ON THE INFLUENCE OF LIGHT OF DIFFERENT WAVE-LENGTHS ON THE GROWTH OF PLANTS

by

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Contents.

I. Introduction — Method.

Literature.

The literature on the influence of light of different wave-lengths on the growth of plants is rather extensive

and has still been enlarged by some important papers during the last few years. I think that I have nevertheless sufficient reason to publish my results on this subject, for, though I have made my researches with entirely different species of plants, they agree in the main with those of other authors, and I further believe that I have discovered some facts, which till now have not met with the attention they deserve.

As Téodoresco in his last paper (48) gave a very complete survey of the literature on the subject, I need not say very much on it, as it would be, for the main part, a mere repetition. On the whole, most authors obtained similair results, notwithstanding the fact that species of plants were used out of many classes and that the methods in getting the different colours varied very much. In most cases only two parts of the spectrum were used, that of the long wave-lengths, red-orange-yellow, and of the short ones, green-blue-violet; the plants which were grown under those conditions being in most cases only compared with specimens, grown in full day light. I hope to make clear that this is not quite right, that in doing so, important facts remain unobserved.

Whether sunlight were used or an artificial source of light, whether the colours were obtained with the aids of prisma, coloured solutions or stained glass, results are always the same so far, that the physiological influences of red and darkness are identical, and that in blue the outer appearance is about the same as that of plants, grown in normal light. This can already be seen in the oldest researches, e.g. of Goethe (19—20—21) and Tessier (49) and equally in the more modern investigations of (to mention only some of the most recent ones) Klebs (26), Koningsberger (33), Popp (39), Schanz (42), Téodoresco (47—48). Sometimes results differ more or less, e.g. those of Foerster (10), but firstly we

need not wonder that the Hepaticae (he experienced on Marchantia polymorpha) react quite differently from the Angiosperms, and secondly it is probable that in his researches the assimilation of carbonic acid played an important role, thus clouding the effect of light upon growth. This question, however, will be dealt with further on.

Pfeiffer (37) too got results, differing from those of the others. In my opinion there was too much disparity of intensity of light in her experiment. When we see that in the blue hothouse there penetrated only 8 % of daylight, in the red partition about 32 %, we need not be surprised that e.g. the length of the stems in blue largely surpasses that in red. The plants in the blue hothouse simply got quite insufficient light to show any normal growth. The conclusion at the end: "both quality and intensity (of light) may be effective in bringing about the changes" is in my opinion too vague.

In the various paragraphs of this paper I will refer to other articles.

Method.

My researches began in June 1927 and have been continued since then, but had to be frequently interrupted by long intervals. Moreover, during the winters, the work was retarded. Results therefore should not be considered as final, as many questions could not yet be answered.

A special cupboard was built for the researches. It is divided into four partitions, measuring 46 × 28 × 28 cm. (see fig. 1). The back of each partition consists of a closely fitting door; all the inner walls are lacquered white. The fronts of the partitions are provided with six stained window panes of 11 × 11 cm. Three holes in each of the horizontal partitions provided ventilation; no direct daylight could penetrate through them. The cupboard was placed

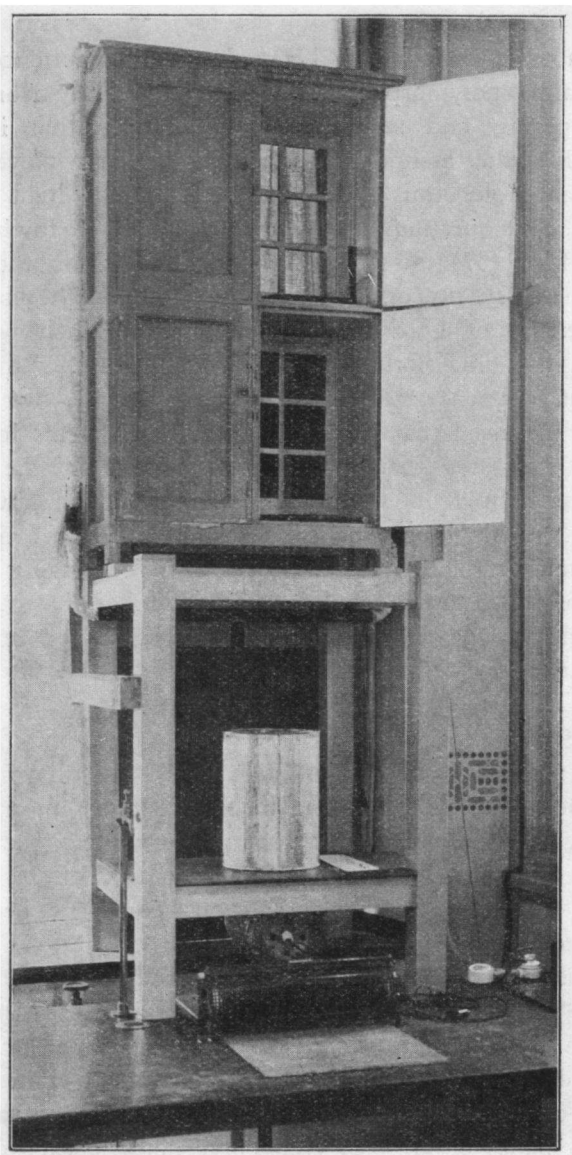

Fig. 1. The cupboard from behind; the grey and red partitions are opened.

quite near a northern window in the Laboratory of Technical Botany at Delft.

As this installation proved to have some inconveniences, an alteration was made this year. The ventilation holes were not sufficient to prevent a too high degree of humidity of the air. This presented, of course, no difficulties, while experimenting on waterplants, but for terrestrial plants it clouded the results, as will be seen later on. Moreover, during spring and autumn the room temperature was often too low, growth being then very slow. A simple installation was constructed, which removed these difficulties. A motor under the table, on which the cupboard was placed, sucks in the air of the room and forces it up vigorously through a large tube. The air passes through a cylinder, in which an electric stove is placed; (this could be tempered by a resistance bank). As in doing so the degree of humidity becomes too low, the air is forced through a second cylinder in which a dish of water, about 10 cm. high, was placed. In this dish stood three screens of gauze so that their upper parts, about 15 cm., were always thoroughly wet. The air brushing past them gets sufficient humidity. In some cases I had even to remove the middle screen. Above the second cylinder the tube ramifies into four parts, those, which go to the upper partitions of the cupboard, being wrapped round with an isolating substance. Inside the partitions the tubes were laid round the bottoms; the air here escapes through a number of little holes. Thus a slow and equal current of air was obtained, which could be further regulated by means of pinch-cocks outside.

In this way the air in the cupboard can easily be kept at about 25° during the day; during the night, temperature was lowered to about 20°. But the most important thing was, that in the four partitions the same temperature always prevailed, deviations never surpassing half a degree.

The degree of humidity varied from about 80 à 85 % during the day to about 95 % during the night. On fig. 1 most particulars of this installation can be seen, except the motor.

The glass panes were obtained from the factory of Schott, Jena. Their particulars are as follows:

No. 12044	subdued day light ("grey")	thickness	3	mm.
„ 20702	red	„	0.6	„
„ 11169	green	„	1.2	„
„ 3653	blue	„	3	„

The exact data of the quantities of light, which are let through for the different wave-lengths, are recorded in the following tables. See page 437.

The data for the relative intensity of the different wave-lengths in sunlight and blue sky have been borrowed from Luckiesh (36); he took the relative energy of μ 0.59 to be 100. The data given by Schott have been multiplied by those of Luckiesh, after applying the formula $Dx = D^x$, for the thickness of the panes (x = thickness.) [1])

Mr. Ir. L. J. N. van der Hulst verified the data of Schott in the Laboratory of Chemical Technology, director professor Ir. H. I. Waterman, Delft, with the aid of the extinctiometer of Keuffel and Esser. Only unimportant deviations from the data of Schott were thus found. [2])

From table 1 it will be seen that the quantities of energy, as far as the visible part of the spectrum is

[1]) The calculations were made by Mr. Ir. H. Eilers, at the time chief assistant of the Laboratory of Technical Botany, Delft. I have great pleasure in expressing my sincere gratitude to Mr. Eilers for his most valuable assistance.

[2]) I also thank Mrs. van der Hulst and Waterman very heartily for their much esteemed help and kindness.

TABLE 1.

Relative distribution of energy in the cupboard; source of light: *blue sky*.

Wave-lengths	Blue sky [1]	Grey [2]	Red [2]	Green [2]	Blue [2]
0.405		28	—	—	172
41	177				
43	185				
436		38	—	3.9	154
45	187				
47	180				
480		45	—	60	7.9
49	162				
509		40	—	84	0.7
51	146				
53	132				
546		34.5	1	73	0.8
55	120				
57	108				
578		31.4	2	40	—
59	100				
61	93				
63	87				
644		28	80	9	—
65	82				
67	77				
69	72.5				
700		27	72	3.6	—
	1908.5	482	394	500	462
		25 %	20½ %	26 %	24 %

concerned, are fairly equal in every partition. The curve of the grey glass slightly deviates from that of daylight. Relatively too much red is transmitted, viz. about 33 %, blue 18 %. Nevertheless the quantity of blue was sufficient

[1] from Luckiesh.
[2] based on Schott's data.

to make some typical facts apparent. Plants which need a great quantity of the short waves, do not get enough of them behind the grey glass, so that their habitus will be more or less similar to that of the plants behind the red glass. If the need of blue rays is only small, they will, on the contrary, resemble more or less those behind the blue glass. Of both cases we shall see distinct instances.

When the same glass panes are used for direct sunlight, the distribution of the quantities of energy is quite different and especially insufficient for blue. See table 2, page 439.

The cupboard was therefore placed before a window facing the north. In my opinion this has still another advantage: it is well known that for experiments like this, daylight is not at all ideal. The composition of sunlight varies very much at different hours of the day; esp. in early morning and in the evening the long waves are relatively more intense (compare Stahl (46)). Though I do not believe that this can have a predominant influence in these researches, it is a difficulty which will be felt much less, when diffuse northern light is used, as in this case the blue occupies a much greater part of the spectrum. The data of Cunliffe (5) also show that northern light, and light from a clouded sky, are more favourable as far as the blue rays are concerned. Moreover it should not be forgotten that most of my researches lasted several weeks or even months. The variations of climatical circumstances during such a period may cause differences of light distribution in the partitions of the cupboard, but they will at any rate not surpass a few per cents at the utmost, and further they will, on the whole, compensate each other.

The average intensity of light in different seasons is very unequal. It increases from January to June and decreases in autumn (compare Cunliffe). The absolute intensity can change from day to day with 100 % and more, but as

those variations have only little influence on the relative distribution of the various rays, we need not trouble very much about them.

TABLE 2.

Relative distribution of energy in the cupboard; source of light: *noon sunlight* (black body at 5000° abs.).

Wave-lengths	sunlight [1])	Grey [2])	Red [2])	Green [2])	Blue [2])
0.405		11	—	—	70
41	72				
43	79				
436		16.5	—	1.7	66
45	84.3				
47	91				
480		23	—	30	4
49	92.5				
509		26	—	55	0.5
51	96				
53	98				
546		28.5	0.9	60	0.6
55	99				
57	100				
578		31.4	11.9	40	—
59	100				
61	100				
63	98.5				
644		32	91	11	—
65	97.1				
67	95.5				
69	93.5				
700		35	92	5	—
	1396.4	403	410	396	215
		29 %	30 %	28 %	15 %

[1]) from Luckiesh.
[2]) based on Schott's data.

One might object that in any case an artificial source of light would have been preferable. I can not quite deny it, although even then slight oscillations in the spectral composition occur. But the measurements of the cupboard would have offered the greatest difficulty, as they require a very strong source of light. Moreover, circumstances forced me to leave the researches for the greater part of the time to themselves, as I could only be present two, at the utmost three times a week.

The very best distribution of light is without doubt obtained by using a prisma, provided one takes care to compensate the strong deviation of the short waves (compare Téodoresco's critic on the researches of Dangeard (7), l.c. page 316). For the same reason as mentioned above, I could not make use of this method. Moreover, it is only available, when researches are made on very small organisms, prothallia, Hepaticae, seedlings, etc. and not when they last several weeks, as did mine.

Infra-red and ultraviolet rays.

A few observations must still be made on the invisible parts of the spectrum. From tables 1 and 2 will be made clear that the red panes transmit large quantities of infra-red rays, the blue panes equally of the ultraviolet ones, till at least μ 0.30; Schott did not give exact data about them.

It must first be observed that the invisible rays have been eliminated in measuring the quantity of light energy of the visible part of the spectrum.

As for the infra-red rays, I think that we may neglect them, for everybody will admit that their physiological influence on the development of plants is only very slight, if it exists at all. Foerster (10) mentions some experiments, l.c. page 344, where he could compare results, got without and with an absorbing layer of water. No difference worth mentioning, could be observed.

Téodoresco (48, l.c. page 219) says: "— car les radiations infrarouges n'ont qu'un rôle tout à fait insignifiant dans la croissance des végétaux". Moreover, his results are there to prove it; he made parallel experiments, putting plants under jars of Senebier and behind panes of Schott. In the first case, nearly all the infra-red rays were absorbed by the 7 cm. thick layer of the solution of potassium bichromate, in the second case they were transmitted. The results were always similar. Klebs (27) expresses the same opinion.

I made parallel experiments myself with and without a layer of water, esp. on the diastase of Aspergillus niger (see II, § 12); also here not the slightest difference could be noticed.

As for the ultraviolet rays, it is known that they can be very injurious to micro-organisms (Bovie, 2—3; Hanssen, 22; Kluyver, 30; Ursprung and Blum, 52); for Phanerogams they only have a superficial influence, damaging the epidermic tissues (30—52). But these facts only concern the very short rays below 300 $\mu\mu$, which occur in quartzlamps, but hardly in sunlight. Moreover, a glass pane absorbs a great part of them and also of those between 400 and 300 $\mu\mu$. Now, my cupboard was placed behind a window of thick plate glass; the waterplants, moreover, grew in thick glass cylinders. And then there were the grey and blue panes of Schott, of 3 mm. each. So I do not think that any appreciable amount of those rays can have been left. Researches on the diastase of Aspergillus niger with and without an extra thick glass jar gave exactly similar results.

Therefore I cannot agree with the conclusions of Schanz (42—43—44) who ascribes a predominant influence to the ultraviolet rays. He relies on observations, made on alpine plants, which grew in the lowland and showed great differences from their normal habitus, esp. on Leontopodium

alpinum. But Bonnier (1) made it sufficiently clear that in such cases there are several factors causing the variations; e.g. the difference of the humidity of the air has an important influence (11; see also fig. 3). Yet experiments of Schanz seem to confirm his observations: plants grown under euphos glass (so without any ultraviolet and the greater part of the blue rays) were more etiolated, at any rate had grown more lengthwise than those under normal glass (so without the greater part of the ultraviolet rays), these last plants in their turn being more elongated than others, which had grown without glass at all. My chief objection is, that under the glasses temperature was much higher than in the open air, which may explain the faster growth. The difference between the plants under normal and euphosglass remained still important. Now euphosglass absorbs a big part of the visible blue rays and I think my results prove that this has an enormous influence (and without doubt also for alpine plants, which very likely need much blue light). If Schanz had made his researches, eliminating the ultraviolet rays, but retaining the blue ones (I think that in using the Schott panes, these conditions are realised), he would probably have agreed with my conclusion, viz. that the visible blue rays are in the first place responsible for the habitus of plants and not the ultraviolet ones. [1])

Plant material — Assimilation of carbonic acid.

Most of the plants which I experimented with, were water- or moorplants. One of the reasons which forced me to choose these, was that I could not be present at the work every day; only two, at the utmost three times

[1]) An elongation of the plants under normal glass, as strong as was found by Schanz, is in my opinion very rarely seen, e.g. in hothouses, unless a high temperature makes its influence felt.

a week I could be at Delft, so that plants which did not need daily care were preferable.

Only once I used seedlings, viz. of Alisma Plantago. As the colour of the light has an influence on the germination (Kommerell, 32; Téodoresco, 48), I made them germinate in full daylight and only placed them into the cupboard after they had reached a height of 1—$1^1/_2$ cm. and their first leaflets had developed.

The utmost care was always taken that the plants in the cupboard and the control-plants were parts of one individual; (if possible, two or three specimens were placed into each partition). This precaution could not be taken for the seedlings of Alisma, but their great number (about 30 to each partition) certainly counterbalanced this difficulty.

The researches were always repeated, sometimes even more than once. Results were the same, with one exception (Alisma), which will be dealt with later on.

In selecting the material for the researches, it often appeared that the quantity of light in the cupboard was not sufficient for many species of plants. This was shown by the fact that either no growth took place and they soon died (Campanula rotundifolia, Ceratopteris thalictriöides, Elodea canadensis, Hieracium Pilosella, Hydrocharis Morsus Ranae, Phyteuma Scheuchzeri, Potamogeton crispum, Ranunculus sceleratus, Salvinia auriculata, S. natans, a.o.) or that they showed equally strong growth in every one of the four partitions, with distinct symptoms of etiolement (Butomus umbellatus, Caltha palustris, Polygonum lapathifolium, P. Persicaria, a.o.). The main cause of this phenomenon was without doubt the insufficient assimilation of carbonic acid, whilst the strong growth in some cases was due to stored-up food, the anatomy of the plants showing every characteristic of etiolement (1—8): few and small intercellular spaces, thin cell walls, weakly developed

palisade tissue, etc. In the investigation on callus formation I met with similar phenomena in Salix Caprea and Sambucus nigra.

Branches of Elodea canadensis produced a rather strong current of oxygene bubbles when placed before a window on the north; this current ceased as soon as they had been placed into the cupboard, in every partition, and reappeared after they had been replaced in full daylight.

I think that it is clear that for the plants above mentioned the assimilation of carbonic acid was not sufficient to surpass the compensation point. Only those species, for which assimilation was stronger than dissimilation, at least for some time during the experiment, (the compensation point does indeed differ for each species of plant (38)), could be used for this research. They will in general be so called heliophobous plants (Ajuga reptans, Alisma Plantago, Ceratophyllum demersum, Glechoma hederacea, Populus nigra, Potamogeton natans, Sagittaria sagittifolia, S. subulata, Sempervivum Funkii, S. tectorum, Vallisneria spiralis. But it must at once be emphasized that these species surpassed the compensation point in only a very slight degree, though sufficiently to reach a certain stage of development. The surplus of assimilation, in other words, was without doubt scanty (somewhat stronger for Ceratophyllum and Vallisneria). This appeared from several facts:

development always ceased after some time, without having reached an adult stage;

flowers were never produced (with one exception); compare Klebs (27) and Vöchting (53);

when the anatomy of the plants was investigated at the end of a research, amylum was hardly ever present in stems or leaves, at the utmost some traces of it, whilst the control-plants of the same age, but grown in full daylight, contained large quantities.

In some cases I measured the dry weight. Most of the

data seem to confirm the views of Lubimenko (35) and Popp (39), viz. in blue relatively the most dry weight was produced. But my observations on this point are too few to justify any definite conclusion, nor do I value them very highly for the following reason: dry weights which have only been determined once during an experiment, viz. at the end of it, do not give enough data for a thrustworthy comparison. It is e.g. possible that for a plant the dissimilation gets the upperhand in the various colours at different stages of development, so that at the end we should get figures which are useless for comparison, the more so as the specimens, grown in full daylight have, at that moment (the end of the experiment) not yet completed their full growth, in other words, their assimilation is still stronger than the dissimilation. Now, it is fairly certain that the opposite is the case for most of the plants, grown in the cupboard; some of them weighed much less at the end than at the beginning. Perhaps it would be worth while to determine the dry weight from day to day, on a large number of specimens, in every colour, but tilll now I have not had the opportunity to do this.

Many of the plants which I investigated, were provided with reserve-food, in rhizomes, tubers, etc. In these cases the scanty assimilation will have had even less influence.

For all these reasons I think I may conclude that, when there appear differences between the specimens in the various colours, these are due to the specific, qualitative action of the different light rays and not to the quantity of light, not to the nutrition. (Compare II, § 11).

Whether we assume, with Kniep and Minder (31) that, the intensity in red and blue (and grey) being the same, the assimilation will be equally strong in these colours, (on green I have to say a few words) or whether we attach more value to the opinion of Warburg and Nägelein (54) (some experiments on Hydrocharis Morsus

Ranae, Phyteuma Scheuchzeri, Polygonum lapathifolium, Ranunculus sceleratus, a.o. seem to point in this direction), the assimilation in my investigations was by no means strong enough to be decisive for the results. This does not hold quite true for the green glasses. Most species do assimilate in this colour, some even fairly strongly (Vallisneria, Ceratophyllum); others, however, apparently do not, development therefore being scarcely possible; in this case we cannot of course ascribe the (lack of) result to the specific influence of these light-rays.

If the cupboard had been turned to the south, I could have made investigations on more species of plants, as the intensity of light would have been greater. But the unequal distribution of energy under those circumstances kept me from doing this.

Téodoresco arranged his researches in such a way, that his plants had full sunlight, at least during part of the day. In my opinion, a rather strong assimilation of carbonic acid must have taken place under these conditions. Therefore I cannot quite agree with him when he says: "Ne pouvant, dans mes expériences, éliminer complètement l'intervention de l'assimilation chlorophylienne, car il faudrait opérer dans une atmosphère entièrement dépourvue de CO_2, je me suis proposé de réduire le rôle de ce phénomène au minimum. A cet effet, j'ai choisi des plantes possédant des quantités suffisantes de substances de réserve: des tubercules, des rhizomes, des graines volumineuses, des spores; en outre, le plus souvent la fin de l'expérience coïncide avec l'épuisement des réserves." It seems very doubtful to me that even a large quantity of stored food could "réduire au minimum" the assimilation of carbonic acid. Nevertheless, his results also prove the preponderant effect of the specific rays. In red, his plants always show most distinct signs of etiolatement.

One other instance may be briefly described to prove

that, even behind dark glasses, more light is transmitted, if the sun shines directly upon them. As has been said above, Hieracium Pilosella could not grow in the cupboard, well developed rosettes no more than seedlings.

A hotbed, facing the south-west, was divided into four partitions, covered with grey, red, yellow and blue glass. Rosettes grown in full daylight, develop a few, short stolons, at the end of which new rosettes are formed; at the end of summer the initial rosette had disappeared; see table 3. When rosettes of the same size were placed under the coloured glass in the hotbed, development is enormous and quite different. Stolons are longer and more numerous; no rosettes at their ends; leaves much bigger. I ascribe this varying growth in the first place to higher temperature, more food and etiolement. For esp. the red and blue glasses were rather thick and dark. Though there are differences between the specimens, grown under red and blue glass (in red: limper constitution, stronger elongation of the stems, leaves erect instead of lying flat, fewer flowers, growth stopping earlier, etc.), their general habitus is very much the same. Assimilation must have been strong. Table 3 gives a survey of the results. The same phenomenon appeared from a similar research on Hieracium stoloniflorum.

TABLE 3.

Hieracium Pilosella; 1926, March 25th—June 17th.

	White	Grey	Red	Yellow	Blue
Average number of stolons	1 à 2	9	6	11	6
Average length of stolons in c.M.	14	39	56	40	37

II. Results.

§ 1. Sempervivum tectorum and S. Funkii.

With these plants only a few experiments were made,

chiefly in order to compare them to those of Téodoresco. The results show that, after the plants had been in the cupboard for resp. 62 and 51 days, those in blue are quite similar to those in white (not elongated), while the plants in red have been elongated strongest of all.

When we compare these results with those of Téodoresco, it appears once more that he has allowed his plants a much greater quantity of light. (The temperature at which we worked was about the same). First the growth of my plants is much less vigorous and esp. in S. tectorum two facts are striking: the strong phototropism of the specimen in red and the epinasty of its leaves, which is almost equally distinct in blue. Téodoresco noticed this only in red, the leaves in blue being on the contrary always normally hyponastic.

§ 2. Glechoma hederacea.

On June 16th 1927 a series of plants was put into the cupboard and remained there till July 25th, control-plants remaining in full daylight. At the beginning the plants were very small, consisted of 3—5 branchlets of a few, at the utmost 6 cm. and with tiny leaves, broad 5—9 mm.

A second series was observed from July 25th till September 12th; these plants were somewhat more robust, leaves with a breadth of about 2 cm.

The results of both investigations were similar. Fig. 2 presents the plants of the 2nd series.

The specimens in grey appear to have been strongly etiolated. The leaves, present at the beginning, have shown some further and normal development; the newly formed ones are quite distinct by their abnormally long petioles and very tiny blades: 5—6 mm. Internodes are also extremely elongated. These phenomena are still more striking in red. The leaves, present at the beginning, are partly dead and have partly remained without further

development; the newly formed ones are still smaller than in grey: 2—4 mm. In green everything resembles the conditions in red, but the development on the whole is very weak; many leaves, moreover, have a sickly yellowish tint.

Quite different, however, is the situation in blue; plants are here very similar to those from full daylight: blades till 27 mm. broad, in white till 33. The conformity is even greater than it could appear from the photograph. It will be seen that there are two features, which in blue are distinctly different from white: petioles are longer and

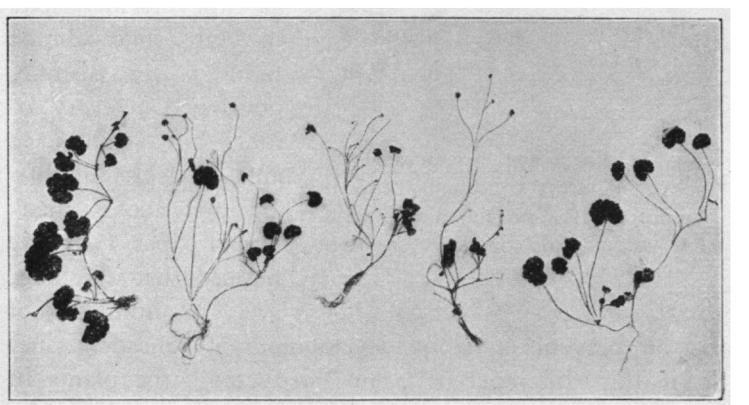

Fig. 2. Glechoma hederacea; 25/7—12/9—'27; f.l.t.r.: white-grey-red-green-blue.

limper and the blades are more reniform. These two variations may safely be ascribed to the, at that time, still too high degree of humidity of the air. For when I made some investigations on this subject in 1922 (11), it appeared that a high degree of humidity of the air makes itself felt in the same way as is shown in fig. 2. Fig. 3 presents two specimens, grown in 1922 at Fontainebleau, one in normal air, one at a high degree of humidity (95—100 %). It will be seen that the differences in both

cases are exactly similar. When this is taken into consideration, in other words, when the deviations from normal growth in the blue plants are "deducted", there remain plants which are normal in every respect.

In tabel 4 I give a survey of some data, concerning the anatomy of petioles and leaf blades. It will not been wondered at, that they agree fairly well for grey, red and green, and that blue approaches the normal state in white. The palisade tissue is very scantily developed in every colour, but again to a smaller degree in blue. In my above mentioned paper I already noticed that a high degree of humidity of the air prevents a strong development of palisade tissue. So if this difference is again "deducted", the plants in blue appear to be very similar to those in white, the others none the less remaining strongly modified.

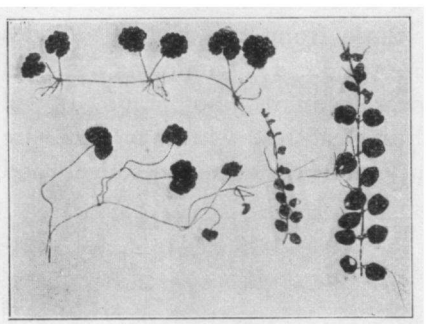

Fig. 3. Glechoma hederacea; 1922; above in normal conditions, below in humid air; (Lysimachia nummularia, left in normal conditions, right in humid air).

From fig. 4-5-6 it appears that in the leaves in red-green-grey the cells are much smaller than in blue and white. The number of cells is the same everywhere. This phenomenon occurs often and will be dealt with further on (§ 11).

The results with Glechoma hederacea teach us some important facts: the portion of the full daylight, which is let through by the grey glass, appears to be insufficient for a normal development. In red and green the same is the case, whilst in blue development is nearly equal to

TABLE 4.

Anatomy of Glechoma hederacea; 1927, July 25th—September 12th.

	White	Grey	Red	Green	Blue
Average thickness of petiole in micra	850	500	500	480	750
Width of vessels	12	10	9	9	11
Amount of starch in petiole	—	—	—	—	—
Development of collenchymatic tissue	++	—	—	(+)	+
Thickness of leaf blade...	150	90	80	80	140
Thickness of palisade tissue	40	16	17	17	28
Amount of starch in leaf blade	++	—	—	trace	—

Fig. 4. Glechoma hederacea; tissue of leafblade in red.

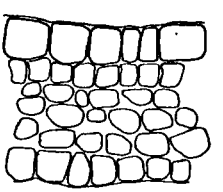

Fig. 5. Glechoma hederacea; tissue of leafblade in blue.

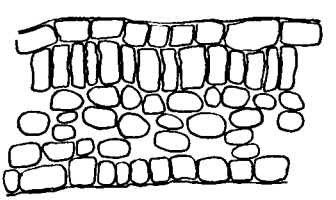

Fig. 6. Glechoma hederacea; tissue of leafblade in white.

that in white. This tallies with the results of most other authors. But the most salient point comes to light when we compare the plants in blue and grey. The predominant importance of the quality of the blue rays will be very clear. The plants in blue have not got more energy than those in grey and yet they do not show the least sign of etiolement. This etiolement in grey is due, not to an insufficient quantum of light in general, but to an insufficient quantum of short-wave rays. Results in green and red are now quite comprehensible.

§ 3. Ajuga reptans.

This investigation was made simultaneously with that on Glechoma. At the beginning, plants were very small, consisted of a rosette of 4 à 6 leaves of a length of about 5 cm. Both series gave a similar result.

After the discussion on Glechoma I can be very brief. The etiolement in grey is not so distinct as in red, it stands midway between red and blue. So the percentage of short-wave rays in the grey partition made itself felt. The development in green was very scanty, perhaps due to lack of assimilation. Blue again approaches the normal state most; influence of humidity must also be taken into account here.

The anatomy shows no important differences between the various colours. The palisade tissue was everywhere poorly developed, when compared with plants in full daylight.

§ 4. Formation of callus by Populus nigra var. italica.

From the same tree a number of branches were taken, long about 20 cm., thick 10—14 mm.; at the lower end they were cut obliquely, at the upper end rectangularly. The lower end was put into water, after they had been entirely immersed for 24 hours. Three of four branches

were put every time into the partitions, and also in full light as well as in darkness. This experiment was repeated six times and, with one exception, always gave the same result: in darkness much callus, no or hardly any shoots; in red the same; in white no or hardly any callus, many shoots; in blue the same, with only somewhat more callus. In grey and green about the same as in blue; esp. for green, this is rather exceptional. So also here red has the same physiological effect as darkness, blue acts similarly as full daylight. Some experiments were made at room temperature in 1930, others in 1931 with heated air. In the last case growth was faster, but the result was the same.

The only exception was seen in a series which was put into the cupboard in the autumn of 1930; first the same phenomena appeared, later on some shoots were also developed in red, so that at the end of the experiment, differences were no longer very distinct. Whether this is

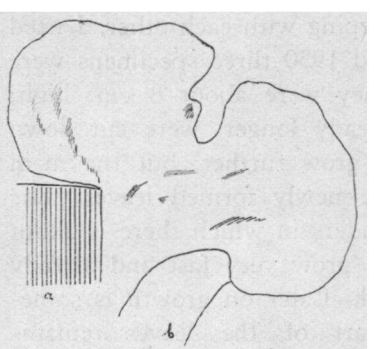

Fig. 7. Populus nigra; radial cut of callus; red.

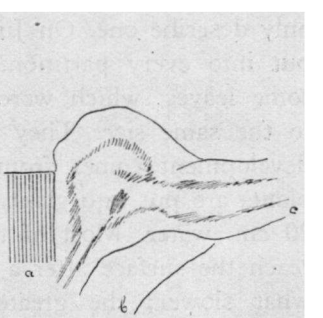

Fig. 8. Populus nigra; radial cut of callus; blue.

due to an influence of season, is a question which must remain unanswered for the moment.

Fig. 7 and 8 present a radial cut through the callus in red and blue (from a series of 1931; it is characteristic for every series, except the case mentioned above). In red

mostly parenchyma and little vascular structure, in blue just the opposite (a means wood, b cortex, c shoot). Simon (45) has also found that in light less callus and more shoots are formed than in darkness. This tallies with my experience. Simon found no anatomical differences, but in this point I cannot quite agree with him. It is true that the differences are not very important, but still it may be expected that where there are many shoots, more vascular tissue will develop and relatively less callus. And this proved to be the case. Moreover, the callus in blue exclusively originates from the cambium, that in red and dark, at least in most cases also, from the inner layers of the phloem parenchyma.

Measurements of the parenchymatic cells did not show any difference worth mentioning.

§ 5. Vallisneria spiralis.

Three investigations were made with this species. As the results were in perfect keeping with each other, I need only describe one. On July 3d 1930 three specimens were put into every partition. They were about 8 cm. high; some leaves, which were already longer, were cut down to the same size. They can grow further, but the main development comes from the newly formed leaves. The plants are put into glass cylinders in which there is about 20 cm. water. Most of them grow very fast and already reach the surface after a week. Later on growth is somewhat slower; the greater part of the leaves remains floating. Table 5 gives a survey of the results. See page 455.

Some specimens in the hothouse of the Laboratory of Technical Botany were used for comparison. Though these were grown at a temperature of at least 25° and the plants in the cupboard at moderate room temperature (summer 1930!), the first were not better developed. The great length, attained by most plants in the cupboard, except in blue, clearly indicates etiolement.

TABLE 5.

Vallisneria spiralis; 1929.

Length in cm. on:	Hot-house	Grey	Red	Green	Blue
July 3rd	9	9	9	9	9
July 17th	29	46	41	33	16
July 24th	37	59	45	49	19
August 8th	41	59	45	49	20
Sept. - 29th	43	63	55	60	20
Amount of starch in stalk on 29/9	++	++	++	+	++
Starch in leaves.....	+	+	+	+	(+)

Most striking here is the strong growth in green. In other series it is still more marked. I think that we must consider this phenomenon in close relation with the amount of amylum in the plants at the end of the investigation. This is exceptionally high, so that we may assume that the supply of light in the cupboard has been sufficient for Vallisneria to assimilate carbonic acid plentifully.

It is true that at the beginning the plants contained rather large quantities of amylum, but it cannot be accepted that after nearly three months this same amount should still be present throughout the plant body. The strong development moreover contradicts this. (In Alisma Plantago we shall see a plant which contains such a large amount of reserve food that it can, even without light, attain a very great development. But in no colour anything of it could be found at the end of the investigation).

In this instance growth must be due, to a much larger extent than in other cases, to assimilation of carbonic acid. But only in blue the quality of light is there to hinder

the excessive height increment, notwithstanding the assimilation. This proves that even when assimilation is strong, the quality of light is a predominant factor for development. (Compare my remarks on the results of Téodoresco).

The thickness of the leaves is equal in every colour to that of hothouse plants. In normal leaves there are outer layers of very small cells, at both sides; the chlorophyll is nearly for 100 % accumulated in them. This holds true also for blue (and green!). In red and grey the situation is different: the outer cells are about the same size of the inner ones and the chlorophyll is spread throughout the whole tissue. It might have been expected that this would also have been the case in green.

§ 6. Ceratophyllum demersum.

This plant was dealt with much in the same way as Vallisneria spiralis. The main results are recorded in table 6.

TABLE 6.

Ceratophyllum demersum; 1928.

Length in cm. on:	White	Grey	Red	Green	Blue
June 1st	10	9	11	12	10
July 19th	29	37	45	41	21

These data recall those of Vallisneria; the same strong development in grey, red and green; in all colours abundance of amylum, proving that CO_2-assimilation must have been rather intense. Yet we observe the same slowness of growth in blue, due to the specific influence of the short-waved rays.

Anatomically there are no modifications worth mentioning.

§ 7. Potamogeton natans.

Glass cylinders of a height of 20 cm. were filled with earth to about 8 cm., the rest with water. In every one were planted two rhizomes with some buds on each. This was twice repeated with similar result.

After about two months growth stopped. Plants, grown in full light get first immersed linear leaves, later-on at the surface floating leaves with well developed blades. In grey and blue the same floating leaves were formed, at about the same time. They remained smaller, were thinner and more limp, but their form is none the less distinct. In red and green only the immersed, linear leaves have developed, the plants could not get beyond the youth-stage, that of the "Primärblätter". Only where a certain amount of short-wave rays was available, this stage was surpassed.

We shall meet with the same phenomenon in the following investigations, which concern also plants with two sorts of leaves.

§ 8. Alisma Plantago.

Glass cylinders of 25 cm. high were half filled with earth and half with water. In each of them one young plant was put. The plants were provided with large rhizomes and had already formed three or four linear, in transverse section triangular leaves, about 6 cm. long. The investigation lasted from January 31th till March 24th 1930. Already after a week most of the plants had reached the surface of the water and had begun to form leaf blades, except in red, where they appeared only about a month later. Growth in red was generally poor, in the other colours very (and equally) strong. On March 24th I noticed the following measurements: See page 458.

The specimens in green and grey are fairly similar to that in blue. The red plant has been very limp from the beginning and gives the impression of having been most

TABLE 7.

Alisma plantago, series I; 1930, January 31st—March 24th.

	Grey	Red	Green	Blue
Greatest length on 24/3 in cm.	60	45	49	55
Number of leaves	12	8	9	13
Dimensions of leaf blade in cm.	15 × 6	7 × 2.3	10.5×4	12×4.5

etiolated, as could be expected. Yet I dared not trust this result, because there had only been one specimen in each partition and the growth in blue was rather too strong.

The investigation was therefore repeated in the same way from March 24th till April 28th. This time specimens were also grown in full daylight and in darkness. On April 28th I noted the following measurements:

TABLE 8.

Alisma plantago, series II; 1930, March 24th—April 25th.

	White	Darkness	Grey	Red	Green	Blue
Greatest length on 25/4 in cm.	12	51	hardly any development	72	71	45
Number of leaves....	10	14		12	9	8
Dimensions of leaf blade in cm.	6 × 2.6	11.5×3.5		12.5×4	9.6×3.1	6.1×2.7

When we compare these measurements with those of the plant in full daylight, it becomes clear that the strong growth is mainly due to etiolement. The enormous development in darkness is especially striking. This plant was of course quite pale, with much anthocyane in stem and leaf veins. But the leaves were fresh and erect. [1])

[1]) Warned by this phenomenon, I also tried other species of plants to see whether they could grow in darkness; in no case this proved to occur to a degree worth mentioning.

The red specimen was again very limp, but this time had big leaves; green was about the same as the former one. Blue had somewhat stayed behind, esp. in the development of the leafblades. This is not to be expected from a specimen in this colour, but at the end of the experiment it appeared that its rhizome was distinctly smaller than of the others.

That the etiolement of the plants was much stronger than the influence of the various light-rays, is clearly shown in the anatomy of the leafblades; (starch could never be found in them). When they are compared with a leaf, grown in full daylight, they differ in the following points: thickness 30—40 % less than normal; palisade tissue poor; intercellular spaces in the spongy tissue very small. These are all typical characteristics of etiolement (1—8); and they occurred, to the same degree, ine very specimen, in both series. The very small leafblades in red of the first series were anatomically perfectly similar to the big ones in the second series.

The differences in the development of the various specimens are therefore certainly due to the different quantities of reserve food in the rhizomes (an exception must be made for the limpness in red; see § 11). Of course it is hardly possible to avoid this difficulty.

This species therefore did not seem to offer a favourable material for this sort of work. Yet I once more repeated the investigation with seedlings and this proved to be better. Seedlings of a length of 1—1½ cm. were planted in shallow bowls, about 30 in each, a few cm. under water.

This experiment lasted from May 5th till June 23rd 1930 and after that till July 14th; the second part will be described in § 11.

On June 23rd the situation was as follows:

white lenght 10 cm. robust leafblades, lifted above the surface.

grey	length	9 cm.	moderately developed leafblades, partly lifted above the surface.
red	„	11 „	linear leaves, floating.
green	„	6 „	poor; some leafblades, but floating.
blue	„	7 „	robust leafblades, lifted above the surface.

Also here the plants in red did not attain the stage of aerial leaves; in green hardly so, because the few developed blades could not lift themselves above the surface. The result is therefore the same as with Potamogeton natans. Grey is again somewhat similar to blue, but more etiolated in its anatomy. Starch was found nowhere, except in the plants in full daylight, where it was formed abundantly.

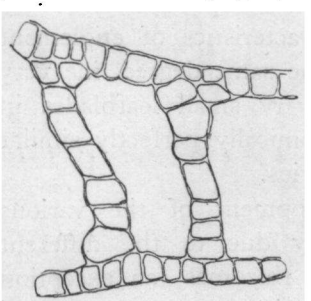

Fig. 9. Alisma Plantago: tissue of immersed leaf in blue.

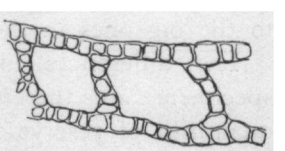

Fig. 10. Alisma Plantago; tissue of immersed leaf in red.

For the rest the anatomy shows that in red, the cells in the leaves are much smaller than in blue; see fig. 9 and 10 (compare Glechoma and § 11). Attention may be drawn to the far-going likeness of the anatomy of aerial leaves in blue to that in white; this holds true also for the immersed leaves.

§ 9. Sagittaria sagittifolia.

On April 28th 1930 some young plants of Sagittaria

sagittifolia were taken from the botanical garden of the Laboratory of Technical Botany, Delft. They consisted of a tuber, out of which had grown a short stem which bore at the end some leaflets, about 6 cm. long. These were planted, two together, in glass cylinders, in such a way that the tops of the leaflets came just out of the earth. Above was a layer of water, 12 cm. thick.

At the end of May the plant in full daylight was so far as to have developed the sagittate leaves; the others did not grow so well. On June 19th every specimen had stopped growing. The result is seen on fig. 11.

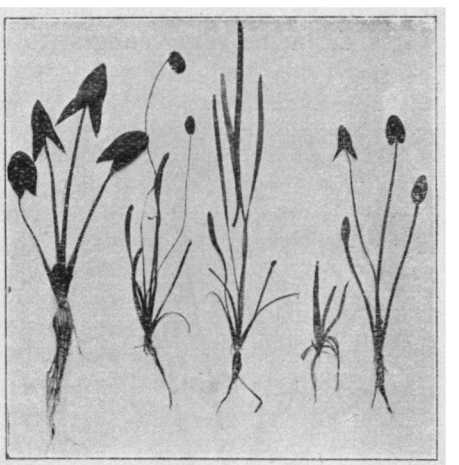

Fig. 11. Sagittaria sagittifolia; 1/5—12/6—'30; f.l.t.r.: white-grey-red-gree-blue.

In grey most of the leaves have remained linear, a few have blades, but without the sagittate form; moreover they are very limp and could not lift themselves above the surface of the water. In red exclusively long, linear leaves of the youth stage; in green hardly any development, most leaves have not surpassed the seedling-stage, which is probably due to the impossibility of assimilation. In blue, however, a habitus which most resembles the normal plants in full daylight with for the greater part sagittate leaves. (Perhaps the humidity of the air has also in this case had some influence, so that in reality the blue plants perhaps still more approach the white ones; compare Schanderl (41).)

Schanderl has observed a distinct difference between plants, grown in full daylight and in the shade. The latter have got shorter and broader leafblades. The palisade tissue is less well developed, the spongy tissue is denser, with smaller intercellular spaces. In all these respects my plants from the blue partition correspond to the shade type. (The compass orientation did, of course, not appear in the cupboard).

The anatomy calls for no further remark; large quantities of starch in the plants in daylight, none in the cupboard.

It may be worth while to compare my plants with a normally developed young plant, which has leaves of all stages, from the very youngest to the adult sagittate ones; see fig. 12. [1])

Fig. 12.

We then see that green corresponds to No. 1, red to Nos. 2 and 3, grey to Nos. 4 and 5, blue to 6, 7 (and 8), whilst only the plant in full daylight reaches No. 9. So, by growing the plants under different wave-lengths, we can separate the different stages of development.

It is well known that Goebel made a plant, which had already developed sagittate leaves ("Folgeblätter"), form linear

[1]) This figure was taken from the Textbook of Botany by professor dr. F. A. F. C. Went, 2nd edition, 1930, page 197, fig. 135. I want to express many thanks to professor Went for his kindly allowing me to make use of this figure.

leaves ("Primärblätter"), simply by giving it less light. He managed this by immersing the plant under a thick layer of water. (In the wild it can often be observed that when Sagittaria grows in deep water, it only develops linear leaves). Now I want to emphasize that in doing so, Goebel probably forgot one thing. I could not find the exact depth of his plants below the surface, but I think it will have been one, at the utmost two meters. Now in this depth there certainly penetrates relatively much of the blue rays, in any case enough for the formation of sagittate leaves. But in my opinion, this thick layer of water is a mechanical hindrance for their formation, because they can only grow in the air (compare Glück, 15). And so it may be strongly doubted if in this case the decrease of light was the primary cause of the formation of linear leaves; in other words, I think that Goebel did not rightly interpret his result.

Moreover, it may be noticed that in my experiment, the layer of water was only thin, 10—12 cm. The leaves in red attained a length of about 40 cm., so that the greater part of them floated on the surface, but yet they did not lift themselves into the air.

Goebel considers the Primärblätter not in the first place as adaptations, in which I agree with him, but as "Hemmungsbildungen", retardation-formations of the Folgeblätter. In the "Einleitung" he says the following (18, page 53): "Die Dunkelpflanzen hatten in derselben Zeit nur bandförmige Blätter gebildet. Dass es sich dabei nicht. etwa um eine durch Nahrungsmangel verursachte Entwicklung handelte, zeigt schon die Tatsache, dass die Dunkelpflanzen Ausläufer trieben, die am Ende zu (natürlich kleinen) Knöllchen anschwollen. Es fehlte also nicht an Bildungsstoffen überhaupt, wohl aber an den spezifischen Bildungsstoffen, welche zur Bildung der pfeilförmigen Blätter notwendig sind; *diese enstehen erst durch die Assimi-*

lationsarbeit der bandförmigen Blätter; (die Bildung der pfeilförmigen wird also unterbleiben, wenn die Assimilationsarbeit infolge Lichtmangels unterbleibt"), (italicized by me) and somewhat further: „Daraus wurde der Schluss gezogen dass Sagittaria durch Lichtmangel auf dem Jugendstadium zurück gehalten werden kann".

I cannot quite agree with this opinion. In no partition of the cupboard the assimilation of carbonic acid can have been very strong, in blue certainly not more than in the other colours. *Yet, only in blue the sagittate leaves were properly developed. They cannot be the effect of the assimilation of the linear leaves.* The "spezifischen Bildungsstoffe" were present; were, however, not formed by assimilation (see also § 11), but by the action of the blue rays. I doubt whether it is necessary to suppose the existence of those "spezifischen Bildungsstoffe". We could equally well express ourselves by saying that only short-waved rays enable the plant to build its organs out of its substances. Both ways of expression give free room to speculation.

I should like to modify the conclusion of Goebel in this way: Sagittaria can be kept back in its youth-stage by lack of blue light or by other means, e.g. by mechanical hindrance (and of course by lack of food also, see the specimen in green). Sagittate leaves will not occur in red, because of the lack of blue rays, but they will neither occur in blue in deep water, owing to lack of air. Once more attention may be drawn to the specimens in grey: the small quantity of blue rays here has caused the formation of leafblades, but it was too small to finish them.

Glück (15) also discusses the influence of the depth of water on the form of the leaves. In the main his conclusions confirm the views of Goebel. So I cannot quite agree with him either, esp. when e.g. he says that the more reserve-food a plant has got, the more chance there will be for the formation of sagittate leaves: a plant in red

light will not develop them, although it should have reserve-food in abundance.

In the wild the plants shoot only late in season, in the beginning of May, out of a thick layer of mud. The surface of the water is in most cases already covered with other plants; the first leaves therefore develop in dim light. By growing them in full daylight from the very beginning, the stage of linear leaves would perhaps be shortened, the sagittate blades would then appear earlier.

§ 10. Sagittaria subulata.

In the hothouses of the Laboratory of Technical Botany there is a basin, filled with Vallisneria spiralis and where there are no other species of plants. Once, when I wanted to make an investigation on Vallisneria, I had material taken from it and put into glass bowls. Though the plants in the basin very probably had originated from one specimen, I yet stuck for all security to the rule that only plants should be taken, which were attached to the same rhizome. It will appear that this precaution was not superfluous. In the beginning the plants grew as normal Vallisnerias, but later, in blue and grey, aerial leaves with well differentiated blade were developed. This seemed strange, as such organs are not known for Vallisneria. The riddle was solved, when in red flowers were formed on two plants. (This was the only case in which, during my researches, plants have blossomed; therefore I will not draw any conclusion from this fact with regard to assimilation, but I only will point out that these plants in red were able to develop flowers, but not to develop aerial leaves). The species could then be determined (4). As no seed was formed and the flowers soon withered, this could not be done with absolute certainty. Yet it is very probable that I had to deal with Sagittaria subulata. In the hothouses this plant had never appeared, that is,

its aerial leaves or flowers had never been seen. At the moment of blossoming in the cupboard I ascertained that in the basin of the hothouse all plants were also in full bloom, but with unmistakable Vallisneria flowers. "Infection" of neighbouring basins proved not to have taken place. The error was in all respects comprehensible, because the immersed leaves of Sagittaria subulata are very like those of Vallisneria, same proportions, three main veins, etc. Only the year after this event, S. subulata was again discovered in the Vallisneria basin; it betrayed its presence by *floating* leafblades; they never lifted themselves above the surface and up till now, blossoming has never occurred. [1])

On January 16th 1930 three young plants were put into each of the partitions of the cupboard under a 10 cm. layer of water. They were about 5 cm. high. On the first of May their length was: grey 14, red 29, green 24, blue 10. Once more the retardation of growth in blue (and here also in grey) is striking; the small quantum of blue rays in the grey partition apparently is important enough for this species, as we will also see later. It seems reasonable that the strong development in green is due to CO_2-assimilation. But, contrary to the case of Vallisneria and Ceratophyllum, at the end of the investigation the leaves contained no starch, or at the utmost traces of it; only in red there was a very small quantum.

On the 5th of June in blue two leaves had lifted themselves 4 à 5 cm. above the surface and had developed blades, distinctly differentiated from the, in transverse section oval petiole. On the 12th of June there appeared two aerial leaves in grey, in blue two new ones were formed.

[1]) An amateur of aquariums informed me that it had often happened to him that plants, bought as Vallisneria spiralis, had formed aerial leaves, quite similar to those in my investigation.

On June 16th there were already five of them in grey. The investigation lasted till July 7th.

Sometimes the differentation between blade and petiole is more distinct in grey than in blue, sometimes the opposite was the case.

The anatomy of the aerial leaves is of course quite different from the immersed ones; well developed palisade tissue, numerous stomata on both sides, etc.; the immersed leaves have a poorly differentiated tissue with big hollows; in this respect, Sagittaria subulata very much resembles S. sagittifolia.

If we may assume that CO_2-assimilation was rather intense, considering the development in red and green, it is even more evident than in the case of S. sagittifolia, that the assimilation is not responsible for the formation of aerial leaves, as these were formed neither in red nor in green. This is another proof that the quality of light is the main cause of the final habitus of the plants.

§ 11. Exchange Experiments — Discussion.

Some additional experiments on Alisma Plantago, Glechoma hederacea and Sagittaria subulata may still be briefly described.

When the specimens of Alisma had reached full development (see page 459—460), I interchanged the bowls in the partitions, between red and blue and also between grey and green. The plants reacted within a few days. The erected leaves with well developed blades from grey and blue, became limp and floating in green and red. In red they soon withered. Afterwards only immersed, linear leaves were formed (sometimes in green a faint indication of blades, but alway immersed).

The plants from red and green formed in blue and grey erected leaves with well developed blades, in blue some days earlier than in grey (compare below Sagittaria subulata).

This change was finished within 12 days. Growth then stopped, probably owing to lack of carbohydrates.

The same experiment was made with Sagittaria subulata after the final result was reached, viz. when in grey and blue aerial leaves had been formed. Within a week the aerial leaves from grey and blue had become limp in green and red, and partly disappeared. In blue (from red) two new aerial leaves were developed. After a few more days, there were four of them and they appeared also in grey, though somewhat later (as before the change), which is due without doubt to the smaller quantity of blue rays.

This result bears a striking resemblance to that obtained with Alisma Plantago.

For the sake of clearness I give the whole of the experiment with Sagittaria subulata in tabular form.

TABLE 9.

Development of Sagittaria subulata; 1930.

Length in cm. on:	Grey	Red	Green	Blue
Jan. 16th	5	5	5	5
March 6th	12	28	23	5
May 1st	14	29	24	11
June 5th				2 aerial leaves
June 12th	2 aer. l.			4 aer. l.
June 16th	5 aer. l.			
June 19th		bloom		
June 23rd	change			
June 30th				2 aer. l.
July 3rd				4 aer. l.
July 14th	2 aer. l.			

Specimens of Glechoma hederacea, which had got the habitus, typical for red and blue (see fig. 2), were also mutually changed. Within a fortnight the very tiny leaves from red had grown to the normal size, while in red the leaves, formed in blue, remained; again the newly developed ones were small.

In my opinion, these results prove distinctly that CO_2-assimilation cannot be the cause of the formation of aerial leaves, "Folgeblätter", typical for the adult stage of Alisma and Sagittaria. It is once more evident that their existence is only made possible by a certain amount of blue rays.

I have already pointed out the fact that the specimens of Alisma Plantago, developed from reserve-food, whether big or small, were always very limp, made the impression of being hardly turgescent. The same phenomenon was, more or less, noticeable in my other investigations; in green it could generally be seen too, in grey only then, when the small amount of blue rays in this colour remained below the necessary minimum for the plants concerned (see e.g. Sagittaria sagittifolia).

Plants which can develop aerial leaves as well as immersed ones, never form them in red, though the immersed leaves can get so long as to be floating for the greater part on the surface.

In the exchange experiments we see aerial leaves get limp in red and green; the cannot maintain themselves in an uplifted position, but become floating.

When we look for an explanation of these facts, we may assume that the limp condition in red, (green and sometimes grey) is caused by a diminished turgescence.

Turgor can decrease by stronger transpiration. But in the partitions of the cupboard I could never notice a difference in this respect, which could be due to the various colours.

A more probable explanation would be, that the evaporated water is less easily supplemented in red than in blue, in other words, that the long-waved rays hinder the waterabsorption or the suction of the protoplasm.

This subject is much discussed and not in the least solved. Most authors contradict one another on this point. It is known that a high temperature lessens viscosity and increases waterabsorption (25). Is it conceivable that blue and white light have an identical influence? Lepeschkin (34) and Tröndle (50) are of this opinion, but the results of Herbert (23), Huber (24) and Weber (55) seem to contradict it. These authors have mainly investigated the permeability of salts (and on various species of plants), but the results obtained do not justify any definite conclusions. Anyhow, I could not get rid of the impression that light does have some influence on the waterabsorption in the above shown way.

In my paper on tropical leaf joints (12) I pointed out that when poured-out leaves lift themselves up, the joints are taking in water, esp. in their lower half and that at the same time, the cells become larger. Kleinhoonte (29) proved that light accelerates the rising of the leaves of Canavallia ensiformis and retards their going down. I will not decide how the distribution of turgescence in the various parts of the joints of Canavallia ensiformis is changed during this process, but only emphasize the main point, viz. that the turgor is very likely influenced by light in the way, indicated above. And when in this connection we remember the experiments of Sachs (40), which show that blue rays have the same influence on plant movements as white light, but that red acts as darkness, I think we may justly suppose that white and blue light further the capacity of waterabsorption of the protoplasm and that other rays and darkness act in the opposite way.

If this be true, many phenomena would be explained: the limper condition of the plants in red, the impossibility for waterplants to develop aerial leaves in this colour, the results of the exchange experiments, etc. But in this case, other proofs of the theory would certainly not be lacking and in this connection I may draw attention to the following: when less water is taken in, the cells on the whole will remain smaller than they are normally. This will not always be the case in a very high degree and we may not expect to see it very markedly in every plant species. Only in Alisma Plantago and Glechoma hederacea the phenomenon was very clear (see fig. 4—5—6—9—10). It can be seen at once, that the smallness of the leafblades in red is due to the dimensions, not to the number of the cells. In the exchange experiments, when the plants come from the red partition into the blue one, the increase in size of the cells could be very well followed, esp. in Glechoma. [1])

[1]) Phyteuma Scheuchzeri belongs to the species which did not grow well in the cupboard. They nowhere formed the long and narrow leaves of the adult stage, but kept to developing the round primary leaves. Only in red these were much smaller (± 9 mm.) than in blue (± 20 mm.) and their anatomy showed the same characteristics as in Glechoma and Alisma; see table 10.

TABLE 10.

Anatomy of Phyteuma Scheuchzeri; 1931.

Average thickness in micra of:	Red	Blue
Middle vein	42	100
Leaf blade	35	60
Parenchymatic cells in middle vein	9	12
Epidermic cells	5 à 6	9 à 10

Everything I have said on this subject is, till now, highly speculative. Minute and exact experiments (e.g. with potometers, on the quickness of plasmolysing, on the influence of long and short wave-rays on the physical and chemical properties of the protoplasm, etc.) are necessary, but I have not yet had time for them. Only some preliminary observations on this point were made, which, for the moment, could perhaps confirm my supposition:

leafbuds of Fraxinus excelsior unfolded quite normally in blue; in red they made a faint attempt and after a few days withered;

specimens of Mimosa invisa with outspread leaves were put into the blue and red partitions; after 45 minutes blue was not changed, in red every leaf was partly folded. After 24 hours the folding had been completed, the leaves in blue were still outspread. The plants were changed and after a few hours, they had reacted very distinctly to the new colour. When this change was repeated, reaction became slower and was limited to the youngest leaves, but still very marked;

similar experiments with Mimosa pudica are being made.

For the moment I venture to conclude that most of the phenomena, described in this paragraph, point to a marked difference in the action of red and blue rays on the water-absorption of protoplasm.

§ 12. Decrease of diastase of Aspergillus niger and A. Oryzae.

A few years ago I observed that the diastase of Aspergillus niger is slowly reduced by light (13), whilst in darkness it keeps its concentration for an indefinite time. The question arose, in how far the various wave-lengths were responsible for it. That they do have a different influence was to be expected from what many authors

already found on this subject (though they experienced with other sorts of enzymes).

Went (56) noticed that in Monilia sitophila carotine is formed in light and not in darkness; and also that in this respect red rays have the same action as darkness, blue ones the same as white light. Moreover, the maltase of this fungus is reduced by white light and keeps its concentration in red and darkness. In blue it decreases, though slower than in white light.

Czapek says about this subject (6): "Während zerstreutes Tageslicht Enzymlösungen meist nur unbedeutend in ihrer Wirksamkeit herabsetzt, kann man durch intensive Sonnenstrahlen oder durch konzentriertes elektrisches Licht stets schon in kurzer Zeit die Enzyme stark inaktivieren". — "Übereinstimmend haben zahlreiche Untersuchungen ergeben, dass der Hauptanteil dieser Inaktivierung auf Rechnung der ultravioletten Strahlen zu setzen ist. Ultraviolettfreies Licht ist nämlich nicht imstande ohne Sauerstoffzutritt zu inaktivieren, so dass hier gewiss Oxydationsprozesse anzunehmen sind".

Euler (9) says: "—; diese Forscher (Jamada and Jodlbauer) konnten feststellen dass die sichtbaren, durch Glas durchtretenden Strahlen des Sonnenlichts, für sich allein imstande sind, Saccharase zu schädigen, aber ausgesprochen nur dann, wenn Sauerstoff zugegen ist". — „Über die Stabilität der Katalase im Licht liegt eine umfassende Untersuchung von Lockemann, Thies und Wichern vor. Die hemmende Wirkung, welche das Licht auf die Blutkatalase, sowohl während der Aufbewahrung der Blutlösung als auch während der Reaktion mit H_2O_2 ausübt, ist in folgender Weise von der Wellenlänge abhängig: Weiss>Blau>Rot>Dunkel. Nach Zeller und Jodlbauer tritt jedoch auch hier eine erhebliche Schädigung durch sichtbare Strahlen nur dann ein, wenn Sauerstoff zugegen ist.

Zu einem analogen Ergebnis kamen Zeller und Jodlbauer und fast gleichzeitig Bach hinsichtlich der Peroxydase; auch hier wirken die sichtbare Strahlen nur in Gegenwart von Sauerstoff".

I can observe to this, that in my researches the ultraviolet rays are practically excluded and that moreover control-investigations were made, in which flasks with the enzyme were put under an extra jar of thick glass. So the light had to penetrate through four glasses before it reached the liquid. No differences could be stated when compared with investigations without the extra jar. (To see whether infra-red rays acted on the diastase, parallel investigations were made with and without a layer of water, but with the same negative result).

In my experiments oxygene was always present. The solutions of diastase were kept in flasks, which were corked and provided with some drops of toluol. To at most 50 c.c. of solution, there were always 50—75 c.c. of air. I did not try whether diastase decreases in concentration without oxygene.

It was my aim to know if, and how, diastase is decreased by the various rays and further how they influence its formation. Aspergillus niger was oculated on a solution of 0.5 % amylum with some salts, mostly in big flasks in which there were 250 c.c. of culture-liquid. As soon as a sufficient quantity of diastase was formed, the solution was filtered off and divided in smaller flasks. These were put into the partitions of the cupboard, also in full daylight and in darkness. The solutions were colourless and perfectly transparent. The results can be seen from tables 11—12— 13—14. They concern only some of the series cultivated, but are typical for all of them.

TABLE 11.

Aspergillus niger; series I; decrease of diastase; original concentration 750; pH 4.—.

Days	Darkness	White	Sunlight
0	750	750	750
1			210
4	750	350	
7	750	210	45
11	750	100	
18	750	53	
25	750	42	15

TABLE 12.

Aspergillus niger, series II; decrease of diastase; original concentration 940; pH 4.—.

Days	Darkness	Grey	Red	Green	Blue	White
0	940	940	940	940	940	940
4	940	940	940	940	650	550
7	940	790	940	880	480	330
14	940	530	940	840	300	200

TABLE 13.

Aspergillus niger, series III; decrease of diastase; original concentration 520; pH 4.5.

Days	Darkness	Grey	Red	Green	Blue	White
0	520	520	520	520	520	520
7	520	520	520	520	330	300
14	470	430	460	470	115	110

TABLE 14.

Aspergillus niger, series IV; decrease of diastase; original concentration 555; pH 5.—.

Days	Darkness	Blue I	Blue II	White
0	555	555	555	555
3	555	500	500	455
7	555	430	430	280
10	555	375	395	150
21	555	185	150	50

In series I (table 11) and II (table 12) it appears that again blue acts as white light, red as darkness. In grey there is also a slight decrease, which may be ascribed to the blue rays it contains.

Sometimes the concentration of the enzyme is not quite stable, so that it is slightly reduced even in darkness and colours other than blue. But in this case, the action of blue presents such a striking difference, that we may assume that the same result is obtained (see series III, table 13). Series I and II had a pH of about 4.—. In series III this was $\pm$ 4.5. The decrease is not so fast here and it is still slower in series IV (table 14), where a pH of $\pm$ 5.— was obtained by buffering the culture-solution with K_2HPO_4. As this experiment was repeated with some dozens of series and as they gave similar results, it may be concluded that there exists a relation between acidity and sensibility to light. (I draw attention to the fact that in series III, the diastase in grey is not decreased as intensely as in series II. Possibly the small amount of blue rays in grey has less influence when pH is 4.5, than when it is 4.—). When the culture solution is still more buffered, Aspergillus niger grows poorly and produces too little diastase to investigate on.

When the fungus was grown in the various colours, results were rather unexpected: the flasks with nutritive liquid were oculated with conidia and then put into the cupboard. The culture-liquid was perfectly transparent, light could freely penetrate it, so that I expected that in white and blue germination would at least be retarded, as the diastase, necessary for the hydrolysis of the amylum (the only carbohydrate available) could not, or hardly, be produced in these colours. But this was not the case; germination was equally strong everywhere. The same can be said of the dry weight of the mycelium, the quantity of the conidia and also of the production of diastase. (I do not think it necessary to give the full data, as they very much resemble those published elsewhere).

But when the culture solution was filtered off and again put into the cupboard, the decrease was as described above. Diastase, originated in blue and white, lost its concentration in these colours, but was quite stable in darkness. See table 15; as there are always individual modifications in the quantities of enzyme, produced by various cultures, I here give the data in percentages, as only in this way they can be compared. The production was at any rate of the same degree of intensity.

TABLE 15.

Aspergillus niger; remainder of original concentration of diastase after 12 days; pH about 4.2.

	Dark-ness	White	Grey	Red	Green	Blue
Kept in darkness .	100 %	100 %	95 %	92 %	91 %	95 %
Kept in own colour	37 %[1])	33 %	75 %	89 %	70 %	33 %

[1]) kept in white.

It might be assumed that in the cultures, grown in the various colours, the diastase was sheltered from the light by the shadow of the mycelium and that this explains the equally strong production of enzyme everywhere. But this cannot hold true for the first week after oculation, as just then most of the diastase is formed and there is not yet any mycelium to shade it. All the same I tried if there is some truth in it and therefore I killed some cultures by adding toluol, without taking away the mycelium. They remained in full daylight, the mycelium covered the whole surface of the culture solution, there was a thick layer of black conidia, so that the liquid was well shaded. Yet the decrease of the enzyme was quite normal, though somewhat slower.

The same investigation was made with Aspergillus Oryzae. This fungus produces the greatest quantities of diastase on buffered solutions, pH 6—7 (14). The enzyme appeared to be perfectly constant in every colour, also in full daylight, even when it was put before a window on the south, where it was exposed to direct sunlight for the greater part of the day. The same holds true for cultures, grown in the different partitions: dry weight, conidia, diastase, everything was similar. Only behind the window on the south, germination and growth were very slow, only little mycelium was formed, but the quantity of enzyme was as large as in other circumstances.

Now for the diastase of Aspergillus niger there seems to exist a relation between acidity and rate of decrease. The latter increases with a higher degree of acidity. This might be possible also for A. Oryzae and it is conceivable that in the strongly buffered culture solutions the pH 6.— à 7.— shelters the enzyme against the influence of light. Therefore A. Oryzae was cultivated on less buffered liquids, pH being about 5.—. The quantity of diastase is then much less, but sufficient for the investigation. At a still

lower pH hardly any enzyme is produced. Parallel to these experiments, some were made on diastase, originated in buffered solutions, which by adding oxalic acid were also brought to pH $\pm$ 5.—. Results were the same in both cases; the diastase is very unstable and strongly decreases in concentration. But a special influence of some sort of light could not be stated. The decrease is very irregular, at one time most distinct in white, but equally often shown in darkness or in one of the partitions of the cupboard. Apparently the influence of acidity is predominant here. It is clear that the diastases of Aspergillus niger and A. Oryzae are very different substances.

III. Summary.

1. The researches were made in a cupboard, which is divided into four partitions. Their fronts are provided with glass filters, which resp. let through red, green, blue and subdued white light, in the same intensity, viz. about 25 % of the energy of diffuse daylight.

   The ultraviolet and infra-red rays, which pass through the filters, can be neglected.

   An installation was made to pass a slow current of air through the cupboard, so that temperature and humidity could be regulated.

   The quantity of light proved insufficient for many species of plants, probably because assimilation of carbonic acid was not intense enough. Results were obtained with plants, where apparently assimilation was stronger than dissimilation, but, with rare exceptions, the surplus of the former was only small.
2. Investigations with Sempervivum tectorum, S. Funkii, Ajuga reptans and Glechoma hederacea make clear that the development in blue is very similar to that in full daylight; red has the same influence as darkness; in green the phenomena resemble those in red, whilst

in grey (subdued white light) they remain about midway between red and blue.

3. Generally the same holds true for Vallisneria spiralis and Ceratophyllum demersum, though these species showed a fairly intense assimilation.
4. Populus nigra forms much callus, without shoots, in darkness and in red, little callus with numerous shoots in white and blue; and also in grey and green, the latter being an exception.
5. Potamogeton natans, Alisma Plantago, Sagittaria sagittifolia and S. subulata form two sorts of leaves: in their youth linear, immersed ones and later on aerial leaves with well differentiated blades. In red and green the plants never develop beyond the linear leaves, never surpass their youth-stage. In blue, and to a smaller degree in grey, also the aerial leaves with blades are formed, quite the same as they appear in full daylight. This is not due to assimilation.
6. Exchange-experiments were made with Glechoma hederacea, Alisma Plantago and Sagittaria subulata; when the stage of development, obtainable by the light in the cupboard had been reached, the specimens from red and blue were interchanged and equally those from green and grey. The result is an immediate reaction, in such a way, that they adapt themselves to the new colours. The aerial leaves, which had been formed in blue and grey, disappear in red and green, whilst only immersed ones are newly developed; and vice versa.

   It is supposed that red light and darkness retard the waterabsorption of the protoplasm, this being furthered by blue and white. If this supposition be true, a great number of phenomena, observed in this research, would be explained.
7. The diastase of Aspergillus niger is destroyed by

white light and equally by blue rays. In darkness, red and green, it is constant. In grey there is a slow decrease. When the degree of acidity is lowered, the decrease is slower in all colours.

The formation of diastase is not influenced by any of the colours, not even by full daylight.

The diastase of Aspergillus Oryzae is not in the least sensible to light at a pH from 6.— to 7.—. At a pH 5.— it becomes unstable, but the decrease in this case is due to acidity rather than to light.

It always appeared that the quality of the light rays has a predominant influence on the outer and inner development of the plants.

In blue, habitus and anatomy are equal to, or approach the normal state in full daylight.

In red, plants are etiolated as in darkness (except of course that chlorophyll is formed).

In green, phenomena are either the same as those in red or development is reduced to a minimum; the latter is undoubtedly due to a total absence of assimilation of carbonic acid.

In grey, development now resembles that in blue, now that in red, depending on the needed quantity of blue rays being small or great.

The cupboard, which was used for this research, was built in the Laboratory of Technical Botany, Delft, thanks to the initiative of the director, professor dr. ir. G. van Iterson. I was allowed to use it throughout my experiments. For this I want to express my heartfelt gratitude to professor Van Iterson, and also for his continual interest in all other respects.

In the laboratory as well as in the botanical garden, there have been many, to whom my investigations caused

much extra word. I have great pleasure in thanking them for their very valuable help and assistance.

Finally my colleague, Mr. C. Deelder, Schiedam, once more had the extreme kindness to help me to correct the translation of this paper. Also to him I owe a great debt of gratitude.

Delft, Laboratory of Technical Botany. October 1931.

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