.*

The influence of the amino acids on the longitudinal growth, celllength, dry weight, etc., appeared to be purely negative, no matter whether the petioles were stimulated by alpha-naphtalene acetic acid or not. The response to the auxin was as rapid as it was in all other experiments.

Thiamin (aneurin or vitamin B_1) was given to *Nymphaea alba, N. jubilé lilacina* and *Limnanthemum,* separately or simultaneously with alpha-naphtalene acetic acid or when this latter auxin had already exerted its influence during a number of days; the concentrations varied between 1/40 and 5 mg/L. I could never detect any action of this important phytohormone on the growth of waterplants.

With a view to the cell division which must take place during the rapid elongation of the petioles, esp. of those of *Limnanthemum,* in solutions of auxins, I wanted to know whether colchicine can interfere with this process and thus disturb the growth. Specimens of *Nymphaea coerulea* and *Limnanthemum,* therefore, were grown in a solution of this substance. The concentration used was 20 mg/L; this is much less than what is usually applied to plants (1,5), but it should be kept in mind that the action was to last during several days. Moreover, I had to choose this dose rather at random because, so far as I know, no experiments up to now have been made with colchicine on phanerogamic waterplants, and I think that in applying it on these organisms the concentration ought to be rather low, because the substance can exert its possible influence on the whole surface of the plants. For the reason mentioned above the colchicine was added to the water simultaneously with the auxin. It goes without saying that both substances were tested separately at the same time.

Neither alone, nor in combination with alpha-naphtalene acetic acid the colchicine showed any influence in my experiments. The response of the plants to the auxin was as usual. Therefore I applied the colchicine also in combination with supra optimal doses of the hormone, in which the waterplants react abnormally, esp. by a strong cell stretching. *Limnanthemum* was grown in a solution of 5 mg/L; last year I stated that this concentration may be considered as injurious on account of the exceptionally long cells which the plant forms in it. This summer my material came from another place and appeared not to be harmed by the strong dose; it grew perfectly well in it and its cells were of the same dimensions as in lower concentrations. This fact once more nicely demonstrates the capriciousness of waterplants and their reactions to different circumstances. An additional dose of colchicine, as was to be expected, had no effect whatever.

Half a milligram per liter of alpha-naphtalene acetic acid is slightly injurious to *Nymphaea* (3 i); when to this concentration colchicine was added the toxicity was strengthened, as was shown by the dimensions of the cells. These were considerably longer than in the petioles grown in the auxin alone, although these in their turn were already longer than in control specimens, owing to the supra optimal concentration. The result of the cell measurements are presented in table 3. This was thus far the only indication of an appreciable action exerted by colchicine in my experiments; abnormal cell divisions, polynuclear cells, etc. were never observed.

TABLE 3. Nymphaea coerulea; aver. length of petioles in cm and of their cells in micra; 4 petioles per group; cells measured at 5 places in each petiole.

	Control	α-napht. acet. acid 0.5 mg/L	α-napht. acet. acid 0.5 mg/L + colchicin 20 mg/L
Length of petioles	44	90	70
Length of epid. cells	115	140	193
Length of subep. cells	122	188	241

IV. *Summary.*

A full grown plant of *Victoria regia* was submitted to the influence of alpha-naphtalene acetic acid in a concentration of 0.25 mg/L; its response was rapid and strong and in many respects paralleled that of other waterplants observed in foregoing years: vigorous renewal of growth of the petioles, hyponasty of the leaves, shortening of the vegetation period.

The leaves of full grown *Nymphaea*'s responded only feebly to the same dose of the hormone, to which young specimens are so uncommonly sensitive.

The flowerstalks of both *Nymphaea* and *Victoria* elongated strongly owing to the addition of the hormone. While in young specimens hardly any reaction of these organs could be observed, this is another example of the diversity of reaction capacity of the plants at different stages of development.

Cut petioles respond exactly in the same way to the addition of alpha-naphtalene acetic acid as those which remain attached to the plant.

Asparagin, tyrosin, cystin, thiamin and colchicine did not show any influence on the growth of waterplants, whether applied separately or in combination with alpha-naphtalene acetic acid. In one case colchicine seemed to strengthen the injurious influence of a supra optimal dose of the auxin.

I want to express my heartfelt thanks to everyone who helped me to carry out these researches in the Botanical Institute of the University of Leyden.

I have great pleasure in thanking the Direction of the N.V. Amsterdamsche Chininefabriek, Amsterdam, who provided me with the alpha-naphtalene acetic acid.

Botanical Institute.

Leyden, October 1942.

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