OBSERVATIONS ON THE GROWTH OF WATERPLANTS

III.

by G. L. Funke.

1) Introduction.

Last year I found that the addition of small quantities of heteroauxin (3 - 7 ½ mgr per liter) to the aquaria had a remarkable effect on the plants which were growing in them. Leafblades of *Limnanthemum nymphaeoides* and of *Sagittaria sagittifolia* roll up hyponastically; their petioles show a more or less marked twisting. In *Vallisneria spiralis* the heteroauxin caused a rolling up of the female flowerstalk, just as if the flower had been fertilised; there was a slight inclination towards parthenocarpy.

This year I have investigated the influence of heteroauxin on other waterplants and at the same time I have compared its action with that of other phytohormones, viz. beta-indolepropionic acid, beta-indolebutyric acid, alpha-naphtylacetic acid and beta-naphtylacetic acid. They were applied in a concentration of 3 mgr per liter, that is 60 mgr per aquarium; in some cases, which will be mentioned when needed, it was less.

The plants were grown in the aquaria which have always been used for this sort of experiments (depth 50 cm); their growth being vigorous and rapid, as has been repeatedly observed and described (2-3-4-5), their reactions to the hormones were quite distinct.

2) Limnanthemum nymphaeoides.

Young plants with petioles about 10 cm long were planted into the aquaria, each time 12 plants which developed some dozens of petioles in each basin. They started growth within one or two days; the hormones were mostly added about that time. It makes no difference whether they are added at the moment of planting or one or two days after growth has started; when the plants are fully grown, however, the hormones have hardly any influence.

The year before I had observed that 3 mgr per liter heteroauxin does not promote the growth and that it causes abnormally looking responses such as twisting of the petioles and rolling up of the leafblades. This concentration is apparently toxic and therefore I applied it once more, but this time I compared it with one of 1 mgr per liter; I did the same with beta-indolebutyric acid.

After about 8 days the plants in all aquaria had developed to the same degree; petioles measured between 60 and 70 cm; in the control basin they were straight; in heteroauxin 1 and 3 mgr per L and in butyric acid 3 mgr per L they were strongly twisted, in butyric acid 1 mgr per L only slightly so; beta-indolebutyric acid apparently is less toxic than heteroauxin, but even in a solution so far diluted as 1/1.000.000 it does not further growth. One week later the petioles had not grown any more; the toxicity of the 1-3 heteroauxin and of the 3 butyric acid solutions was further demonstrated by the fact that the leaves in them had turned yellow and the plants began to decay, whilst in the 1 butyric acid solution and in the control they still looked perfectly fresh.

Three mgr. L. of beta-indolepropionic acid had hardly any influence; petioles remained straight, leafblades showed a slight hyponastical curvature.

Alpha and beta-naphtylacetic acid gave striking differences in response; in alpha the twisting is so strong, that most petioles did not grow to the surface; the leafblades are rolled up hyponastically; in beta, however, the petioles remain straight and the only influence of this substance consists in a slight epinastic bending of the blades. In all other solutions I noted a hyponastic curvature, strong (heteroauxin, butyric acid, alpha-naphtylacetic acid) or weak (propionic acid).

Figure 1, a and b, represent photo's of *Limnanthemum* in alpha and beta-naphtylacetic acid, taken about one day before the petioles reached the surface in the beta acid; the enormous difference between the influences of these nearly related substances on *Limnanthemum* is clearly shown.

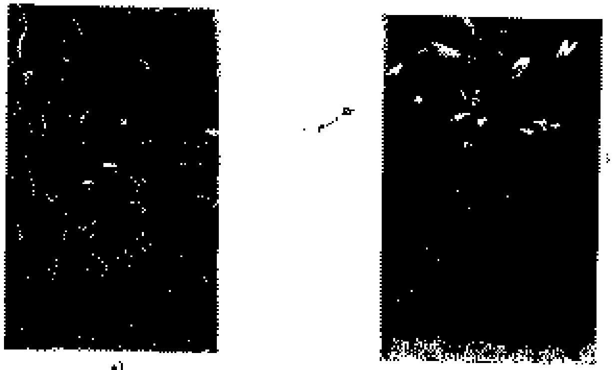
a) b)

Fig. 1. — *Limnanthemum nymphaeoides*:
a) in solution of alpha-naphtylacetic acid.
b) in solution of beta-naphtylacetic acid.

The results obtained with *Limnanthemum* are recorded in table 1:

3) Vallisneria spiralis.

My observations on the female flower stalk of *Vallisneria spiralis* confirm those of last year and I can summarize them with the same words as I did at that time: «The growth » of the female flower stalk of *Vallisneria spiralis* is an ex» traordinarily rapid one; slow in the beginning, it may » reach a pace of more than 2 cm an hour, which pace may » be kept on for a whole day; the depth of the waterlayer » is of no influence. This growth is mainly accomplished » by cell stretching. » I lay stress on this point because in the mean time a paper, published by KAUSIK (8), makes clear that this does not always hold true. KAUSIK studied the behavior of the flower stalk of an Indian form of *Vallisneria spiralis*. He states: « A change in the depth of wa» ter,..., brings about a corresponding change in the length

Table 1; Limnanthemum nymphaeoides

responses	control	α - napht. ac. acid	β - napht. ac. acid	β - indole ac. acid	β - indole prop. ac.	β - indole butyr. ac.
twisting of the petioles	none	very strong	none	strong	none	strong
bending of the blades	none	strong hypon.	slight epin.	strong hypon.	slight hypon.	strong hypon.

» attained by the scapes of the developing pistillate flowers »; so e.g. in an aquarium of 16 cm depth the stalks hardly ever grew to more than about 18 cm; this is quite different from what European and American forms show; but: « Sometimes, however, the length of the scape may » continue to increase afresh even after the pistillate flower » is completely exposed on the surface of the water and has » been lying thus for some time; in *one* instance it was » found to have developed a scape fully 40 cm long while » the water in the aquarium was only 16 cm deep ». One instance of « fully 40 cm » is rather meagre when compared with the numbers of stalks which in our shallow basins grow up to 100, 150 cm and more. The cell measurements of KAUSIK, however, seem to tally fairly well with mine.

Six aquaria were planted with a dozen young plants of *Vallisneria* each; they unfortunately developed a small number of female flowers so that results can be valued only qualitatively.

Coiling of the flower stalks occurred in the solutions of alpha-naphtylacetic acid, beta-indoleacetic and -butyric acid; in beta-naphtylacetic acid and in beta-indolepropionic acid no reaction was to be observed. The rolling up was always irregular, as has been represented in figure 6 of last year's paper (5); however, when a stalk of some 80 - 90 cm long, one half of which is floating on the surface, rolls up after the addition of the hormone till the flower is pulled far below the surface of the water, this reaction may be called coiling.

Parthenocarpy was observed in alpha-naphtylacetic acid and in heteroauxin; not, or, exceptionally, in a very small degree, in the other solutions. The length of the control flowers, when opened, is between 16 and 18 mM; in heteroauxin I measured fruits of 28 mM, in alpha-naphtylacetic acid, however, of as much as 56 and 65 mM.

A few measurements are recorded in table 2:

The data of the controls represent averages of 12 samples; those of heteroauxin of 4; those of alpha-naphtylacetic acid bear on individual cases; therefore they can hardly be compared with each other and have only a limited value; the cell measurements were done in microtome slides. Although these data are insufficient I still think that they allow us to deduce from them that the parthenocarpic development of the fruit consists in the uptake of water and of solid

Table 2: Parthenocarpy in Vallisneria spiralis

	length in mM	diameter in micra	length of cells in micra				fresh weight in mGr.	dry
			epid.	1st cort l.	2d c layer	3d c. layer		
control	17	1850	14	23	26	25	47	4.7
heteroauxin	27						63	6.7
α-napht. ac. a.	33						125	13.5
—	58	2150	42	78	72	83		
—	65		55	104				

Table 3: Vallisneria spiralis.

responses	control	α - napht. ac. acid	β - napht. ac. acid.	β - indole ac. acid.	β - indole prop. ac.	β - indole butyr. ac.
coiling of the stalk	none	strong	none	strong	none	strong
parthenocarpy	none	very strong	none	rather strong	none	very slight

matter, that the longitudinal growth is accomplished by means of cell stretching and that growth in thickness hardly takes place. Many of these phenomena resemble very much those observed by HUBERT and MATON in their experiments with orchids (6).

The results obtained with *Vallisneria* are recorded in table 3:

4) Nymphaea.

In each aquarium were planted two corms of three species, viz. *N. Devoniensis, N. Boucheana* and *N. terminalis.* The development was satisfactorily uniform. When the leafblades were nearing the surface, consequently when the petioles had a length of 40 - 45 cm, the hormones were added to the water. Two days later the first observation showed that a response of the plants had been obtained, and that it consisted in a few twistings of the petioles, esp. in alpha-naphtylacetic acid and in heteroauxin, hyponastic bending of the blades in alpha-naphtylacetic acid, beta-indolebutyric and -acetic acid, and a very strong longitudinal growth of *N. Boucheana:* longest petiole in alpha-naphtylacetic acid 158 cm, in butyric acid 148 cm, in heteroauxin 125 cm and in propionic acid 110 cm.

All petioles longer than 50 cm were cut away and the further leaves practically developed, from the beginning, in the solutions of the hormones.

Figures 2 and 3 give an impression of the form of the leafblades, typical for the different hormone solutions, of resp. *N. Devoniensis* and *N. terminalis.* We see that alpha-naphtylacetic acid, beta-indolebutyric and -acetic acid have the strongest influence in rolling up the leaf and reducing its size; propionic acid also exerts a marked action, whilst beta-naphtylacetic acid does not change the blade at all; the same difference between the influence of the several hormones is seen in *N. Boucheana.*

It was observed several times that the leafblades which developed so poorly in the hormone solutions, showed a sagittate form, resembling very much the juvenile sagittate form of the first leaves which are formed in practically every species of *Nymphaea* and *Victoria*, and which never grow to the surface of the water; the influence of the hor-

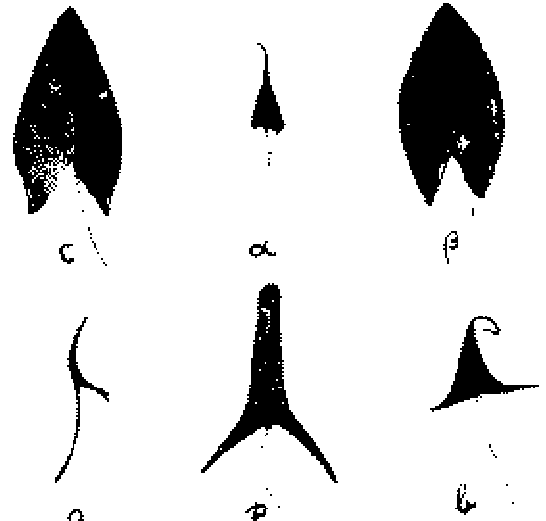

Fig. 2. — Leafblades of *Nymphaea terminalis* in different hormones; from left to right: toprow: control — alpha-naphtylacetic acid — beta-naphtylacetic acid; bottomrow; indoleacetic acid — indole propionic acid — indolebutyric acid.

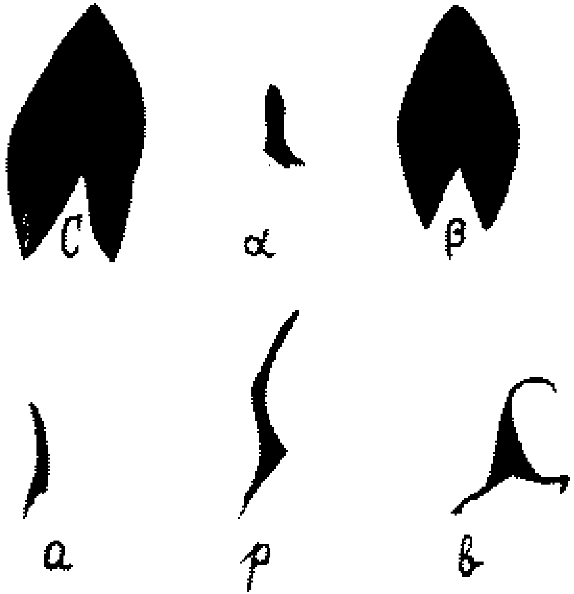

Fig. 3. — Leafblades of *Nymphaea Devoniensis* in different hormones; from left to right: toprow: control — alpha-naphtylacetic acid — beta-naphtylacetic acid; bottomrow: indoleacetic acid — indole-propionic acid — indolebutyric acid.

mones in this detail, therefore, seems to consist in preventing the leaves from reaching maturity. I had no opportunity to go deeply into anatomical details; a few observations gave me the impression that the dimensions of the cells were of the same order as those in control leaves.

Alpha-naphtylacetic acid causes strong twisting of the petioles in all three species and in this respect it surpasses by far even indolebutyric and -acetic acid; figure 4 gives an impression of some petioles of *N. Boucheana* in this solution; on the right is a controlleaf; the 4th petiole from the left measured 62 cm, so it could easily have lifted its blade up to the surface of the aquarium; but it hardly reached half of its height, owing to the spirallike coiling.

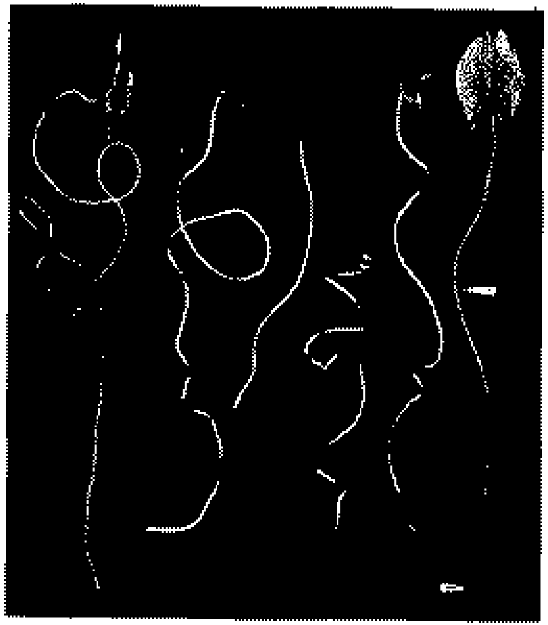

Fig. 4. — Leaves of *Nymphaea Boucheana* in solution of alpha-naphtylacetic acid; controlleaf on the right.

In this experiment I have noticed for the first time a growth promoting influence of the hormones on the petioles of waterplants, esp. on those of *N. Boucheana*. As mentioned above, in the first days of the experiment it were

esp. alpha-naphtylacetic acid, heteroauxin, indolepropionic and -butyric acid which proved very active, beta-naphtylacetic acid once more showing no action; the second set of leaves gave the same response, but this time it was butyric acid which proved the most active hormone; the maximum lengths reached in the aquaria were resp.: control 57 cm, alpha-naphtylacetic acid 120, beta-naphtylacetic acid 70, beta-indoleacetic acid 127, — propionic acid 155, — butyric acid 227. A petiole of 227 cm in an aquarium which has a depth of about 46 cm is certainly uncommon; and this petiole was not the only one which reached this length; several of them attained two meters and something more.

In tables 4, 5 and 6 are recorded part of the cellmeasurements made on a number of petioles of each of the three species. When we make allowance for specific characteristics and for the individual differences between the single petioles, we may deduce two facts from these data: first that the growth of the very long petioles is accomplished by a strong cell stretching, and secondly that petioles, grown in the hormone solutions and which are equally long as those in the control basin, are constituted by much longer, and therefore by a smaller number of cells than the control specimens. An exception must again be made for beta-naphtylacetic acid which shows cell dimensions equal to those of the controls.

In former papers (3,5) I have pointed out that abnormally long cells may always be expected in petioles which, in some way or other, can be looked upon as pathological (in the case of ageing, wounding, sickliness, etc.); it is clear that every growth substance used in this experiment is more or less toxic for the waterplants, except beta-naphtylacetic acid; therefore, we need not be astonished when we see that the outward features of this intoxication are accompanied by a corresponding abnormal cellstretching.

A very striking example of such pathologic cell stretching is shown by a petiole of *N. terminalis* in indolepropionic acid; it was only 18 cm long, but at the time of cellmeasurement it happened that all other leaves of *terminalis* had disappeared in this basin; the remaining one looked sickly and so I was not at all astonished to see that its cell dimensions surpassed by far those which were found in much longer healthy control petioles; these cellengths may of course not be compared with the other ones.

Table 4; Nymphaea Devoniensis; cellengths of the petioles.

(a means above; 1/4: at 1/4 from the top; m: in the middle; 3/4: at 3/4 from the top; b: below; e = epidermis; s = subepidermis; cells in micra)

	control		α - napht. ac. acid		β - napht. ac. acid		β - indole acet. ac.		β - indole prop. ac.		β - indole butyr. ac.	
length of petiole in cm	50		53		56		84		47		50	
	e	s	e	s	e	s	e	s	e	s	e	s
a	27	92	134	200	104	150	79	110	60	92	155	135
1/4	100	110	380	315	215	175	160	205	125	180	285	245
m	151	212	385	405	180	210	310	310	170	220	310	270
3/4	134	160	350	325	157	240	210	330	175	215	310	265
b	136	193	230	370	140	240	210	400	170	265	355	365

Table 6; Nymphaea terminalis; cellengths of the petioles; compare table 4.

	control		α - napht. ac. acid		β - napht. ac. acid		β - indole acet. ac.		β - indole prop. ac.		β - indole butyr. ac.			
length of petiole in cm	53		41		54		68		18		62		136	
	e	s	e	s	e	s	e	s	e	s	e	s	e	s
a	70	90	65	100	125	185	50	75	160	250	145	240	115	175
1/4	155	155	150	200	260	210	185	230			275	310	300	275
m	190	175	170	255	285	225	350	295	195	300	375	315	440	400
3/4	150	180	300	380	190	160	330	265			300	380	390	425
b	130	170	290	460	95	110	215	395	250	315	425	315	490	500

Table 5; Nymphaea Boucheana; cellengths of the petioles; compare table 4.

	control	α - napht ac. acid.		β - napht. ac. acid	β - indole acet. ac.		β - indole prop. ac.		β - indole butyr. ac.	
length of petiole in cm	57	67	126	56	49	134	48	165	63	225
	epidermis									
a	40	180	310 475 1)	47	50	70	75	160	60	135
1/4	90	240	265	70	95	160	140	300	200	330
m	110	260	250	110	170	235	180	330	250	415
3/4	85	125	180	100	200	340	155	360	200	395
b	80	170	130	60	265	300	175	170	270	470
	subepidermis									
a	65	170	245 340 1)	67	95	90	95	200	100	165
1/4	170	235	310	95	250	190	210	315	230	365
m	200	275	355	110	260	300	215	365	270	355
3/4	175	170	290	125	240	325	215	335	270	350
b	160	280	230	130	280	425	185	270	340	460

1) on two spots, quite near each other, 1 cm under the blade.

Table 7; Nymphaea

responses	control	α - napht. ac. acid	β - napht. ac. acid	β - indole acet. ac.	β - indole prop. ac.	β - indole butyr. ac.
Nymphaea Devoniensis						
twisting of the petioles	none	strong	none	slight	none	slight
bending of the blades	none	strong hypon.	none	strong hypon.	strong hypon.	strong hypon.
long. growth	normal	normal	normal	rather strong	normal	normal
cellengths	normal	very long	normal	long	about normal	very long
Nymphaea Boucheana						
twisting of the petioles	none	very strong	none	slight	none	rather strong
bending of the blades	none	strong hypon.	none	strong hypon.	strong hypon.	strong hypon.
long. growth	normal	strong	normal	strong	strong	very strong
cellengths	normal	very long	normal	long	long	long
Nymphaea terminalis						
twisting of the petioles	none	strong	none	slight	none	none
bending of the blades	none	strong hypon.	none	strong hypon.	strong hypon.	strong hypon.
long. growth	normal	normal	normal	rather strong	normal	strong
cellengths	normal	long	normal	long	?	long

The results obtained with the three species of *Nymphaea* are recorded in table 7:

5) Blyxa echinosperma.

Young, vigorously growing leaves reached the surface of the water within a few days, as I have described formerly (3). No response whatever to the addition of the hormones was seen; the petioles remained straight, the leafblades did not curl. However, one peculiar phenomenon was observed which may be worth while mentioning here. In my last year's paper I described the development of a tremendous lot of bacteria which was sometimes seen as an aftereffect of the addition of heteroauxin; the bacteria at first cause a white or grey cloudiness of the water; later on they settle down in thick films on the petioles and on the blades; sometimes I observed this opaqueness also after addition of alcohol in the same quantity as is used to solve hormones, but in this case no settlement followed. In the experiment with *Blyxa* a strong development of bacteria was seen in the aquaria to which alpha-naphtylacetic acid, indolepropionic and -acetic had been added; there was hardly any cloudiness, the bacteria were seen from the beginning as white, rapidly growing plugs, clustering to the leaves. They apparently were harmful to the plants because after a few day the leaves to which they clung turned brownish and the plants died; those in the control basin, in beta-naphtylacetic acid and indolepropionic acid remained fresh.

I cannot say for certain that the hormones alpha-naphtylacetic acid, indolebutyric and -acetic acid are responsible for this phenomenon, but on the other hand it seems striking that it occurs just in those aquaria where the substances are added which in all other cases proved to have the strongest influence on waterplants.

6) Discussion and summary.

a) The aim of this research was to investigate which influence several phytohormones may have on the development of waterplants. The following substances were used: alpha- and beta-naphtylacetic acid, beta-indoleacetic, -propionic and -butyric acid; they were applied in concentrations of 3/1.000.000, sometimes of 1/1.000.000.

b) *Limnanthemum nymphaeoides:* a strong twisting of the petioles was caused by alpha-naphtylacetic acid, indoleacetic and -butyric acid; hyponastic bending of the leafblades was seen in the same three substances and slightly in indolepropionic acid, whilst in beta-naphtylacetic acid a slight epinastic curvature appeared.

c) *Vallisneria spiralis:* coiling of the female flowerstalk was strong in alpha-naphtylacetic acid, indoleacetic and -butyric acid; in other solutions it was not seen at all. Development of parthenocarpic fruits was very marked in alpha-naphtylacetic acid, much less so in heteroauxin and hardly worth mentioning in other solutions.

d) *Nymphaea Devoniensis, N. Boucheana, N. terminalis:* twisting of the petioles is caused by alpha-naphtylacetic acid in all three species. Hyponastic curvature of the blades and inhibition of their full expanding is shown very markedly by all three species in the solutions of alpha-naphtylacetic acid, indoleacetic and -butyric acid, somewhat less in propionic acid. The petioles attain abnormal lengths in alpha-naphtylacetic acid, indoleacetic, -propionic and -butyric acid in all three species, but by far the most markedly in *N. Boucheana:* butyric acid had the strongest influence on this phenomenon. Abnormally long celllengths are observed in alle three species and in all solutions, with the exception of beta-naphtylacetic acid.

e) *Blyxa echinosperma:* no response of the plant itself could be observed; there is a strong development of bacteria in alpha-naphtylacetic acid, indoleacetic and -butyric acid.

We see that, on the whole, alpha-naphtylacetic acid, indoleacetic and -butyric acid exert the strongest influence on the various phenomena of waterplants, among which three substances alpha-naphtylacetic acid surpasses the other two; indolepropionic acid, as a rule, has much less action and beta-naphtylacetic acid has hardly any influence at all.

There is a marked parallelism between these results and those obtained by HUBERT and MATON with orchid flowers as far as the swelling of the gynostemium is concerned; in their experiments as well as in mine, alpha-naphtylacetic acid is the only substance which causes distinct parthenocarpy. Whether this parallelism holds true for rootformation (7) also, cannot be said for certain, because experiments with alpha-naphtylacetic acid are so far too scarce to draw conclusions from them; in any case it is a fact that indoleacetic and -butyric acid have a much stronger rootforming capacity than other substances investigated. A certain parallelism with the *Pisum* test (curvature of slit stems) (9) seems to exist, whilst for the *Avena* test (curvature) the relation between the action of the various hormones is of a perfectly different nature.

Alpha-naphtylacetic acid, indoleacetic and -butyric acid cause a coiling of the female flowerstalk of *Vallisneria* of the same character as that which occurs after pollination. BORESCH (1) applied heteroauxin to the tendrils of *Vitis*, *Cucumis* and *Passiflora* and obtained coilings which resembled very much the haptotropic rolling up after contact; he assumes that this is accomplished by a strong cell stretching on the convex side of the organ under the influence of the heteroauxin, whether in one plane or not; he, however, did no cell measurements to confirm this assumption; in my last year's experiments I found no change of celllength after the rolling up of the flowerstalk of *Vallisneria*. This side of the question certainly needs further investigation.

It is a remarkable fact that hormones are capable of causing spiraling in organs which possess a natural tendency to it, but that at the same time, they can do so to organs which normally never roll up; see figures 1 and 4; this is also a problem worth while considering.

I feel indebted to my assistants dr. B. HUBERT and dr. J. RAPPAPORT for their valuable help in taking the photographs; I have also great pleasure in thanking my assistant J. MATON who has made the microtome slides of the parthenocarpic fruits of *Vallisneria*.

Ghent, Botanical Institute. July 1939.

7) Literature.

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9) **Thimann** K. V. and **Schneider** C. L., *Amer. J. of Bot.*, 26, 1939, « The relative activities of different auxins ».

PROEVEN OVER PHOTOPERIODICITEIT BIJ VERSCHILLEND GEKLEURD LICHT

door G. L. **Funke**.

IV

With a summary in English.

1) BESPREKING DER LITTERATUUR.

In het laatste jaar is weer een groot aantal publicaties verschenen over photoperiodiciteit; ook zijn enkelen van iets ouderen datum mij bekend geworden. De voornaamsten daarvan zullen hier besproken worden.

De bloeihormonen komen steeds meer in het middelpunt van de belangstelling te staan. **Kuyper** en **Schuurman** (20) zetten de proeven van **Kuyper** en **Wiersum** van 1936 voort (14, N° 27); zij werken met twee varieteiten van *Glycine Max*, « soja à graines jaunes » van **Vilmorin** en « Ked. 29 Java », die bij de lange zomerdagen in Nederland niet bloeien. Zij enten een korte-dag-top op een lange-dag-onderstam en omgekeerd een lange-dag-top op een korte-dag-onderstam; als contrôle gebruiken zij gewone lange-dag-exemplaren, intact, van den top ontdaan, en met eigen top geënt. Het resultaat van 1936 werd bevestigd; het florigeen wordt naar beneden getransporteerd, maar niet naar boven. Belangrijk is de waarneming dat soms ook het transport naar beneden niet plaats heeft, n.l. wanneer de K D ent zich krachtig ontwikkelt; deze gebruikt dan zelf het florigeen; dit was vnl. het geval met de varieteit Ked. 29, die als K D top goed groeide, maar nooit een L D onderstam tot bloei bracht.

Cailachjan en **Yarkovaja** (4) (zie 14, N° 7) hebben opnieuw aangetoond dat florigeen ook naar boven getransporteerd kan worden en wel met een proef op *Perilla nankinensis:* wanneer L D enten van deze stricte korte-dag-plant