WAVE LENGTH DEPENDENCE OF PHOTOMORPHOGENESIS IN PLANTS

J. A. J. STOLWIJK

Dit proefschrift met stellingen van

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De Rector Magnificus der Landbouwhogeschool,
W. F. EIJSVOOGEL

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS IR W. F. EIJSVOOGEL,
HOOGLERAAR IN DE HYDRAULICA, DE BEVLOEIING,
DE WEG- EN WATERBOUWKUNDE EN DE
BOSBOUWARCHITECTUUR
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
DER LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG 10 DECEMBER 1954 TE 16 UUR

DOOR

J. A. J. STOLWIJK

H. VEENMAN & ZONEN – WAGENINGEN – 1954

STELLINGEN

I

De photoperiodiciteit is slechts een van de vele processen, die via een en hetzelfde photoreceptor systeem onder invloed van het licht verlopen.

Dit proefschrift

II

De formatieve reacties van planten op licht van hoge intensiteit berusten op een lichtgevoelig systeem dat verschillend is van het systeem dat de reacties op licht van lage intensiteit bepaalt.

Dit proefschrift

III

Bij aanvullende belichting van planten met licht van lage intensiteit is de werking van het golflengte gebied tussen 500 en 700 mμ antagonistisch ten opzichte van de werking van blauwe en infrarode straling.

Dit proefschrift

IV

De argumenten die BORTHWICK c.s. aanvoeren ten gunste van een photochemisch evenwicht van twee in elkaar overgaande pigmenten zijn niet dwingend.

H. A. BORTHWICK *et al.* Bot. Gaz. **115**, 205–225 (1954)

V

De theorie van LANG en LIVERMAN dat het groeistofgehalte via een morphogenetische photocyclus bepalend zou zijn voor het al of niet bloeien van korte en lange dag planten, is in deze vorm niet houdbaar.

A. LANG and J. L. LIVERMAN, Proc. 8th Intern. Bot. Congr., section 11, 12, p. 330–331, Paris 1954

VI

Een gunstige invloed van een wortelsnoei als cultuurmaatregel bij gewassen als koffie, cacao, rubber, is niet een gevolg van de verwijdering van een deel van het wortelstelsel doch duidt op een uitgesproken ongunstige physische bodemconditie in de aanplant.

VII

Er bestaat een „vrije ruimte" in de plantenwortel waarin opgeloste stoffen ongehinderd, door vrije diffusie, kunnen binnendringen.

G. W. BUTLER, Physiol. Plantarum **6**, 617–635 (1953)

VIII

Het verdient aanbeveling de practische bruikbaarheid van daglengte behandelingen als hulpmiddel bij de veredeling van de aardappel te onderzoeken.

IX

Het streven naar stichting van internationale centra voor fundamenteel wetenschappelijk onderzoek dient gestimuleerd te worden.

Diss. J. A. J. Stolwijk (Wageningen, 1954)

Aan mijn ouders

VOORWOORD

Gaarne benut ik de gelegenheid om op deze plaats mijn dank te betuigen aan allen die op enigerlei wijze hebben bijgedragen tot mijn wetenschappelijke vorming.

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De wetenschappelijke medewerkers van het laboratorium voor plantenphysiologisch onderzoek vormen door hun grote belangstelling en spontane hulpvaardigheid een milieu waarvan een zeer stimulerende invloed uitgaat. Ik ben hen allen hiervoor zeer erkentelijk.

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CONTENTS

[1]) *Laboratory for Plant Physiological Research, Agricultural University, Wageningen, Netherlands: 128th Communication.*

CHAPTER I

THE STUDY OF THE FUNCTIONS OF LIGHT IN GROWTH AND DEVELOPMENT OF HIGHER PLANTS

Light supplies the energy required for the assimilation of carbon dioxide in the photosynthetic process. This, however, is not the only function of light in plant growth. Light is also responsible for the outward appearance of plants, it determines the height, the dimensions of the leaves, the length of the internodes, and many other aspects which together constitute the "normal appearance" of a plant, as opposed to the "etiolated appearance" acquired in the absence of light.

These light functions can roughly be divided into two groups, according to some characteristics that the functions of one group have in common. Provisionally these groups may be distinguished as photoenergetic and photostimulus processes (73).

Photosynthesis belongs to the first group. This process requires rather high light intensities for measurable responses, and at light intensities below the saturation level the rate increases linearly with light intensity. The over-all reaction is endothermic, and at light intensities below the saturation level a constant, and relatively high fraction of the light energy is converted into chemical energy. The compounds in which this chemical energy is stored, supply the energy for the remainder of metabolic activities and are used as raw materials in the growth and expansion of the organism.

The second group contains a large number of processes, such as phototropism, photoperiodism, internode inhibition, leaf growth promotion, seed germination, etc. These processes are characterized by relatively low light energy requirements, in general by a non-linear relation between light intensity and effect, and by the fact that the over-all process is endothermic. The amount of energy released or directed may be accepted to exceed strongly, in most cases, the energy input by the light. Under certain conditions, the effect is linearly related to log energy. In photosynthesis, carbon dioxide is used and carbohydrates are formed, so that the rate can be measured, either by the disappearence of carbon dioxide or, *e.g.*, the formation of carbohydrates. In photo-stimulus processes, there is no reason to suppose that the nature of the

initial photoreaction is fundamentally different from that of the initial photoreaction in photosynthesis. However, in photostimulus processes the primary effect of the light reaction seems to be destructive rather than synthetic. The extremely low light intensities required (*cf. e.g.*, [48], [26]), entail great difficulties for the measurement of any product formed in stoechiometrical relation to the input of light quanta. Therefore, so far, in most studies of these processes the response of the plant rather than the substances immediately formed or destroyed, has been used to estimate the effect of the light. In some cases, especially with auxins, separate, biological test methods have been used but the relation between energy input and auxin concentrations was found to be complicated (*cf.*, *e.g.*, [45] and [46]). The method of using the plant itself as a measuring device has, of course, the disadvantage that in case the condition or substance involved, and the mechanism of the reaction of the plant are insufficiently known, it is difficult to establish anything quantitative about the over-all process. This procedure has analogy in such studies on photosynthesis, in which the rate of this process is estimated by measuring an indirect effect. An example of such sort, is found *e.g.*, in some experiments by WENT (80), in which photosynthesis was estimated by measuring the rate of stem elongation in tomatoes, but only after the relation had been verified by the comparison of sugar content data and stem growth measurements. This kind of verification, however, is usually impossible in photostimulus processes, because the nature of the process and its products are unknown. The long chain of reactions between the first photoreaction and the change finally observed in the plant (length growth, leaf growth, flower initiation etc.) is extremely vulnerable to correlative influences of processes which are regulated either by the same or by different environmental factors. This necessarily implies that studies of these stimulus processes must be made under rigidly controlled conditions, otherwise the results will be irreproducible.

Many controversies in the literature on photostimulus processes could be ascribed to differences in methods, and inadequate control of environment.

In the present study some of the earlier observations on qualitative effects of light of various wave length regions have been repeated and extended under carefully controlled conditions, using equipment which is provided with a combination of selective light sources that have become available, and suitable glass filters.

Most studies of photomorphogenetic processes have been made with etiolated material. Although this method has the advantage that the experiments can be of relatively short duration, and require a relatively small space, it has a big disadvantage in that the result cannot always be applied to light grown plants (see Chapter II). Several attempts have been made to study photomorphogenetic reactions in light grown plants but in many of these the light intensity, the spectral purity of the light, or other environmental factors were insufficiently known or controlled for a quantitative evaluation of the results.

The object of this study has been to quantitatively investigate some morphogenetic effects of different well defined wave length regions on plants, applied at known intensities, either supplementary to white light, or as the only source of light energy. Special attention has been given to leaf shape, internode elongation, and flowering. We have made use of two equipments, to be described in Chapter III.

During the course of the experiment and often also previously, the plants

have been exposed to light of known duration, constant intensity, and well defined spectral composition, while temperature and humidity were controlled.

CHAPTER II

REVIEW OF LITERATURE

1. INTRODUCTION

A complete review of the extensive literature dealing with the effects of light on plants, as was given in 1936 by BURKHOLDER (11, 12), is beyond the scope of this paper. The review given below will be limited to certain light effects other than photosynthesis, in higher plants. Special attention will be given to the spectral dependence, the characteristic that has been studied most in many of the morphogenetic effects of light in higher plants.

Etiolated, as well as light grown plants have been used in studies on the effects of light. Many indications have been obtained that light grown plants somehow adapt themselves to light, resulting in modified responses as compared with the same plants in etiolated condition. VAN OVERBEEK (46), and THIMANN and SKOOG (67) showed that the sensitivity to auxin, and its production is different in plants, grown in light and in darkness. More recently, GALSTON and BAKER (25), working with stem sections of *Pisum*, and also VAN DER VEEN and DAAMS (71) found that the combined effects of light and auxin were quite different in etiolated and light grown sections. The nature of this adaptation is still uncertain, although TRUMPF's results suggest that there is no direct relationship with photosynthesis, or even with the green leaf pigments (69). TRUMPF obtained plants without chlorophyll that did not differ morphologically from normal green plants, by exposing *Phaseolus* seedlings to light from a carbon arc, given daily at high intensity during 30 minutes or less. It may be assumed that under these circumstances some chlorophyll might have been formed, which, however, may have disappeared in the dark periods. TRUMPF did not do chlorophyll determinations.

Internode growth in etiolated *Avena* is inhibited most effectively by red light (*cf.*, *e.g.*, GOODWIN and OWENS [26]). In light grown plants red light can have an inhibitory (STOLWIJK [65]) or a promoting effect (WASSINK and STOLWIJK [79]) on internode elongation depending on the experimental conditions.

In photoperiodism another example is found of differences between light and dark grown plants. LEOPOLD (39) observed flower initiations in complete darkness, in etiolated plants of several species that once they are light adapted, require a certain day length for flower initiation. DE ZEEUW (85) observed, however, that *Perilla* flowered even in continuous light, provided the light intensity was very low.

In phototropism, PRIESTLEY (52) found differences in the energy required for phototropic response of light and dark grown plants. ATKINS (2) has found that green *Avena* plants are phototropically sensitive to red light, whereas etiolated *Avena* seedlings show no phototropic response to red light (4, 29). In view of these differences between dark grown and light adapted plants with respect to their light response, it is felt that their photomorphogenetic reactions should be discussed separately.

2. PHOTOMORPHOGENETIC REACTIONS IN ETIOLATED PLANT MATERIAL

In some plants seed germination is light sensitive. Some seeds, such as *Phacelia tanacetifolia* (REMER [55]) and *Nigella sativa* (KINZEL [31]) require darkness for germination, whereas many others, such as some varieties of *Nicotiana tabacum* (HONING [27]) and *Lactuca sativa* (FLINT [15]) show a light promoted germination. The action spectrum for the effect of light on germination of lettuce seeds was first measured by FLINT and MCALISTER (16). Their results have been confirmed by recent experiments of BORTHWICK *et al.* (9). The action spectrum for promotion of seed germination in lettuce shows a peak at about 640 mμ, and decreased activity in the yellow and green wave length regions. Near infrared radiation inhibits seed germination, with a maximum inhibition at about 730 mμ. BORTHWICK and coworkers did not confirm the action spectrum in the blue region as it was measured by FLINT and MCALISTER. As will be discussed in Chapter VIII, these discrepancies could perhaps be explained by differences in the experimental techniques. EVENARI and NEUMANN (13) ascribed the inhibitory effect of blue light to the infrared transmission of blue filters often used for this type of experiments. This criticism, however, cannot be applied to the work of FLINT and MCALISTER, who discovered the inhibitory effect of the infrared by investigating discrepancies between green filters with different infrared transmissions. Moreover, the accuracy of FLINT and MCALISTER's spectrographic method was not inferior to that of the technique used by EVENARI and NEUMANN. The mechanism of the reaction governing seed germination is still very obscure. HONING (27) could demonstrate that the factor responsible for the light requirement of tobacco seeds is situated in the embryo, and not in the seed coat. Many investigations have shown that temperature, oxygen pressure, carbon dioxide pressure, removal of the seed coat and other factors have an effect on dormancy of lettuce seeds.

Phototropism also has been studied in many investigations with etiolated plants. BLAAUW (4) already obtained an action spectrum of the phototropic reaction in the *Avena* coleoptile. He found maximum effectiveness near 450 mμ, and little or no effectiveness in the red and infrared regions of the spectrum. JOHNSTON (29) repeated these measurements using a compensating light source and found maxima at 440 and 475 mμ, with a sharp drop towards the green (5% of maximum effectiveness at 500 mμ), and more gradual decrease in the violet (40% at 400 mμ). More recently, GALSTON and BAKER (24) also measured the action spectrum for the phototropic reaction in *Avena* coleoptiles and obtained similar results. OPPENOORTH (45) obtained evidence that the curvature in *Avena* coleoptiles could not be correlated with differences in auxin extracted from illuminated or shaded halves of coleoptiles, although he found photo-inactivation of auxin in light. There seems to be no absolute certainty about the pigment sensitizing the photoreaction in phototropism. GALSTON (23) reviewing the literature on this subject, failed to collect conclusive evidence for either the carotene or the riboflavin theory. SCHURINGA (61) demonstrated that carotenoids sensitize photodestruction of auxin; it seems likely that other plant pigments also can have this effect.

Internode elongation in etiolated plants is much more pronounced than in light grown plants. This excessive elongation is inhibited by light. In a recent investigation GOODWIN and OWENS (26) confirmed previous observations showing that red light was most effective in inhibiting the elongation of the mesocotyl

of *Avena* much more than blue light. The action spectrum for internode inhibition in etiolated, but potentially green, and in albino barley seedlings was determined by BORTHWICK *et al.* (7). These authors found that this action spectrum was very similar to that for photoperiodic control of flower initiation. Again red light was most effective, much more than blue light. Because acarotenoid albino barley seedlings, and potentially green seedlings showed approximately the same action spectrum, it was concluded that screening by other pigments did not occur. From absorption measurements it was estimated that the pigment responsible for photoreception in the light-induced inhibition process absorbs less than 0.001% of the energy incident on a barley seedling, even in the region of maximum absorption. The nature of the pigments is still unknown.

A similar action spectrum was found by PARKER *et al.* for the effect of light on leaf growth of etiolated pea seedlings (48). In leaf growth, however, red light has a promoting effect. In the blue, again, a far greater energy is required for a given leaf growth promotion.

Recently, LIVERMAN and BONNER (41) demonstrated that the growth promoting effect of red light on *Avena* coleoptile sections cannot be reversed by infrared, unless auxin is added. In the latter case, the promotion by red light is even stronger, and this extra growth can be inhibited by subsequent irradiation with infrared. These findings will be discussed in greater detail in Chapter VIII. LIVERMAN and STARR (unpublished, quoted in [41]) demonstrated the same phenomenon for leaf growth in *Phaseolus*.

Concluding, it can be said that apart from phototropism, all light reactions in etiolated plant material seem to show more or less the same spectral dependence, although in some cases the reversibility of the red light reaction by infrared has not been demonstrated so far; LIVERMAN and BONNER found reversibility only when auxin was added to the system.

Pigment formation also largely depends on light. However, with the synthesis of the green leaf pigments probably a very important step is made towards light adaptation so that this reaction is typical of etiolated plants only as far as the primary formation is concerned. In light grown plants the amount of leaf pigments also depends on factors such as light intensity and light quality. Most higher plants do not form chlorophyll in darkness. KOSKI *et al.* (36) found that formation of chlorophyll in maize seedlings has an action spectrum similar to the absorption spectrum of protochlorophyll; the long wave length limit is somewhere near 680 mμ. WITHROW *et al.* (84) were able to demonstrate that light of wave lengths beyond this limit was active in internode inhibition and leaf growth promotion in bean and maize seedlings. Although in most cases detectable amounts of chlorophyll were synthesized, there was no quantitative relationship between photomorphogenetic response and chlorophyll content. WASSINK *et al.* (74) found that in light inhibition of potato sprouting, also, there was no correlation between inhibition and chlorophyll content. WITHROW and co-workers also observed that the amount of carotenoids increases upon irradiation with moderate intensities of near infrared, an observation also made by the present author, in similar experiments with peas (65)

Up till now there seems to be no evidence that the leaf pigments such as chlorophyll and carotenoids have a primary function in photomorphogenetic reactions, apart from the possible relation between carotenoids, and phototropism, which, however, is perhaps not a photomorphogenetic process *sensu stricto*. The photoreceptive system that induces enhanced carotenoid formation

when etiolated plants are irradiated in the near infrared still deserves further investigation.

3. PHOTOMORPHOGENETIC REACTIONS IN LIGHT GROWN PLANTS

The reactions of light grown plants are much more complicated than those of etiolated plants. The number of investigations is very large, especially on some of the aspects such as, *e.g.*, photoperiodism. The experiments involve more methodical difficulties than those with etiolated plants, light grown plants growing slowly but finally becoming far bigger than dark grown plants. The light intensity required for photomorphogenetic reactions in light grown plants usually is much higher than that necessary for etiolated material, it has to be given over a larger area, and during longer periods. The differences between individual plants are greater than in etiolated plants, and it is not possible to have many replicates in the limited space usually available for irradiation under controlled conditions.

Apparently, the morphogenetic reactions of light in green plants are not coupled to the presence of chlorophyll. BATALIN (3) and later TRUMPF (69) showed that normal light-adapted development can occur without formation of chlorophyll or photosynthesis, provided a sufficient amount of reserve substances is available, and the light treatment given is such that no appreciable chlorophyll formation can occur. SPOEHR (63) cultured albino maize, by applying sugar to the leaves; some of the plants grew quite well. His albino plants developed normal stems and leaves in intermediate light intensities, although they did not contain any chlorophyll. LEBEDEFF (38) also found that the phototropic reaction and the development of leaves was the same in green and albino maize seedlings, indicating that these processes can occur independent of chlorophyll.

The phototropic response in green plants has been extensively studied. PRIESTLEY (52) found that green plants require much higher energies and more time for developing a phototropic curvature than etiolated plants. This probably accounts for some of the controversies that seem to have arisen about the effectiveness of red radiation in this respect. ATKINS (2) found effectiveness of the red region, in green plants of *Avena* and *Lepidium*. These observations were confirmed by BÜNNING (10) who worked with *Sinapis* and *Helianthus*, and VAN OVERBEEK (46), working with *Raphanus*. The present author, in some unpublished experiments with tomato and potato, found that red and yellow light was completely inactive in the phototropic reaction. Blue light was most effective in producing phototropic curvatures, followed by violet and green light around 400 and 550 mμ respectively. FORTANIER (17) observed that *Arachis* curved phototropically towards blue light, although red light was given from the other side at an intensity that was 25 times higher. It would seem that the spectral dependence of the phototropic reaction in light grown plants needs more investigation before a definite conclusion can be drawn.

In many plants the initiation of flower buds is day length dependent. Some plants show this phenomenon in a qualitative way, and will not flower unless they are exposed to the required day length, but in many photoperiodically sensitive plants the reaction is of a more quantitative nature, so that flowering is only speeded up by a suitable day length. The light energy required for day length extension is relatively low, as was found, *e.g.*, by PARKER *et al.* (47).

The spectral dependence of this light reaction has been studied by several investigators. Most of the results obtained are in accordance with the action spectra for the photoperiodic reaction as measured by BORTHWICK *et al.* (6,8, 47,49,) since 1946. RASUMOV (54) found red light to be very effective. WITHROW *et al.* (81, 82,) confirmed this result and found that blue light was ineffective, except in *Callistephus*. FUNKE (18, 19, 20, 21) from 1936 to 1943, investigated a very large number of species with respect to the spectral dependence of the photoperiodic reaction. Apart from the photoperiodically indifferent plants he distinguished four groups. In the first group, red light was effective, and blue light was not, while the second group was sensitive both to red and blue light. The greater part of the plants investigated belonged to these two groups. In FUNKE's group III the plants reacted to white light, but not to blue or red light. His fourth group contained plants, reacting to blue light, while red light was ineffective; all plants in this group belonged to the *Cruciferae*.

KATUNSKY (30), WALLRABE (72) and KLESHNIN (34) found that all wave length regions were effective, provided the energy was sufficiently high. WALLRABE also described experiments in which the entire light period and not, as usual, the day length extension, was given as coloured light. She found that green light was ineffective in such a treatment.

FUNKE's first group of plants could be explained on the basis of the action spectra of PARKER *et al.* (47) especially since the method of filtering day light through coloured glass during part of the day was rather primitive. The intensity factor might explain the second and third group. However, it was very difficult to assume that the strongly deviating fourth group might be attributed to methodical shortcomings only.

In order to investigate this matter further WASSINK *et al.* (77, 78) and STOLWIJK (64, 65) studied the spectral dependence of the photoperiodic reaction of several *Cruciferae* under more critical experimental conditions and confirmed FUNKE's results. They also found that light of wave length regions in the near infrared had the same effect as blue light. The action of blue and infrared wave length regions was associated with an elongating effect on stem and leaves. This elongating effect was also found in other plants not belonging to the *Cruciferae*, and which show a different spectral dependence of the photoperiodic reaction.

WASSINK *et al.* (78) found that the promotion of flowering in *Brassica Rapa* var. by blue and infrared was independent of day length to a much greater extent than that in white light. They concluded that, in white light, the remainder of the spectrum has an antagonistic effect, destroying the promotion caused by the blue and near infrared components in the white light. Later on BORTHWICK *et al.* (8) demonstrated that in the red-sensitive plant *Xanthium* the effect of red light could be reversed by subsequent infrared irradiation. It can be assumed now that the photoreceptive pigments in both *Cruciferae* and other plants are the same, as the spectral dependence is identical; it remains obscure so far which is the cause of the difference in the effect of the two groups of plants.

The primary light reaction in photoperiodism initiates a chain of reactions which eventually leads to flowering. Not much is known about this chain of reactions. So much seems certain, that the initial light reaction must be similar to that governing many reactions in etiolated plants, and also to that controlling elongation as affected by supplementary light of low intensities (64, 65, 78).

Length growth in green plants is a much more complicated phenomenon than

it is in etiolated plants. Part of this complexity is due to photosynthesis and its influence on growth. In experiments on morphogenetic reactions the supply of carbohydrates should not limit growth. With etiolated plants this condition usually can be fulfilled rather easily by the reserves in the seed, because only young stages are normally used. Green plants, however, are dependent on photosynthesis for their carbohydrates, so that variation in light conditions has a twofold influence on growth: through photosynthesis, and through morphogenetic reactions.

In our experiments, the intensity of illumination providing photosynthesis was the same in all treatments. In the supplementary light treatments the light energy of the supplementary light is negligible as compared with that in the basic light period. When spectral regions were given at high intensity, the intensities in the various spectral regions were the same.

As has been shown in investigations of WASSINK and STOLWIJK (79), and of FORTANIER (17) another complication arises from the intensity dependence of stem growth reactions. At low intensities red light is most effective in internode inhibition, at higher intensities blue light is more effective in this respect. In view of these findings, it is evident that unless experiments are made on a quantitative basis, the interpretation of the results is very difficult. Many of the earlier investigations, made at a time in which no suitable light sources, filters or methods for light measurement were available, have little quantitative value at present. In many of the older papers, but also in comparatively recent ones it can be noticed that the light intensity measurements are unsatisfactory. In many cases illumination meters are used, and consequently the light intensity is given in foot candles or lux, whereas plant response is a function of the energy or the number of quanta of the light, and the relative spectral effectiveness curve for the particular reaction studied. The luminosity value, as expressed in lux or foot candles is correlated with the energy value and the sensitivity of the human eye at a particular wave length of the light.

In general it is found that absence of the short wave length regions results in increased stem elongation in green plants. Most of the earlier experiments have been made using day light, filtered through coloured glasses. KLEBS (32) probably was the first who made an attempt at measuring absolute light intensities. A review of the results obtained till 1936 has been given by POPP and BROWN (51). Since then advances in the development of gas discharge and fluorescent lamps have made selective light sources available. This has made possible to grow plants in artificial light of increased intensity and higher spectral purity. Moreover, it became easier to keep other environmental factors such as temperature and humidity under control.

ROODENBURG (56) and ÅBERG (1) used neon, sodium and mercury discharge lamps as artificial light sources for plant growth. ROODENBURG (57) concluded that blue light was responsible for inhibition of internode growth. Lack of blue light caused excessive internode growth. ÅBERG did not obtain such clear results in his plants grown under mercury light. His results could perhaps be explained by the presence of the yellow and green mercury lines, and even more probably by the relatively low intensities he used. An intensity of about 8000 ergs/cm²/sec may have been too low for satisfactory internode inhibition. ÅBERG also noticed epinasty of tomato leaves in plants grown in the absence of violet blue wave length regions, a phenomenon also noticed by SHIRLEY (62), ROODENBURG (56), and WASSINK and STOLWIJK (79).

VAN DER VEEN (70) grew a number of plants in coloured light obtained from fluorescent lamps with a single fluorescent coating, without any filtering. He found inhibition of internode elongation in blue light, and excessive elongation in red and green light. MOSHKOV (44) grew *Perilla* plants in violet-blue, yellow-green, and red light; the violet-blue and green light was obtained from mercury lamps, the red light from incandescent lamps. The *Perilla* plants elongated most in yellow-green light, very little in violet-blue light, but also very little in red light. As the dry weight was very low in plants under red light, the absence of elongation must probably be ascribed to the large amount of near infrared transmitted by the water filter (see also Chapter VII).

WASSINK and STOLWIJK (79) used special, concentrated sets of coloured fluorescent lamps with additional filtering, so that several spectral regions were obtained which each showed a combination of spectral purity and intensity as had not been achieved before. In experiments in which plants grew in equal energies it was found that in yellow and red light elongation was greatest, whereas in blue-violet it was reduced to below that in the white controls. The importance of the intensity factor is illustrated by results obtained by WASSINK and STOLWIJK (79) and by FORTANIER (17). These authors found that blue radiation at low intensity results in increased stem elongation, whereas violet and blue light at high intensity inhibit stem elongation; on the other hand red, yellow and green light at low intensity inhibit stem elongation in some plants, whereas stem elongation is increased by the same spectral regions when given at high intensity. ÅBERG (1) also concludes that at low light intensities the longer wave lengths of the visible spectrum are most effective in inhibition of elongation, whereas at high intensity the shorter wave lengths are most effective in this respect. WASSINK *et al.* (77, 78), STOLWIJK (65), and FORTANIER (17) found that near infrared radiation, when given supplementary to a short day in white light of high intensity also promotes stem elongation (see also Chapter VI).

CHAPTER III

MATERIAL AND METHODS

1. PLANT MATERIAL

Experiments were made with the following species: *Brassica Rapa* f. *oleifera* subf. *annua*; *Spinacia oleracea* var. Nobel; *Cosmos bipinnatus*; *Lactuca sativa* var. "Meikoningin", "Attractie" and "Wonder van Voorburg"; *Solanum Lycopersicum* var. Vetomold 121; *Sinapis alba*; *Iberis coronaria* var. Empress and some others. Seeds of *Brassica Rapa* var. were obtained from a rather variable population by selection of the individuals with pronounced long day requirement. Self-pollination during several generations increased the uniformity of the seeds. The seeds of the other species were obtained from commercial sources.

About 10–14 days before the start of an experiment, the seeds were sown in flat seed pans, in sand that was regularly watered; these seed pans were placed in the greenhouse. After emergence, the young plants were transplanted into pots or square dishes with fertile soil to which a suitable amount of fertilizers had been added. As soon as the young plants had regained their vigour they were selected for uniformity; usually about half of them were sufficiently uniform to be used in the experiment. These plants were brought into the laboratory under controlled conditions of day length, light intensity and temperature. Plants used for

photoperiodicity experiments were exposed to a day length inhibiting flowering from the very beginning up to the start of the experiment. Usually, the experimental treatment started 3–4 days after the plants were brought under controlled conditions. In most cases 4 plants were used in each treatment, and the results given are averages of at least 4 plants. Experiments in which the plants showed irregular growth were discontinued, and the results were discarded.

2. THE WHITE LIGHT EQUIPMENT

This equipment is in a basement room of about 6 × 4.5 m. In winter this room can be well controlled as far as temperature is concerned, however, it has no cooling outfit. Therefore in summer the average temperature must be set higher in order to provide a suitable control (see below).

The white light equipment is used for irradiating plants at a light intensity sufficient to yield normal growth. Usually, a 10 h. illumination per 24 h. cycle is used which may or may not be supplemented by a low light extension for the photoperiodic reaction.

The white light equipment is built up of panels of 120 × 70 cm, each equipped with eight 40 W daylight type fluorescent tubes which are mounted at 8.5 cm distance. The panels are suspended by chains allowing different heights of the lamps above an eternite-topped bench on which the plants are placed. The maximum light intensity which can be maintained over long periods is about 6000 lux (600 f.c.). With new fluorescent tubes and the mains voltage at its nominal value this maximum is about 8000 lux (equivalent to about 30.000 ergs/cm²/sec.). All light intensities given here are averages; they are subject to short term changes from —9 % to + 6 % owing to fluctuations in the mains voltage. The white light equipment is operated by an electrically wound spring-driven time switch. The temperature in the equipment is kept constant in controlling the room temperature by means of a thermoregulator which operates a fan drawing in outside air. The humidity was not controlled, but it was checked regularly with wet and dry bulb thermometers, and only rarely fell below 70 %.

3. THE EQUIPMENT FOR SUPPLEMENTARY COLOURED LIGHT

The equipment for supplementary coloured light is the one described by WASSINK and VAN DER SCHEER (75). It consists of 6 compartments, separated by white-washed hard board walls. The compartments are 120 cm high, 45 cm wide and 210 cm long. Part of the top consists of hard board, the rest is covered by coloured glass filters of 30 × 110 cm. Under the glass filters a sheet of colourless glass is mounted at a distance of 5 cm, acting as a heat filter. A simple diagram of one compartment is given in fig. 1. A small 25 W fan is mounted light proof in the front wall of each compartment, removing warm air from the space between the filters and the glass. The front wall of each compartment is partly removable, the back is closed by dark blankets. Two zinc trays, half filled with vermiculite and water on the floor of every compartment maintain a nearly constant relative humidity of about 75 %. The temperature in the equipment is controlled by the same mercury relay that controls the room temperature by operating a fan drawing in outside air during winter, and blowing out warm air during summer. The normal working temperature of the equipment is 20 ± 1 °C. In cases

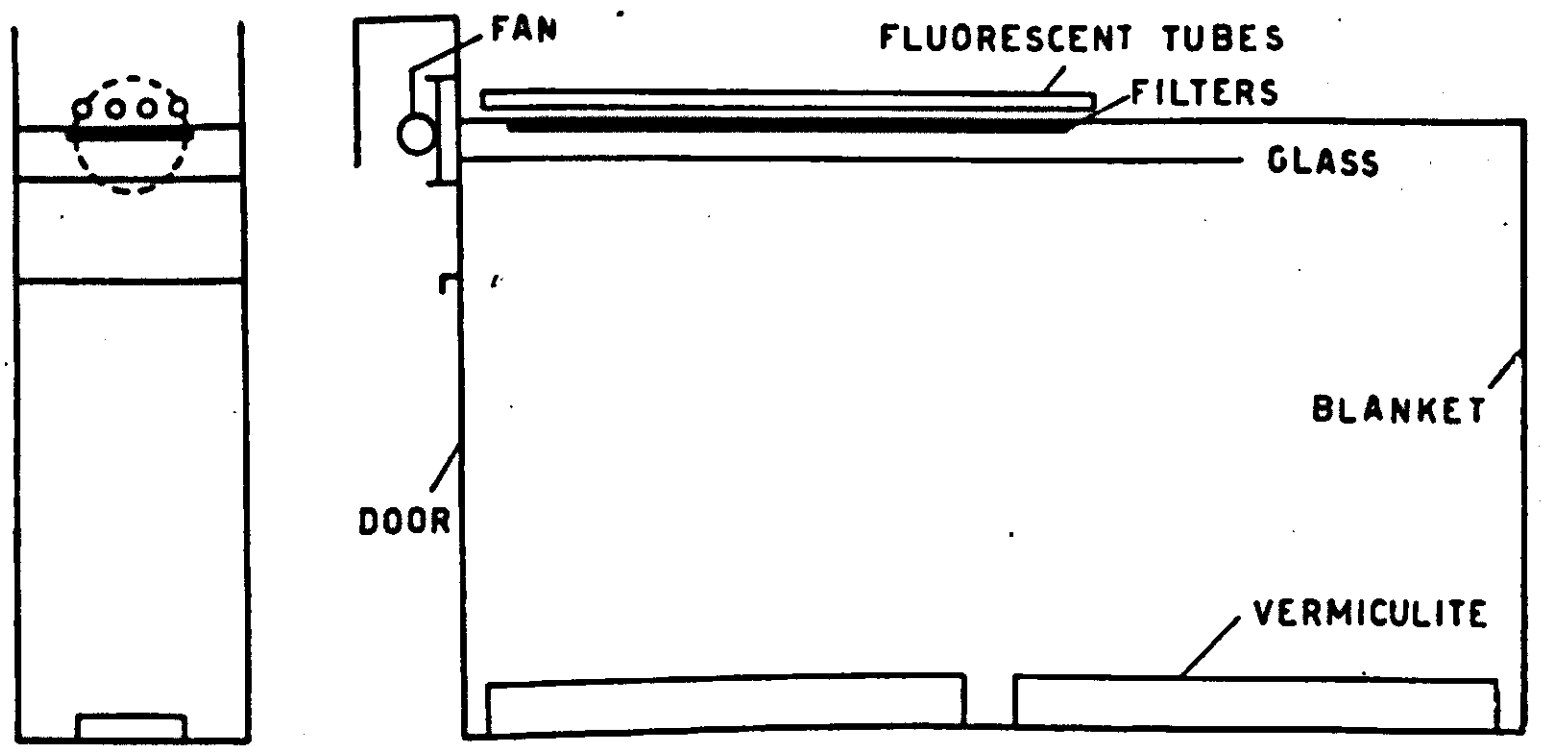


FIG. 1. Simplified diagram of one of the compartments of the supplementary light equipment. Left: front view; right: side view.

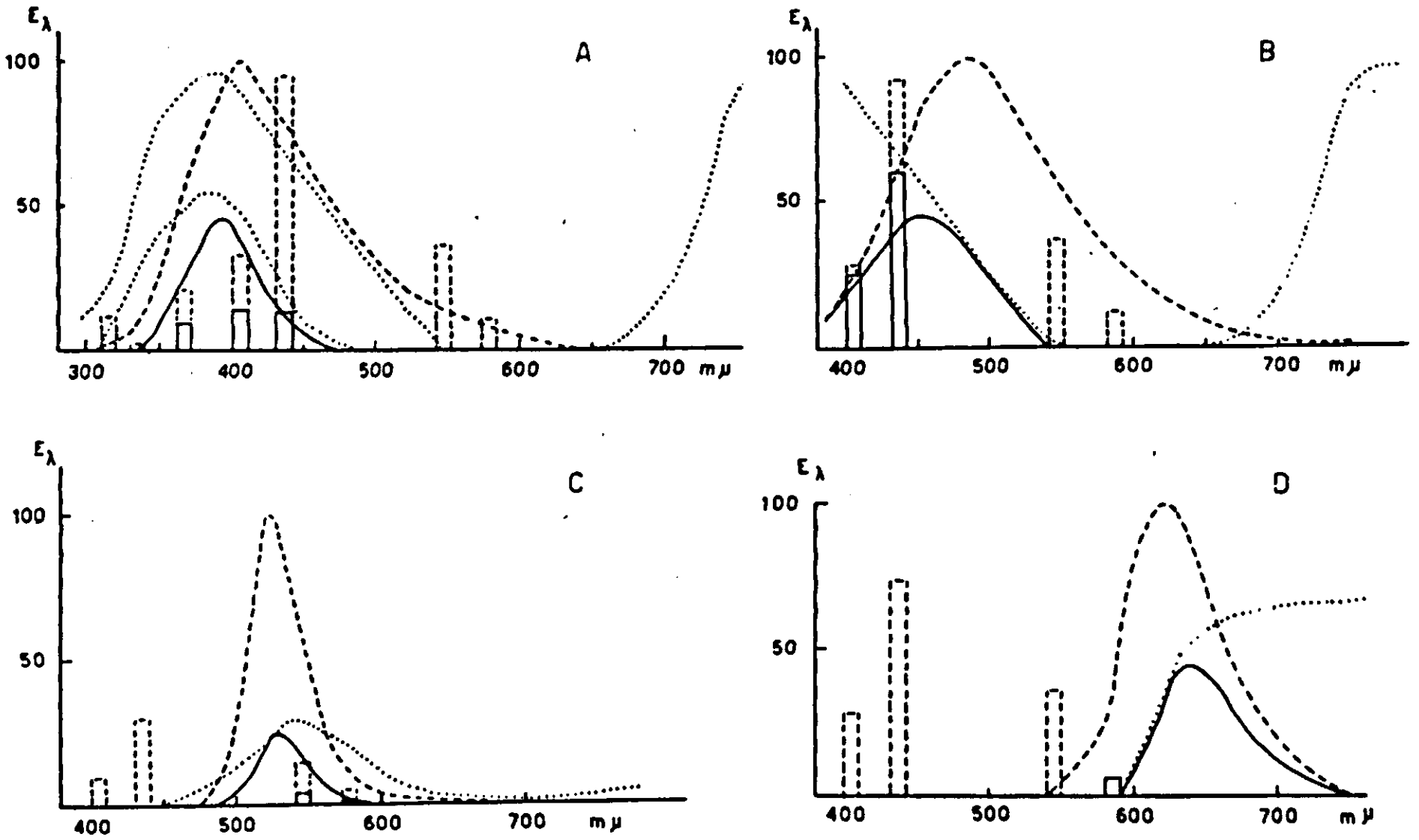


FIG. 2. Spectral characteristics of the compartments in the supplementary light equipment. Broken lines: emission spectra of the light sources, dotted lines: transmission curves of filters. full-drawn lines: spectral energy distribution of the filtered light.
A: violet light compartment,
B: blue light compartment,
C: green light compartment,
D: red light compartment,
E: infrared compartment.

that the outdoor temperature rises to high values during summer it was sometimes necessary to set the relay at higher temperatures, up to 25 °C, because at the lower settings the temperature control was no longer effective. If so, this was done during the whole of an experiment, and it will be mentioned in the description.

Since the first description of the equipment (*l.c.*) a few changes have been made in the light sources and the filters, in order to improve the spectral purity and to increase the intensity of the coloured light. Some of these alterations will be briefly discussed.

In the violet compartment, the 7 blue fluorescent tubes used originally have been replaced by 4 fluorescent tubes of the "blue-actinic" type, which have become available since. The peak of the fluorescent emission of these tubes is around 400 mμ, which results in a higher intensity in the compartment, because a larger part of the emission is transmitted by the blue and violet filters. Also, a higher spectral purity is obtained since much less radiation of wave lengths from 450–500 mμ is present. Fig. 2A gives the curves of the ultimate emission in the violet light compartment as it is at present. The lamps in the violet compartment were changed on 18–4–'52. In all experiments after that date the improved violet was used. It was found that the effects of the original violet were due to contamination with blue light. After the improvement in spectral purity the similarity in the effects of blue and violet was less obvious.

The blue light compartment still has the original combination of 4 blue fluorescent tubes, and a blue glass filter, yielding blue light of satisfactory intensity and spectral purity (fig. 2B).

The green light compartment also is used in its original form, having 4 green fluorescent tubes and a narrow green filter, the spectral characteristics of which are given in fig. 2C.

In the yellow light compartment that has two 140 W sodium lamps as light sources, another filter (no 7) and a green filter have been added to the no 7 filter that was in use before. The

latter filter mainly absorbs the near infrared. The addition of a narrow green filter reduced the relative intensity of the red Ne-lines in the visible part of the spectrum, while the second no 7 filter results in a further reduction of the near infrared. Since the visible radiation is mainly concentrated in the sodium doublet at 589 mμ, a graphical presentation of the spectral characteristics of the yellow light seems unnecessary. The red compartment is used in its original form, having 4 red fluorescent tubes and a red filter. The spectral characteristics of the red light are given in fig. 2D.

The infrared compartment, with six 60W incandescent lamps as light sources has been improved by the addition of a 5 cm layer of water (65); this served to reduce the long wave length infrared, without an appreciable decrease of the intensity of the near infrared of wave lengths of 700–800 mμ. A combination of a red and a blue filter is used to cut off the visible radiation. The spectral characteristics of the infrared compartment are given in fig. 2E. The filter *c* in this figure is a no 7 filter, absorbing far infrared.

The maximum light intensity obtainable in the compartments is about 2000 ergs/cm²/sec. The size of the equipment easily allows variation of the intensity of irradiation by changing distance between plants and lamps. Moreover, sometimes, part of the lamps were switched off.

In graphs presenting experimental results, the various spectral regions used are indicated by their optical centres. These points are as follows: violet 400 mμ, blue 460 mμ, green 550 mμ, yellow 590 mμ, red 660 mμ, and infrared 760 mμ.

Each experiment in which supplementary coloured light was applied, had two controls: one lot of plants received additional white light of the same intensity as the supplementary coloured light, and one lot was placed in darkness during equal periods. The white light was obtained from a single daylight type 40 W fluorescent tube, without any filtering.

4. THE EQUIPMENT FOR COLOURED LIGHT OF HIGH INTENSITIES

The light intensity in the equipment described in the preceding section is not sufficiently high to grow plants in this light only. To this purpose another type of equipment was built that has already been described in (79). It also received some extension since.

At present it consists of 7 light cabinets set up in a separate building of 3 × 8 m. The windows of this building are screened, and the walls and ceiling have been painted black, to avoid reflections that would decrease the spectral purity of the light in the cabinets.

The cabinets are uniform and consist of two parts (fig. 3). The inner part is the plant chamber, 110 cm long, 35 cm wide and 85 cm high. The plant chamber consists of an iron frame, the lower 10 cm of which is sheet iron. The remainder of the side walls and the top have colourless glass, mounted air tight. Metal doors were made in the front and back which are air tight also. As in normal use the doors are kept ajar, screens have been mounted around the doors to prevent stray light from entering the cabinets. Glass filters are mounted in rims along the edges of the colourless glass of side walls and top.

The outer part carries the light sources and their ballasts and is placed over the plant chamber, but not connected to it, so that it can be taken away or replaced without much difficulty.

At present, the seven units are set up in a row; they are separated by board screens suspended from the ceiling and reaching to the bottom of the cabinets.

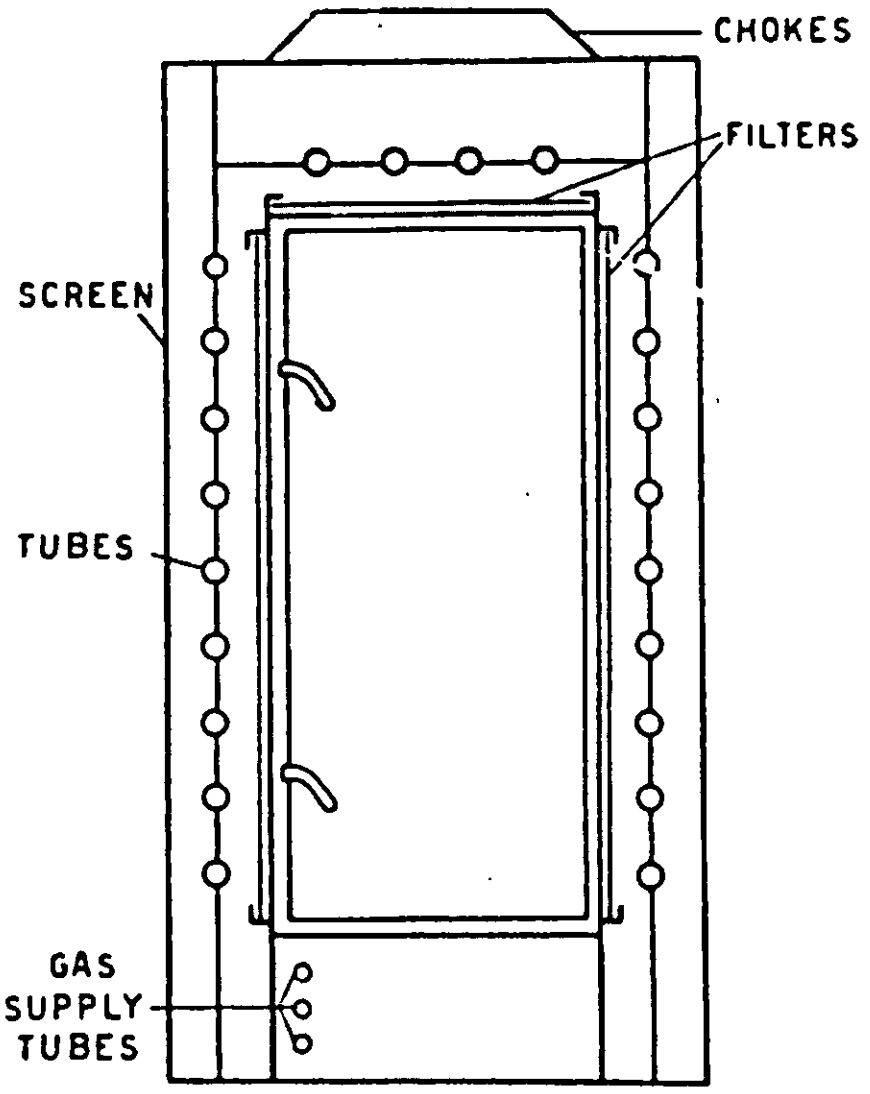
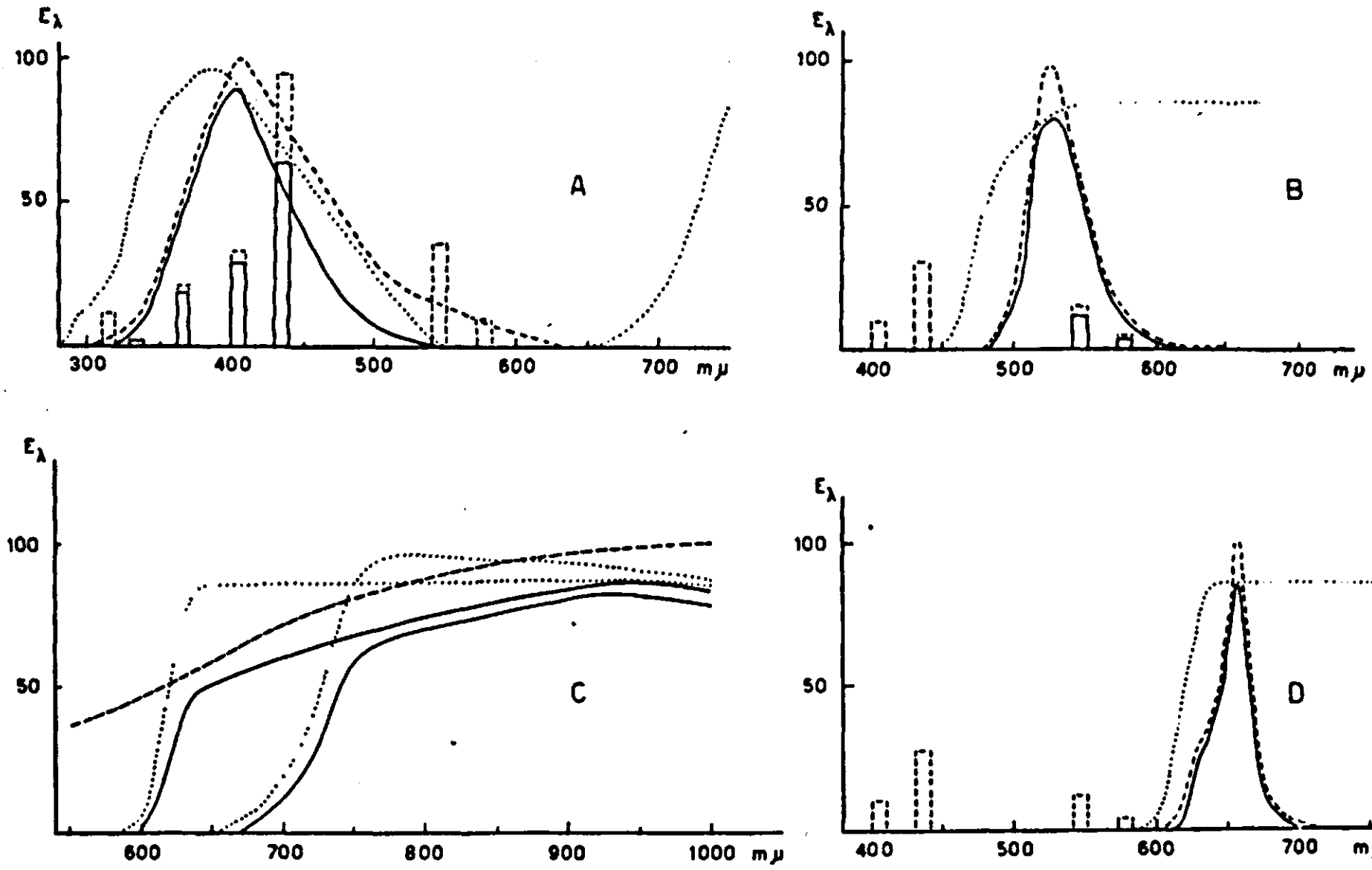


FIG. 3. Simplified diagram of a light cabinet of the high intensity equipment.

The blue-violet light cabinet has 20 fluorescent tubes of the "blue-actinic" type and blue filter glasses. The maximum energy in the transmitted light is about 400 mμ. The spectral characteristics are shown in fig. 4A. A "blue" light cabinet has been added since the first description. It has 30 blue fluorescent tubes and blue glass filters. The number of tubes is higher than in the other cabinets because blue filters of satisfactory spectral characteristics usually

have low transmission values. The maximum energy in the transmitted light is at about 460 mμ. Fig. 2B shows the spectral characteristics of the blue light cabinet.

The green light cabinet has 20 green fluorescent tubes, combined with yellow glass filters. The yellow filters absorb the violet and blue mercury lines present in the emission of the fluorescent tubes. The spectral characteristics of the green light cabinet are given in fig. 4B.



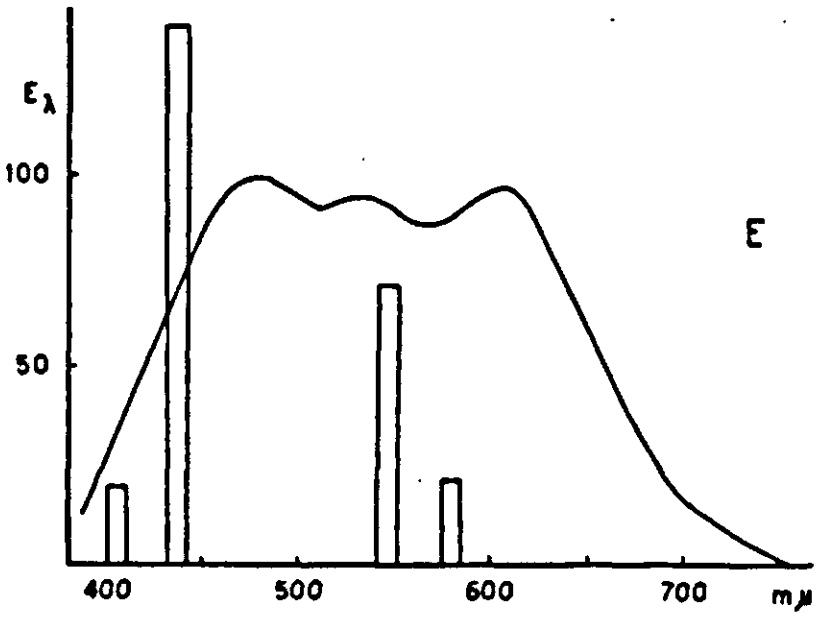


FIG. 4. Spectral characteristics of the cabinets in the high intensity equipment. Broken lines: emission curves of light sources; dotted lines: transmission curves of filters; full-drawn lines: spectral energy distribution of the filtered light.
A: violet light cabinet,
B: green light cabinet,
C: red + infrared cabinet,
D: red light cabinet,
E: white light cabinet.
Blue light cabinet: spectral characteristics as in fig. 2B.

The yellow light cabinet has five 140 W sodium lamps as light sources and orange filters to eliminate the blue and green lines of sodium and neon, the latter gas also occurring in the discharge tubes. Thus, almost all visible radiation in this cabinet is concentrated in the Na-doublet at 589 mμ, so that it seems superfluous to give further spectral characteristics. The red light cabinet can be fitted out with 2 types of red fluorescent tubes, of which 20 can be mounted. One type has its fluorescence maximum near 620 mμ, the other at about 660 mμ. The latter has been used in all experiments reported in this paper. A red glass filter cutting off at about 610 mμ absorbs the blue, green and yellow mercury lines present in the emission of the red fluorescent tubes. The characteristic curves are presented in fig. 4D.

The infrared cabinet can be used in two ways. At present, thirty 40 W incandescent lamps are used as light sources. Since plants cannot grow in infrared radiation alone, a suitable amount of red light, in the wave length region from 610 to 700 mμ is also allowed to pass the filter, so that the intensity of the photosynthetically active light is the same as in the other cabinets. Besides this, the light has a high content of near infrared. The cabinet can be used in a second way by adding blue glass filters to the red ones. This results in near infrared, from 700–1200 mμ, without photosynthetically active red radiation. So far, this method has not been used, since in this set-up the plants would have to be fed sugar to enable their growth, which introduces

experimental difficulties, and moreover would not guarantee plants quite comparable to those grown in the other compartments. Fig. 4 C shows the spectral characteristics of the infrared cabinet with and without the blue filter.

The seventh cabinet serves as a white control. Eight daylight type fluorescent tubes without filtering yield white light of the same intensity as the light in the coloured cabinets. Fig. 4E shows the spectral energy distribution of the white light.

The equipment is operated by electrically wound spring-driven time switches permitting any desired combination of photoperiod and dark period, provided the cycle is 12 or 24 hours. The plants are placed in earthenware dishes or pots and they are watered twice daily. Recordings of relative humidity showed that it varied from 71–78 %. The temperature control is twofold. During the photoperiod, the temperatures in the plant chambers tend to increase and without control settle at about 15 °C above room temperature. By means of a mercury relay, connected to a thermoregulator, a powerful fan removes air from the room, so that the temperature increase is largely prevented. The thermoregulator is placed in one of the cabinets and the temperature in the other cabinets can be adjusted by opening the doors more or less, for which different catches at the doorhandles are provided. Screens have been mounted around the doors to prevent stray light from entering.

In this way, in the various cabinets the temperature during the photoperiod is kept constant to within 1 °C. During the dark period, the temperature tends to drop, and a lower limit is set up by a second thermoregulator that switches on electric heaters when the night temperature drops below a certain pre-set value. In this way, day and night temperatures can be set independently, provided the outside temperature is below the photoperiod temperature during the corresponding part of the 24-hour cycle. For this reason the photoperiod was given during the natural night in most of the experiments.

The construction of the units is such that the total light intensity in the plant chambers is virtually the same everywhere, so that this equipment affords the possibility to grow plants in light of well defined quality and equal intensity and under constant conditions of day temperature, night temperature, light intensity, relative humidity, over long periods.

Up to now, for practical reasons the cabinets are adjusted to equal energy values, so that in the various spectral regions the plants receive different numbers of quanta per unit time. The intensity in the violet, *e.g.*, should be increased by about 55 % over that in the red to equalize the quantum intensity in both regions. However, since the intensity in the blue is the limiting factor in aiming at still higher light intensities, quanta intensity equalization is only possible by sacrificing still more of the potential intensity in the red, yellow and green regions, which, so far, was considered impracticable, although equal quantum intensity would have to be preferred above equal energy intensity.

5. METHODS OF LIGHT MEASUREMENT

All light measurements are being made with a calibrated thermopile first, later on light intensities are mostly checked with barrier-layer photocells, calibrated against the thermopile for the various spectral regions. The thermopile is a MOLL large surface thermopile, and has been calibrated at the Physical Laboratory of the University of Utrecht; its sensitivity is 0.36 V/Watt/cm². A KIPP portable galvanometer is used to measure the thermopile output. The sensitivity of the galvanometer was checked regularly during measurements by calibration voltages from a voltage divider. The circuit is shown in fig. 5. The sensitivity of the thermopile is essentially independent of the wave length of the light within the range transmitted by the glass cover, and it can be used for measuring energies in various spectral regions. All light intensities given here, are obtained in this way. They are intensities in the range of 400–700 mμ, unless stated otherwise. The infrared was separated from the visible by giving "dark" with an RG 8 filter of SCHOTT (Jena) which filter has a steep short wave length cut-off very near 700 mμ, and freely transmits infrared radiation of wave length region from 700–2800 mμ. A correction for the reflection losses introduced by the use of the RG 8 filter was made by giving "light" with a WG 3 filter. In this way, by using other filters, the percentage radiation in narrow wave length bands in the visible region has also been measured.

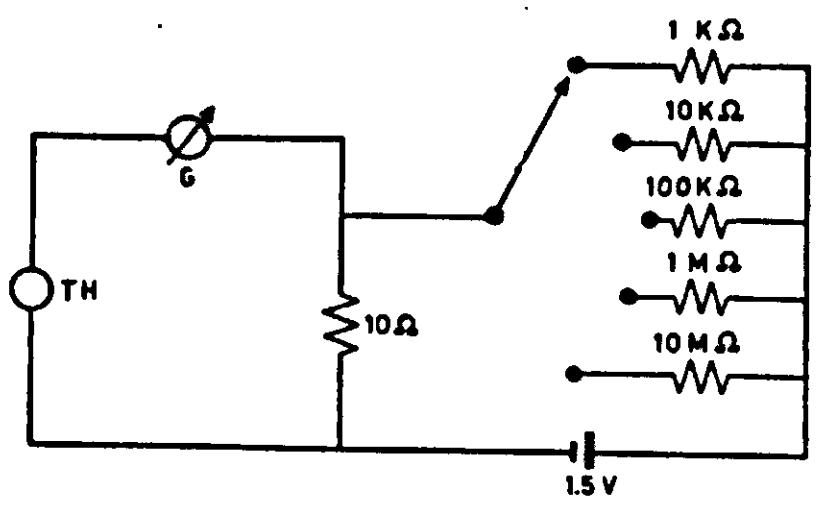


FIG. 5. Circuit used for light measurements with thermopile. TH = thermopile, G = galvanometer.

Two types of radiation meters with photocells are used. One, a flat meter, has one barrier layer photocell covered by a slightly spherical opaline glass cover, so that the apparatus obeys the cosine law. The other is a apherical light meter, with two barrier-layer photocells back to back, each covered by opaline glass consisting of a hemisphere on a short cilindrical part, and dimensioned so that the meter is near to complete independence of the direction of the light. It has been described in detail by WASSINK and VAN DER SCHEER (76). Both light meters are used with micro-ammeters having 50 μ amp full scale deflection, and 1500 Ohms internal resistance. Of course, they have to be calibrated for every individual type of light source, because of the dependence of sensitivity on spectral energy distribution.

In the white light equipment, intensities were measured as incident on a flat surface since most of the light comes from above. The same applies for the supplementary light equipment. In the equipment for coloured light of high intensity, however, the measurement of light intensities is somewhat complicated. The light intensity is the same at almost every place in the plant chamber, but at each position the light comes from nearly all directions. Since the receiver of our thermopile is flat, a large number of measurements at evenly distributed angles were made to simulate a spherical receiver. These measurements were averaged and then gave the influx of radiation into a sphere of 1 cm² surface. For reasons of conformity this expression is converted into: the light influx into a sphere with a cross section area of 1 cm²; this involves multiplication with a factor 4. This way of measuring light intensity requires much time and work, and cannot be used when the greater part of the space in the cabinets is occupied by plants. For these reasons the spherical light meter with photocells, calibrated in every spectral region is used in routine checks of light intensity. Because of the dimension in which the light energy is expressed (ergs/sec/cm² ø sphere) and because the light is monochromatic the intensity cannot be given in terms of lux or foot candles. Even the comparison between white light of an intensity of, *e.g.*, 4000 ergs/cm²/sec. and 4000 ergs/sec/cm² ø sphere is not very easy, but it is quite sure that a plant in the latter intensity absorbs much more energy than in the first. A plant growing in the first light intensity absorbs 4000 ergs/sec/cm² of vertical projection. In the second light intensity a plant absorbs 4000 ergs/sec/cm² of total superficial area; this is much more than the vertical projection in plants with an appreciable vertical extension.

When two wave length regions were mixed, thermopile measurements of light intensity were made, first with the light source supplying one wave length region, then with the source supplying the other region. In this way percentages of both components could be computed accurately at the same spot at which the plants were exposed.

CHAPTER IV

PHOTOPERIODIC AND FORMATIVE EFFECTS OF SUPPLEMENTARY LIGHT OF VARIOUS WAVE LENGTH REGIONS IN *CRUCIFERAE*

1. INTRODUCTION

The results obtained in experiments in which a short day in white light of high intensity was supplemented by coloured light of low intensities show that the effect of the supplementary light can be divided roughly into two reactions: a photoperiodic reaction as a result of the change in day length by the supplementäry irradiation, and a number of formative effects not primarily dependent on length of day, but due to the presence or absence of specific wave length regions in the supplementary light. Of course the photoperiodic effects only occur when photoperiodically sensitive plants receive supplementary irradiation with light of wave length regions active in the photoperiodic reaction. In some cases experiments have been designed to determine whether there is a direct relationship between formative and photoperiodic effects.

So far, in almost all cases in which the spectral dependence of the photoperiodic reaction was investigated it was reported that the spectral region 520–700 mμ was very active, with a smaller activity of the blue and violet regions. FUNKE

(18, 19, 20, 21) was the first to observe that in many plants, mostly belonging to the *Cruciferae* blue radiation was active, and red was not. However, these observations stood quite alone, and in view of the limitations in his methods their value was open to some doubt. In the course of the present investigation this observation was confirmed and extended. No activity of the infrared was ever found except in the work started in this laboratory (77), and more recently, by BORTHWICK *et al.* (8). Some of the earlier results mentioned below have been been described in preliminary communications (65, 77, 78) but will be given here also for the sake of completeness.

2. SPECTRAL DEPENDENCE OF THE PHOTOPERIODIC REACTION

The first plant tested for the spectral dependence of its photoperiodic reaction was *Brassica Rapa* f. *oleifera* subf. *annua*, (77, 78). This plant, as all *Cruciferae* tested so far in this laboratory, is not qualitative in its long day reaction but initiates flower primordia under short day conditions in the course of time; however, these flower primordia grow out only very slowly. Flower primordia initiation was found to be quicker in blue, violet, and infrared additional irradiation (77). Table 1 shows that in this plant blue supplementary light is most active in prolonging the short day; violet and infrared show a slightly smaller effectiveness. The light of the spectral region 520–700 mμ shows no promotion of flowering, the plants are the same as the controls, receiving only a short day. This experiment was made many times before and after the one of which the results are summarized in Table 1.

TABLE 1

Photoperiodic response to supplementary light of various wave length regions in the long day plant *Brassica Rapa* L. var., at an intensity of 1000 ergs/cm²/sec., given during 8 hours per day, after a short day in strong white light (10 + 8 hours treatment). Number of days after the start of the treatment until flower buds (resp. flowers) were observed in 50 % of the plants. Taken from (77).

Wave length region of supplementary light	Flower buds	Flowers
Violet	13	25
Blue	12	22
Green	> 37	–
Yellow	> 37	–
Red	> 37	–
Infrared	23	36
White	15	28
Dark	> 37	–

In one of the experiments the growing points were dissected and examined microscopically after 2, 5, and 8 days of treatment. For this experiment very young plants were used, older plants having inflorescence primordia even when the usual precaution of growing them in a 10 hour day is taken. After 8 days some of the plants in every treatment had inflorescence primordia. This indicates that the differences obtained in the flowering behaviour probably are mainly due to differences in the speed with which the primordia develop further; with this limitation concerning the nature of the reaction, FUNKE's observation could thus be reproduced and extended to the infrared sensitivity, under better defined

conditions of controlled environment and spectral purity .It will be demonstrated later on that the flowering reaction of this cruciferous plant, and probably of all *Cruciferae*, shows deviations in its mechanism that do not occur in plants of the red-sensitive group. It was of considerable interest to see how other *Cruciferae* would react under our experimental conditions.

Sinapis alba L. and *Iberis coronaria* L. var. Empress were taken as further representatives of this family.

Sinapis alba was sown on 10–1–'52. The supplementary irradiation, in a 10 + 8 hours treatment started on 10–2–'52. Measurements were made after 32 and 39 days of treatment. Some of the results are given in Table 2.

TABLE 2

Degree of flowering in *Sinapis alba* L. as dependent on wave length of supplementary light in a 10 + 8 hours treatment. Measurements of 13–3–'52 and 20–3–'52 (after 32 and 39 days of treatment respectively). Averages of 8 plants. Taken from (65).

Wave length region of supplementary light	Degree of flowering after 32 days of treatment	Degree of flowering after 39 days of treatment
Violet	3.30	7.50
Blue	7.25	9.50
Green	0.37	2.75
Yellow	0.75	2.75
Red	0.75	3.00
Infrared	3.75	5.50
White	3.75	5.50
Dark	0.75	2.00

In Table 2 and in some subsequent Tables, the stage of development of the growing point is expressed as "degree of flowering". This is the averaged score of the plants on a scale, ranging from 0, completely vegetative, to 10, open flowers; (0: vegetative, 1: just visible microscopical inflorescence primordia, 2: inflorescence primordia, 3: floral primordia, 4: advanced floral primordia, 5: flower buds only just visible to the naked eye, 6: flower buds visible, 7: many flower buds easily visible, 8: elongated flower stalk, 9: flower buds opening, 10: open flowers). Especially in the case of *Cruciferae*, in which photoperiodism seems manifest only as a quantitative reaction, or when limitations in the number of plants did not permit the microscopical examination of the growing point in the early stages, this arbitrary scale proved quite useful.

The data, compiled in Table 2, clearly indicate that in *Sinapis alba*, too, the blue, violet and infrared wave length regions are active in the photoperiodic reaction, while the region 520–700 mμ hardly showed any activity at all, as compared with the dark controls,

In the experiments with *Iberis coronaria* var. Empress the same treatment was given as in the previously described experiments. The treatments started on 21–4–'52. Measurements were made after 8, 24, and 38 days of treatment. Some of the results are summarized in Table 3.

The reaction of *Iberis coronaria* is very similar to that of *Sinapis alba*, with this difference that the long day requirement is even less pronounced. Even so, there is no promoting effect of the region 520–700 mμ on flowering, as compared with the dark controls.

TABLE 3

Degree of flowering in *Iberis coronaria* L. var. Empress as dependent on wave length of supplementary light, in a 10 + 8 hours treatment. Observations of 29–4–'52, 15–5–'52 and 29–5–'52, after 8, 24 and 38 days of treatment respectively.

Wave length region of supplementary light	Degree of flowering		
	after 8 days	after 24 days	after 38 days
Violet	2.25	5.37	9.37
Blue	6.25	8.75	10.00
Green	3.75	3.12	4.50
Yellow	2.00	3.80	5.87
Red	2.50	4.37	6.50
Infrared	5.75	6.62	8.87
White	3.50	4.24	8.00
Dark	2.00	3.30	5.33

3. FORMATIVE REACTIONS

The first evidence of formative effects was obtained with *Brassica Rapa* L. f. *oleifera* subf. *annua*, in the same experiments that were made to determine the spectral dependence of the photoperiodic reaction (77). Stem elongation, and a change in leaf form occurred in the plants receiving violet, blue or infrared supplementary irradiations. Later experiments have shown that the formative effects in this plant are at least partly correlated with the development and growth of the floral primordia. In section 4 of this chapter it will be shown that it is impossible to separate the formative and photoperiodic reactions completely. Fig. 1 of (77) demonstrates moreover that with violet, blue or infrared supplementary irradiations the first leaves do not show the elongation, which is typical in *e.g.*, the 6th leaf. However, elongation of the stem starts immediately when violet, blue or infrared is given. This is especially evident in the plants under infrared supplementary irradiation; these plants flower fairly late, but the elongation starts very early. For this reason the aspect of the plants is different from all others: from the flowering plants because of the "vegetative" leaves, and from the non-flowering plants because of the stem that is absolutely suppressed in these plants. The elongating effect of infrared on the leaves starts at about the same time as flowering. It seems justified to conclude from these facts that change in leaf form is coupled with the flowering reaction, but that stem elongation in this plant can be considered as a primary formative effect. This is also supported by the fact that the white controls are also shorter than the "violet", "blue" or "infrared" plants notwithstanding the fact that they are about equally advanced in flowering. Some more arguments for the mentioned assumption will be given in later sections of this paper.

Sinapis alba also showed elongation of the stem when given supplementary irradiation in the violet, blue, or infrared wave length regions. In this Crucifer, also, stem elongation started before a reaction of the growing point was visible. This is evident from Table 4, in which the stem length data are compiled, obtained in the experiment, the photoperiodic data of which have been given in Table 2.

From a comparison of these data it is quite evident that apart from the increase in stem length owing to the formation of a flower stalk, there is a marked elongating effect of blue and infrared. This is seen at once, when, *e.g.*,

the stem lengths and degree of flowering of the plants in infrared, white and dark after 39 days are compared. The white and dark controls have about the same stem length, *viz.* 237 and 207.5 mm respectively, whereas there is a marked difference in the degree of flowering (5.5 and 2.0, respectively). However, the plants in infrared have the same degree of flowering as the white controls, *viz.* 5.5, but the stem length is 365 mm, which is much more than that of the white controls.

TABLE 4

Stem length in *Sinapis alba*, as dependent on wave length of supplementary light, in a 10 + 8 hours treatment. Measurements of 13–3–'52 and 20–3–'52 (after 32 and 38 days of treatment, respectively). Average of 8 plants. Taken from (65).

Wave length region of supplementary light	Stem length in mm	
	after 32 days of treatment	after 38 days of treatment
Violet	221	265
Blue	377	478
Green	176	218
Yellow	198	233
Red	175	224
Infrared	296	365
White	194	237
Dark	168	208

Iberis coronaria L. var. Empress shows much the same reaction, as is indicated by the results in Table 5. This plant also has a certain stem length, even in non-flowering condition. It can be concluded from Table 3, that the photoperiodic reaction in this plant is even less clear than in *Sinapis alba* or *Brassica Rapa* var., but there still is a distinct promoting effect of blue and infrared supplementary irradiation. The difference between the dark and white controls is smaller. Notwithstanding this weak reaction, the same reasoning as in the case of *Sinapis alba* can be applied to demonstrate that in this plant also distinct stem elongation results from blue and infrared supplementary irradiation, apart from the flowering reaction.

TABLE 5

Stem length in *Iberis coronaria* var. Empress, as affected by supplementary light of various spectral regions in a 10 + 8 hours treatment. Measurements of 29–4–'52, 15–5–'52, and 29–5–'52, after 8, 24 and 38 days respectively.

Wave length region of supplementary light	Stem length in mm		
	after 8 days	after 24 days	after 38 days
Violet	49.5	138	207
Blue	79.5	233	304
Green	48	120	172
Yellow	47	155	234
Red	43	155	221
Infrared	79	209	292
White	42	140	197
Dark	47	127	188

4. RELATIONSHIP BETWEEN PHOTOPERIODIC AND FORMATIVE EFFECTS

As has been emphasized in the preceding sections, some of the formative reactions have to be correlated with flowering. This is especially true for the change in leaf form in *Brassica Rapa*, in the upper leaves of plants receiving supplementary irradiation from violet, blue or infrared wave length regions. The elongation and the change in shape always coincide with the growth of a flower stalk. On the other hand the stem elongation was shown to be a primary formative effect occurring before the flower primordia grow out. Experiments were designed to investigate the correlation of these phenomena. It was thought that the formative reactions might be independent of day length, so that supplementary irradiation extending a very short day to a total day length of 12 hours, would inhibit flower formation, but might still promote the elongation of the stem in violet, blue and infrared.

Brassica Rapa var. was subjected to such a treatment of 8 hours of white light of high intensity (22.500 ergs/cm²/sec) supplemented by 4 hours irradiation with various spectral regions at an intensity of 1000 ergs/cm²/sec. Thus the total photoperiod per daily cycle did not exceed 12 hours. The stem length of the plants grown under these conditions has been given in Table 6.

TABLE 6

Stem length of *Brassica Rapa* var. in an 8 + 4 hours treatment, as affected by wave length of supplementary light. Measurements of 10–5–'50, 19–5–'50, 1–6–'50 and 6–6–'50 (after 22, 31, 43, and 48 days of treatment respectively).

Wave length region of supplementary light	Stem length in mm, after different durations of treatment			
	after 22 days	after 31 days	after 43 days	after 48 days
Violet	26	60	137	179
Blue	32	69	165	215
Green	12	19	21	32
Yellow	6	16	26	35
Red	4	13	18	28
Infrared	24	58	133	149
White	6	20	29	38
Dark	3	20	44	42

From these data it is quite evident that the elongating effect of blue and infrared radiation still exists when the supplementary light is given within the short day. However, in this experiment, visible flower buds developed in blue after 36 days, in violet after 40 days and in infrared after 42 days of treatment. After 48 days of treatment open flowers were observed in the plants receiving blue supplementary light. No flower buds or flowers could be observed in red, yellow, green, or in the white and dark controls. The white control received in its supplementary white light about $\frac{1}{3}$ of the energy in the blue that the blue plants received.

This remarkable behaviour that a long day plant flowers under short day conditions, provided an irradiation with light of suitable wave length regions was included in the short photoperiod, gave a good reason to attempt at a further reduction of the day length.

In subsequent experiments it was tried to grow *Brassica Rapa* var. on a photoperiod of 5 + 4 hours. This was not very successful since the total amount of

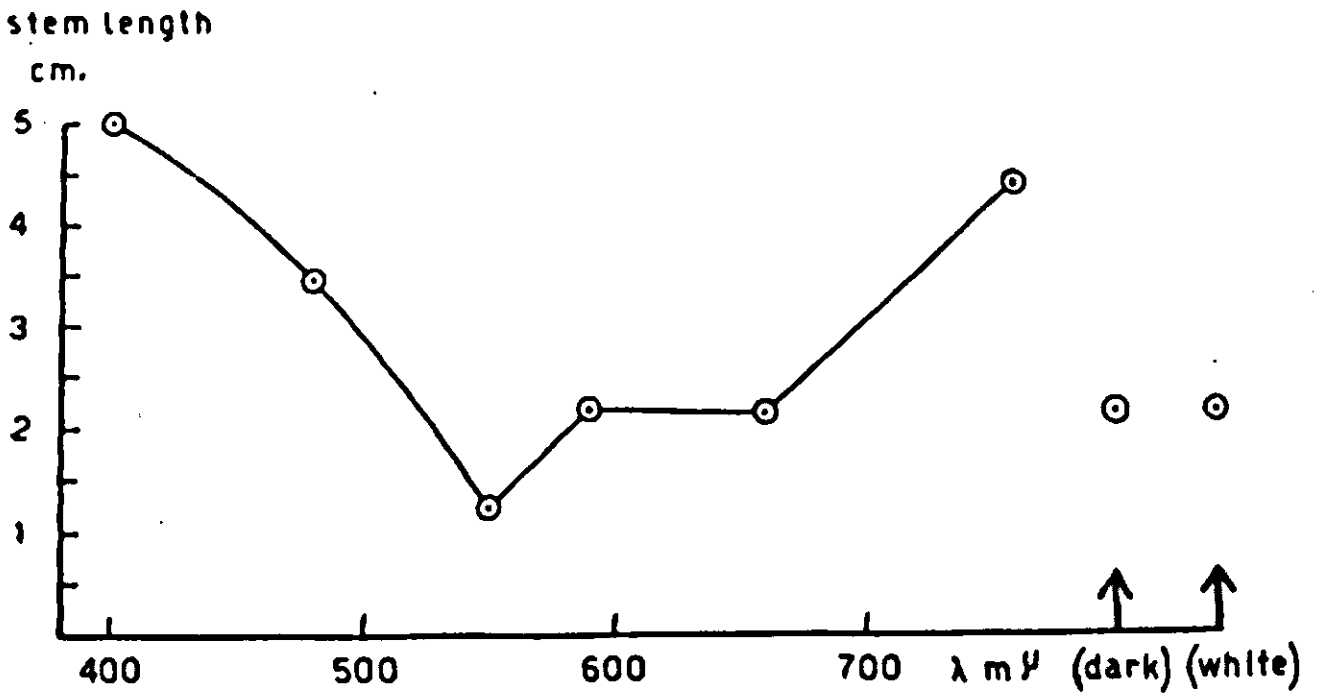


FIG. 6.
Stem length in *Brassica Rapa* var., as affected by a 5 + 4 hours treatment with supplementary light of various spectral regions at an intensity of 1000 ergs/cm²/sec. Measurements of 6-3-'51.

light probably was too low for normal growth. However, as can be seen in fig. 6, it was quite clear that the elongating effect of blue and infrared supplementary irradiation still existed under the conditions of this experiment. The growth of the plants was rather irregular, and the treatment had to be continued for a relatively long time because of the slow growth so that not much absolute value can be attached to the following counts of flower buds after 79 days of treatment: violet: 7, blue: 16, green: 2, yellow: 10, red: 9, infrared: 19, white: 7, dark: 2 flowerbuds per 4 plants. The flower buds did not open and dried out after some time. There is reason to suppose that the difference in the number of flower buds between the plants in blue and infrared, and in the other treatments was significant since this was found both times the experiment was made.

5. DISCUSSION

From the results given in this chapter it seems justified to conclude that in most *Cruciferae* the photoperiodic reaction is governed by a mechanism of which the photoreceptive part should absorb light of wave length regions from 400–500 and from 700–900 mμ. The combination of the results of the present investigation and the fact that to the author's knowledge no *Cruciferae* have been found to react photoperiodically to the red, yellow and green regions gives sufficient ground for this assumption. As has been pointed out in preliminary papers (78, 65) an antagonistic effect of light of the wave length region 500–700 mμ is involved in the overall reaction to white light, but for the discussion of that phenomenon reference is made to Chapter VI of this paper.

It has been shown in a number of experiments described above that the formative effect of stem elongation must be considered as a primary effect of the supplementary violet, blue and infrared irradiation. However, it has not been possible to separate the stem elongation from a promotion of flowering, not even under short day conditions that are strongly inhibitory to flowering normally. As will be shown later on, the stem elongation in red-sensitive plants like spinach, is brought about by the change in the growing point and it has proven impossible to bring out the stem by a formative effect. It is suggested that this difference might offer an indication as to the nature of the photoperiodic reaction in *Cruciferae*. It seems that in representatives of this family flowering is promoted by an elongation of the stem brought about by the action of specific wave length regions, more or less independent of the day length. Only when white light is given there seems to be some importance of the length of day. It has been suggested previously that this effect of the length of day could

be explained by the antagonism mentioned above (65), assuming that the red-reaction is subject to a time effect, which might be due to variations in the amount of substrate at the disposal of the pigment system, or to a saturation of secondary reactions. If the blue-infrared-reaction in the *Cruciferae* does not have such a time effect, the length of day would be decisive for the position of the equilibrium between the red- and blue-infrared-reactions.

CHAPTER V

PHOTOPERIODIC AND FORMATIVE EFFECTS OF SUPPLEMENTARY LIGHT OF VARIOUS WAVE LENGTH REGIONS IN NON-CRUCIFEROUS PLANTS

1. INTRODUCTION

The spectral dependence of the photoperiodic reaction has been investigated in many plants. However, the methods vary considerably: from the simple experiments made by FUNKE (18) who used day light, filtered by coloured glasses, to the elaborate spectrographic method employed by BORTHWICK *et al.* (47) is a long way. Between these two extremes many other methods have been used. It is surprising that nevertheless most investigators reached the same conclusion or obtained data that could be explained on the basis of this conclusion. Generally, it was found that expecially the red part of the spectrum had the greatest activity, closely followed by the yellow and green regions. Sometimes, when high amounts of light energy were used, or when the energy requirement of the photoperiodic reaction of the plant was very low, it was found that the blue region was equally active, but in most cases this did not disprove the smaller efficiency usually attributed to the blue region. BORTHWICK *et al.* (47) using a large size spectrograph, have been able to measure action spectra of the photoperiodic reaction in a number of plants. The initiation of flower primordia was employed as a criterium. Following the wave length scale from red to violet, their action spectra show a maximum effectiveness in the red (about 640 mμ), a gradual decrease of efficiency up to about 550 mμ, then a steep fall, resulting in a minimum at about 470 mμ, followed by a small rise up to the short wave length limit of the equipment (400 mμ). At the red end of the spectrum they found a very steep fall in effectiveness beyond 680 mμ, and no effectiveness in the infrared (*cf.* also discussion in Chapter VI).

2. SPECTRAL DEPENDENCE OF THE PHOTOPERIODIC REACTION

Two plants have been tested which could be expected to show a definite reaction to day length, *viz. Cosmos bipinnatus* CAV., a short day plant, and *Spinacia oleracea*, var. Nobel, a long day plant. Both plants have been used in several experiments, but only the results of one of them will be given, as the results proved to be exactly reproducible. Table 7 summarizes the results obtaind in a typical experiment with *Cosmos bipinnatus*, in which a 14 hour dark period was interrupted by 2-hour exposures to light of various spectral regions, at an intensity of 1000 ergs/cm^2/sec. The interruptions were applied in the beginning, near the middle, or at the end of the dark period.

TABLE 7

Degree of flowering of *Cosmos bipinnatus* CAV. receiving 2-hour exposures to supplementary light at the beginning (I), near the middle (II), or at the end (III) of a 14 hour dark period. Intensity of supplementary light: 1000 ergs/cm²/sec. Observations of 13–3–'52, after 40 days of treatment. Taken from (65).

Wave length region of supplementary light	Degree of flowering		
	I	II	III
Violet	7.25	4.25	6.75
Blue	8.00	5.75	8.00
Green	0.75	0.00	1.25
Yellow	0.00	0.50	0.50
Red	0.75	0.00	1.75
Infrared	8.25	9.00	7.50
White	0.00	0.00	1.00
Dark	8.00	8.00	8.00

From the observations presented in Table 7 two conclusions arise. In the first place there is a slight increase in effectiveness of the light interruption if it is given near the middle of the dark period, as has been found before by several other workers *cf*, *e.g.* (47). It is also obvious that in this plant the middle region of the spectrum, from 520-700 mμ is very active in the photoperiodic reaction. The violet, 390–430 mμ is slighly more effective than the blue region in the suppression of flowering, whereas the infrared does not inhibit flowering at all, as compared with the dark controls. The region 520-700 mμ shows the greatest effectiveness. This picture is in complete accordance with the action spectra obtained by BORTHWICK *et al.*, with some other plants (67, 47, 49). In later experiments BORTHWICK *et al.* (8) found an antagonistic effect of the infrared in the photoperiodic reaction of *Xanthium*. This antagonism will be discussed in Chapter VI, but here attention is drawn to the fact that under the conditions of this experiment the increase in flowering of the infrared plants that should be expected, is absent or in any case very weak. However, this does not necessarily contradict the concept of an antagonism, as will be seen in a later discussion.

The long day plant *Spinacia oleracea* L. var. Nobel has also been tested for the spectral dependence of its photoperiodic reaction. It was run in some routine experiments in a 10 + 8 hours treatment. The supplementary light was given at an intensity of 1000 ergs/cm²/sec. In the experiment, the results of which are given in Table 8, the treatment started on 11–11–'51. From this Table it is evident that this long day plant also shows the spectral response normally encountered in the photoperiodic reaction. In *Spinacia oleracea*, stem length can also be used as an indication of the flowering reaction, since, in the vegetative condition, the leaves form a rosette. The elongation of the stem is directly coupled with the developmental condition of the growing point (see, *e.g.*, WITHROW *et al.* [81, 82, 83]). Again the spectral region 520–700 mμ is very active in this respect, and the regions 400–500 and $>$ 700 mμ only show a very slight promotion of flowering, if any at all, especially if the rather high intensities used are taken into consideration.

Two more plant species have been used in this type of experiments: *Lactuca sativa* L., var. "Wonder van Voorburg" and *Solanum Lycopersicum* L., var. Vetomold 121.

TABLE 8

Condition of the growing point, and stem length of *Spinacia oleracea* L., var. Nobel after 33 days of treatment with 10 hours white light and 8 hours supplementary light of the indicated wave length regions. Intensity of supplementary light 1000 ergs/cm²/sec. Measurements of 14–12–'51. Taken from (65).

Wave length region of supplementary light	Number of plants			Average stem length in mm
	Dissected	Reproductive	Vegetative	
Violet	20	11	9	8.9
Blue	12	8	4	15.0
Green	15	15	–	45.5
Yellow	18	18	–	37.0
Red	15	15	–	53.6
Infrared	16	2	14	4.4
White	12	12	–	50.5
Dark	16	2	14	5.1

Lactuca sativa has been shown to be photoperiodically sensitive for flowering but varietal differences are known to exist. The variety used here showed no such dependence on length of day under the prevailing conditions and at least for the duration of the experiments: it did not initiate flower primordia at all. Therefore, the results obtained in this investigation are not connected with the photoperiodic reaction for flowering, although it is not certain that this variety does not have photoperiodic requirements under different experimental conditions.

Solanum Lycopersicum has been found day neutral by previous investigators. This plant was included to see whether the formative reactions found in plants that are sensitive to day length, also occurred in day neutral species, and also to investigate whether these plants possibly showed a mechanism similar to that found in *Cruciferae*, *i. e.*, relative insensitivity to length of day in white light, but sensitivity to isolated spectral regions. It turned out that under the conditions of the experiment, consisting of a 10 + 8 hours treatment, no effect on flowering of either day length or wave length of supplementary light could be demonstrated; all plants produced flowers at the same internode, and there was no significant difference in the time of flowering.

3. FORMATIVE REACTIONS TO SUPPLEMENTARY LIGHT OF VARIOUS WAVE LENGTH REGIONS IN NON-CRUCIFEROUS PLANTS

In the experiments on the photoperiodic reaction that have been dealt with in the preceding section, a number of formative reactions were observed. It was found, *e.g.*, that stem length, leaf length, and petiole length, as well as the angle between leaves and stem are dependent on the wave length of the supplementary irradiation.

The first reaction of this kind was observed in the 10 + 8 hours experiments in the short day plant *Cosmos bipinnatus*. This plant reacted to supplementary irradiation in the blue and infrared regions with a marked elongation of the internodes growing out after the beginning of the treatment. This elongation started more or less immediately after the beginning of the treatment and was evident in all internodes formed from that moment up to the end of the experiment, or up to the formation of a flower bud, which formation stopped terminal

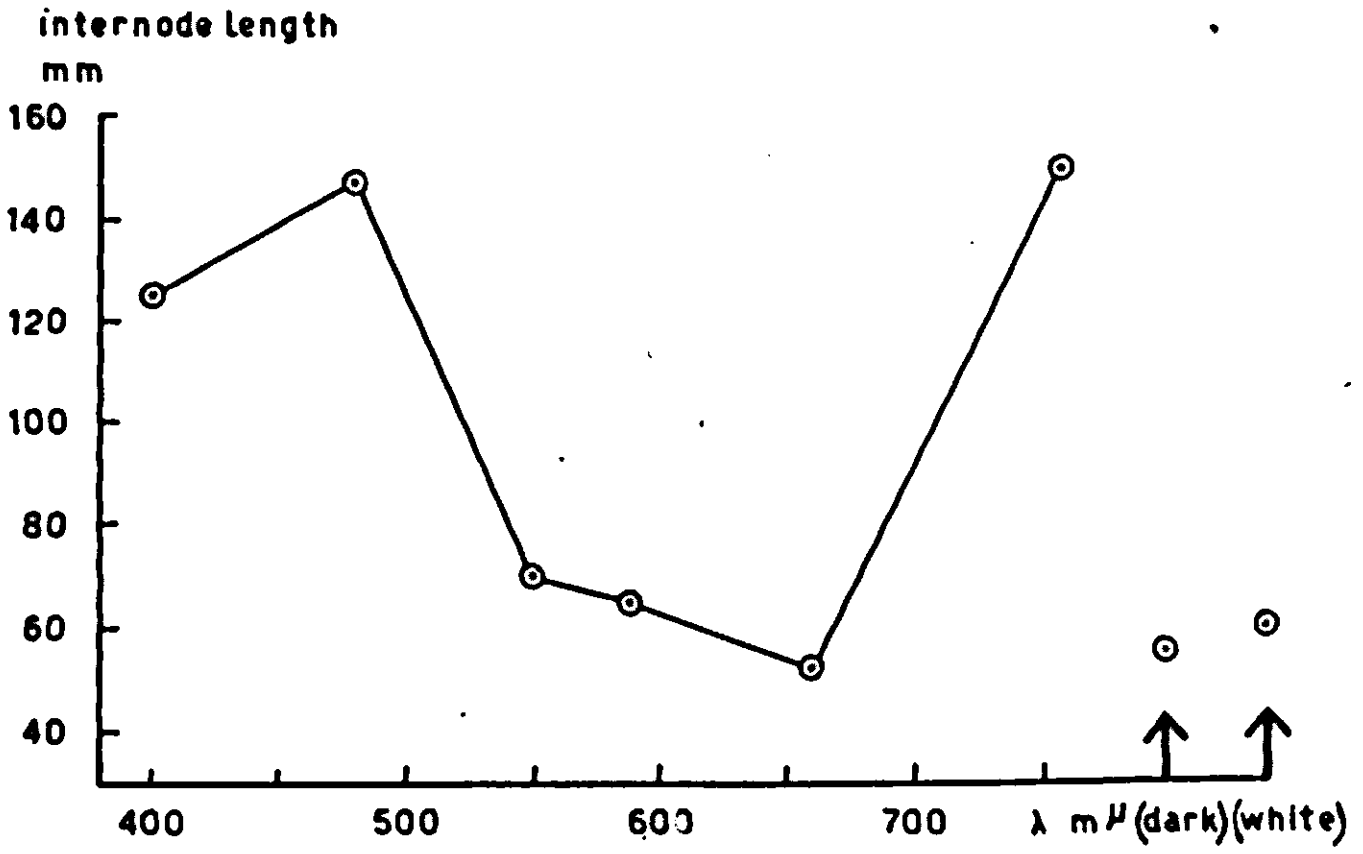


FIG. 7. Length of the second internode in *Cosmos bipinnatus*, as affected by a 10 + 8 hours treatment with various spectral regions at an intensity of 1000 ergs/cm²/sec.

growth. This reaction was so consistent and quantitative that the internodes of *Cosmos* have been used in most of the experiments on the nature and properties of the formative reaction in the non-cruciferous plants. Because of the uniformity in the results of the routine experiments, the data obtained in an arbitrary one can be considered representative and consequently only one set of measurements will be given here. Fig. 7 gives the length of the second internode of *Cosmos* plotted as a function of wave length of supplementary light in a 10 + 8 hours experiment (taken from [78]). Apart from this elongation, another difference was noticed, which, so far, has not been investigated in greater detail. Normally, the stems of *Cosmos bipinnatus* contain a certain – rather variable – amount of anthocyanins, localized in the epidermis. However, as soon as such a plant is exposed to blue or infrared additional irradiation, the subsequently formed internodia are quite free from anthocyanins, in contrast to the plants receiving supplementary white, red, yellow, or green light, or to the dark controls. In *Cosmos*, the leaves did not show a clear and consistent reaction to the wave length of the supplementary light.

The long day plant *Spinacia oleracea* L., var. Nobel also showed marked growth responses to the supplementary irradiation. In this plant, however, that in vegetative condition has a rosette of leaves and a very short stem, stem length was not increased by blue or infrared additional irradiation. The stem elongated only after the growing point had become reproductive. Since, under the conditions of the experiment this only happened in the spectral region 520–700 mμ, one might conclude that in this plant the middle region of the spectrum promoted elongation of the stem, a type of reaction that has not been found in any other plant used in the present work. That this conclusion is not justified is demonstrated by the fact that in this species no elongation of the stem occurred when the growing point still was vegetative, and also by the slow start of the elongation as compared with the elongation resulting, *e.g.*, from blue or infrared that starts right away. Thus, it must be concluded that the stem elongation is a secondary effect, related to the flowering process.

However, there is a distinct effect of the blue and infrared supplementary radiation on petiole length, as is shown in fig. 8 and in Table 9, in which the pe-

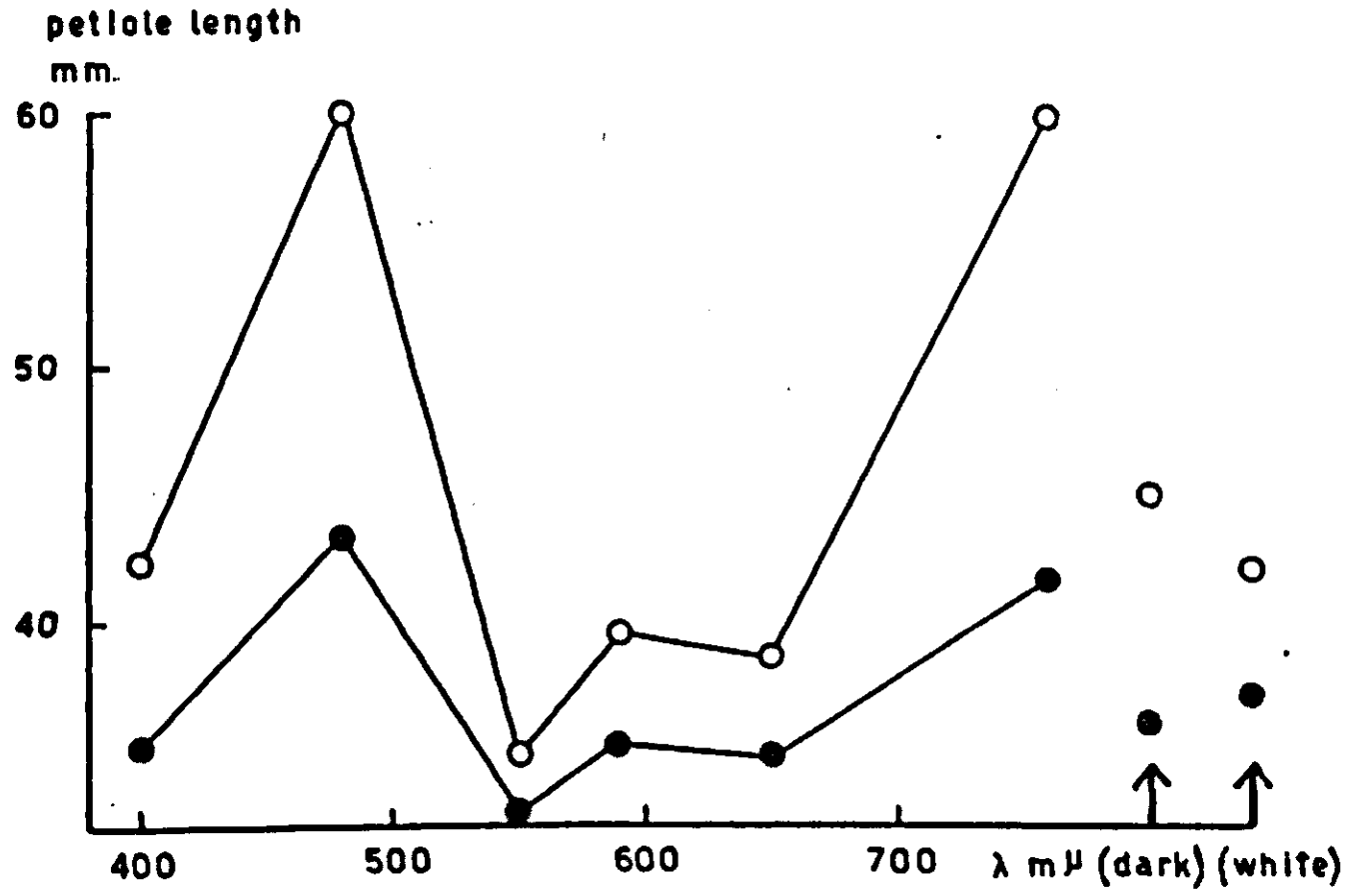


FIG. 8. Average length of the petioles of the 4th (●) and the 6th (○) leaf of plants of *Spinacia oleracea* var. Nobel, as affected by a 10 + 8 hours treatment with various spectral regions at an intensity of 1000 ergs/cm²/sec., after 33 days of treatment. Measurements of 14-12-'51.

tiole length of the 4th and the 6th leaf are given. This indicates that this plant species also contains the mechanism responsible for the blue-infrared reaction, only the location of the effects differs from that so far found in most other plants. The petioles of these leaves have already reached their final length before the flower stalk starts to elongate. Moreover, the independence of flowering shown by this effect is especially evident from the fact that petiole length in the dark and white light controls was very similar, although the white control flowered about as quickly as the plants in red, yellow, and green supplementary light, whereas the dark controls did not flower for the duration of the experiment. It is evident from Table 9, that, owing to this difference in flowering tendency, the stem lengths in the dark and white light controls show conspicuous differences.

It should be remarked that the phenomenon discussed can only be made clear by measuring petioles of separate corresponding leaves, not by taking average petiole lengths of all leaves present, because, along with the development

TABLE 9

Stem length and length of petioles in *Spinacia oleracea* var. Nobel after 22 and 33 days of a 10 + 8 hours treatment. Intensity of supplementary light 1000 ergs/cm²/sec. Measurements of 4–12–'51 and 14–12–'51.

Wave length region of supplementary light	After 22 cycles		After 33 cycles	
	Stem length mm	Petiole length (4th leaf) mm	Stem length mm	Petiole length (6th leaf) mm
Violet	2.3	35.5	8.9	42.3
Blue	4.6	43.3	15.0	60.1
Green	10.0	32.3	45.5	34.8
Yellow	12.6	35.2	37.0	39.5
Red	17.0	34.8	53.6	38.6
Infrared	2.0	41.7	4.4	59.9
White	18.6	37.2	50.5	42.0
Dark	2.2	36.0	5.1	45.1

of a flower stalk, a flowering plant develops sessile leaves, whereas a plant in non-flowering condition, continues producing leaves with petioles so that the average petiole length in a flowering plant for this reason is likely to be smaller than the average petiole length in a vegetative plant of the same age.

Also in *Lactuca sativa* L., var. "Wonder van Voorburg", marked formative effects occurred upon supplementary irradiation with blue and infrared wave length regions. As was already remarked in the preceding section, this plant did not initiate flower primordia in any of the treatments under the prevailing experimental conditions. Thus it cannot be doubted that the formative effects observed were due only to the spectral quality of the supplementary light and had nothing to do with the duration of the photoperiod, or with flowering. The influence of the blue and infrared wave length regions became manifest as an elongation of the stem, contrasting with the normal rosette aspect of the plant, and as elongation of the leaves, resulting in a change in shape. In this species it proved possible to bring about stem elongation in plants that normally have leaf rosettes, without the necessity of disturbing the vegetative condition of the vegetation point. The results of a typical experiment are presented in Table 10.

It will be noticed that after very long duration of the treatment the elongation also starts in the white and dark controls, but at that stage the blue and infrared plants have elongated to a very much greater extent (three or four fold). It must be emphasized that these very elongated plants had to be handled very carefully as they were very weak. Because of this, it is very well possible that towards the end of the experiment the elongation was limited by other factors than light, *e.g.*, translocation of photosynthates.

It must also be observed, that two other varieties of *Lactuca*, *viz.* "Attractie" and "Meikoningin" did not show an appreciable stem elongation with blue or infrared supplementary irradiation. In these varieties, the only formative effect was found in leaf length, that was affected in a way very similar to that, demonstrated in Table 10 for the variety "Wonder van Voorburg".

Solanum Lycopersicum L., var. Vetomold 121, was chosen as a representative of the group of photoperiodically indifferent plants. It was subjected to a 10 + 8 hours treatment under the usual conditions. As is evident from Table 11, this plant species also responds to blue and infrared supplementary irradiation with

TABLE 10

Stem length and leaf length in *Lactuca sativa* var. "Wonder van Voorburg", as affected by wave length of supplementary light in a 10 + 8 hours treatment. Intensity of supplementary light 1000 ergs/cm²/sec. The treatment started on 19–4–'50.

Wave length region of supplementary light	Stem length in mm		Average length of leaves in mm	
	after 49 days	after 67 days	after 24 days	after 31 days
Violet	120	212	105	106
Blue	192	434	143	143
Green	21	94	84	96
Yellow	28	65	84	92
Red	30	101	93	106
Infrared	137	287	119	129
White	21	61	91	109
Dark	30	44	98	103

TABLE 11

Stem length in *Solanum Lycopersicum* L., var. Vetomold 121, as affected by wave length of supplementary irradiation in a 10 + 8 hours treatment. Intensity of supplementary irradiation: 1000 ergs/cm²/sec. Measurements of 15–5–'52 (after 12 days of treatment) and 30–5–'52 (after 27 days of treatment). Taken from (79).

Wave length region of supplementary light	Stem length in mm	
	after 12 cycles	after 27 cycles
Violet	66	120
Blue	110	228
Green	67	125
Yellow	63	126
Red	66	127
Infrared	93	194
White	72	120
Dark	71	117

an increase in stem length over that of the dark and white controls, and the other treatments, which all reached about the same length.

Again this behaviour of a photoperiodically indifferent plant is an indication that the formative reactions can occur independently of the flowering reaction.

Apart from the differences in stem length, no significant differences were found in number of internodes (variation from 8.87 to 9.75 after 27 days) or in number of leaves, leaf length or petiole length. It should be remarked that in this species infrared radiation was not quite as effective in producing elongation as blue. For the length of the 5th petiole it was found that the average petiole length in blue was 53.0 mm, compared with lengths varying from 38.0 to 41.5 mm in the other treatments, including infrared. More or less the same reaction was found in total leaf length. Average total leaf length in plants receiving blue supplementary radiation was 188.0 mm, as compared with a range of 148 to 161 mm for the other treatments.

4. RELATIONSHIP BETWEEN FORMATIVE AND PHOTOPERIODIC EFFECTS OF SUPPLEMENTARY LIGHT OF VARIOUS SPECTRAL REGIONS

In *Cruciferae*, it is impossible to separate the flowering reaction from the elongation occurring in blue and infrared, as has been shown in experiments described in Chapter IV. In cruciferous species a treatment enhancing elongation also enhances flowering, even when given in a daylength that in itself inhibits or delays flowering. It seemed worth while to investigate whether in plants in the red-sensitive group a similar relationship occurs.

In the case of the short day plant *Cosmos bipinnatus*, the indirect evidence was in favour of the concept of independent reactions, and a crucial experiment was designed, with a double purpose: to investigate whether in this species the formative reaction could also be produced under short day conditions, and at the same time to verify whether flowering was affected by the spectral composition of supplementary irradiation given within a short day.

Cosmos plants, germinated and raised under long day conditions were subjected to a daily cycle of 8 + 4 hours, so that they received 8 hours of white light of high intensity, 4 hours of supplementary light of various wave length regions, and 12 hours of darkness daily.

TABLE 12

Length of the second internode of *Cosmos bipinnatus* CAV., in mm, when grown in an 8 + 4 hours treatment, as affected by wave length of supplementary light. Measurements of 11–4–'51.

Wave length region of supplementary light	Length of second internode in mm
Violet	73
Blue	75
Green	37
Yellow	25
Red	32
Infrared	82
White	43
Dark	37

Table 12 gives the length of the second internode of the plants in this experiment. As only a minimum number of plants per treatment was used, *viz.* 4, it is not possible to give accurate data on the flowering reaction; on 11–4–'51, 57 days after the start of the treatment, 4 plants out of 4 flowered in dark and green, 3 out of 4 in yellow and white, 2 in red, and only one in violet, blue and infrared. All plants had macroscopic flower buds.

The results of this experiment, combined with the indications obtained in 10 + 8 hours experiments, prove that the formative reaction can occur independent of flowering. In 10 + 8 hours experiments the second internode completes its growth before the initiation of flower primordia starts. In 8 + 4 hours experiments flowering seems independent of elongation in all treatments.

From the experiments discussed in this section, further arguments for the existence of an antagonism have been obtained (*cf.* [78, 65]). From a comparison of the *Cosmos* plants receiving 10 + 8 h. white and 8 + 4 h. blue it is evident that the absence of excess elongation in the 10 + 8 h. white treatment cannot be due to lack of blue light as compared with the 8 + 4 h. blue plants, but must be ascribed to the action of the remainder of the spectrum that was given simultaneously to the 10 + 8 h. white plants.

5. SOME CHARACTERISTICS OF THE FORMATIVE REACTION

To obtain more information on the formative reaction to blue and infrared supplementary irradiation, a number of experiments have been made in which the influence of the temperature during the dark periods following the supplementary irradiation was studied.

These experiments have been made with *Cosmos bipinnatus* and *Lactuca sativa* var. "Wonder van Voorburg". The plants received 10 hours of white light of high intensity and 8 hours supplementary light, both at a temperature of 20 °C, followed by 6 hour dark periods at 5°, 15° or 25 °C.

From Tables 13 and 14, in which the results of the elongation measurements in two of these experiments have been summarized, it is evident that in all treatments an increase of temperature during the dark period caused an increase in extension growth of internodes and stems, in both *Lactuca* and *Cosmos*. Representative plants of the *Lactuca* experiment are shown in Plate 1.

Although these measurements cannot be considered on a quantitative basis because it is not fully certain that at these various temperatures the elongation

TABLE 13

Stem length, in mm, of *Lactuca sativa* L., var. "Wonder van Voorburg", as affected by wave length of supplementary light in a 10 + 8 hours treatment and by temperature during the 6-hour dark period. Measurements of 30–6–'51, after 30 days of treatment.

Wave length region of supplementary light	Dark period temperature		
	5 °C	15 °C	25 °C
Violet	138	203	292
Blue	83	135	294
Green	39	48	76
Yellow	45	46	109
Red	51	37	104
Infrared	160	176	253
White	62	106	166
Dark	68	70	140

was determined by the same factor, they suggest that in any case a large portion of the length growth occurs during the dark period, and that the elongating influence of blue and infrared radiation is not limited to the irradiation period but is carried over into the subsequent dark period. This has been shown quantitatively by direct growth measurements for *Cosmos* internodes in experiments described on page 213. It is not at all certain whether under the conditions of the experiment the wave length region or the temperature limit the elongation. In fact a comparison of the lengths of the 4th and 5th internode in Table 14 suggests that the differences due to different dark period temperatures in a not fully elongated internode are bigger than in a full grown internode.

It could be expected that the temperature has an effect on the rate of growth during the dark period. This would result in differences in internode length in the various temperatures before growth is completed in all temperatures. This, however, would not necessarily affect the final internode length, but only the rate of growth would be influenced by temperature. From the data in Tables 13 and 14, it can be seen that growth rate as well as final internode length is increased by increasing the temperature during the dark period.

TABLE 14

Internode length, in mm, of two internodes of *Cosmos bipinnatus*, the 4th being full grown, and the 5th not yet fully elongated, as affected by wave length of supplementary light in a 10 + 8 hours treatment, and by temperature during the daily 6-hour dark period. Measurements of 22–9–'51, after 23 days of treatment.

Wave length region of supplementary light	4th internode, mm			5th internode, mm		
	Temperature dark period			Temperature dark period		
	5 °	15 °	25 °	5 °	15 °	15 °
Violet	53	68	77	22	47.5	59
Blue	75	80	85	51	58	82
Green	33	47.5	38.5	22	34	29.5
Yellow	34	38	40	22.5	25	40
Red	41.5	43	55	28	33.5	41
Infrared	68	76	100	46	56.5	94
White	53	59.5	74.5	39	32	65
Dark	38.5	45.5	56	27	30	36

As the elongation is also promoted by blue and infrared radiation, and as the two effects are additive to a large extent, it seems logical to conclude that the blue and infrared radiation exert their influence by stimulating a process occurring in darkness. This dark reaction is sensitive to temperature. The elongation in the white controls that was not found in other experiments, is due to the fact that in this experiment incandescent light was used for these control plants, for supplementary irradiation.

In order to obtain more information on the process of elongation a large number of direct short term measurements were made. After some preliminary experiments, the internodes of *Cosmos* were found to be the most suitable material for this type of measurement (65a, 65b).

An S.I.P. horizontal microscope was used. This microscope has a scale of 0–75 cms, which can be read to 0.02 mm, and eyepiece cross-threads and micrometer that can be read to 1μ. The view field is about 4 mm in diameter. The microscope was equipped with a small lamp in a brass tube; the light of the (incandescent) lamp was concentrated by a lens, in the focal plane of the microscope. By this arrangement it was possible to measure growth during dark periods with a minimum of light that, moreover, only touched a small portion of the stem. The leaves remained in darkness. The light from this lamp passed through a G.A.B. Calflex interference filter that filtered out all radiation of wave lengths between 690 and 1000 mμ. The cross threads were set on small dots of India ink, spaced at regular distances on the internode to be measured. It was established that the India ink dots did not interfere with growth.

In first measurements it was tried to establish in how far extension growth should be attributed to cell division and to cell elongation, and how these two types of growth are distributed over light and dark periods. A number of *Cosmos* plants that had been growing in an 18 hour day, and that had a young internode of about 5 mm length were selected and brought under a 12 hour day, at a light intensity of 25.000 erg/cm^2/sec and a constant temperature of 20 °C. On every

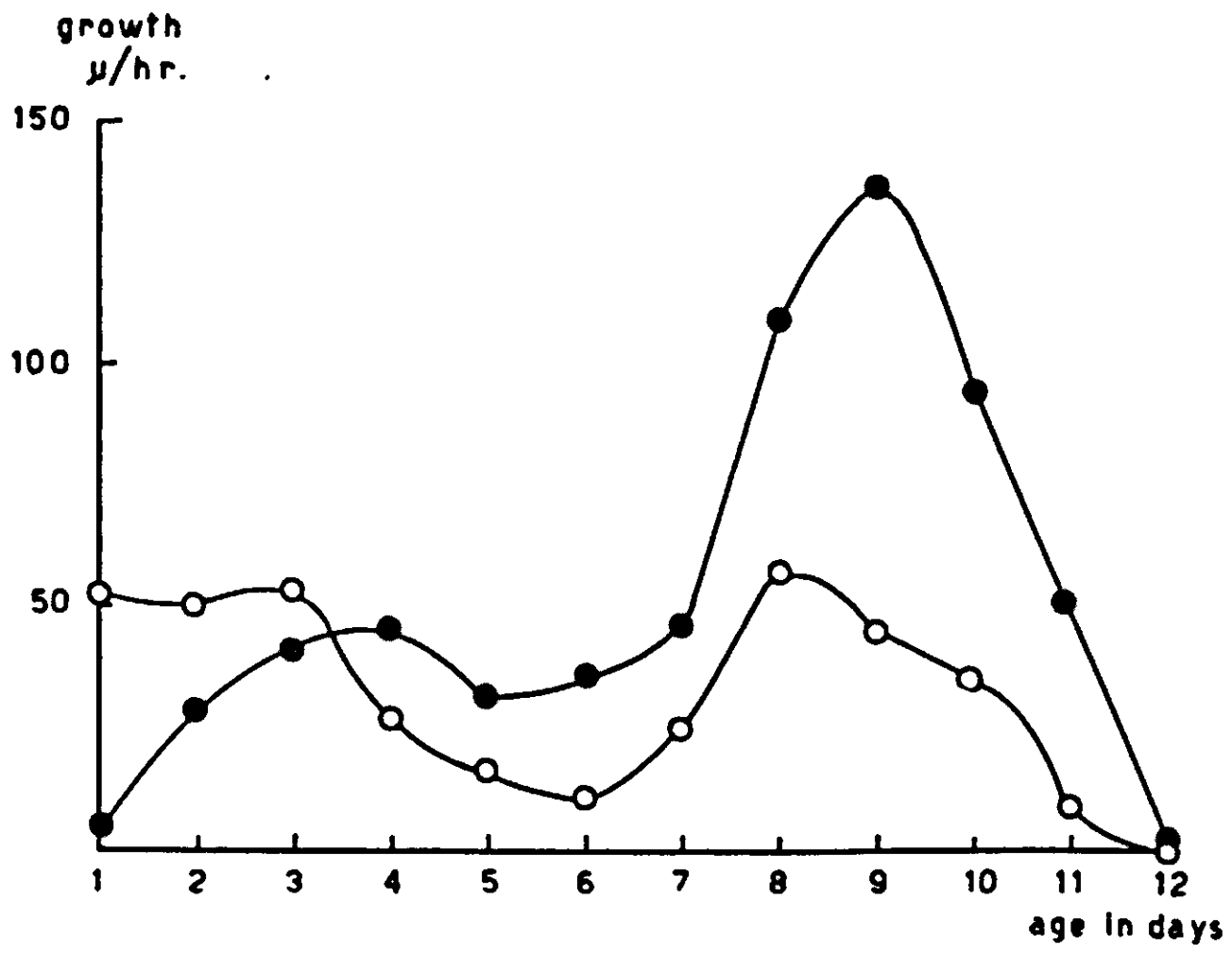


FIG. 9. Growth rate, in μ/hour of a *Cosmos* internode, as a function of age of internode. Open circles: growth rate during the photoperiod; closed circles: growth rate during the dark period. Measurements of 5–18–5–'53.

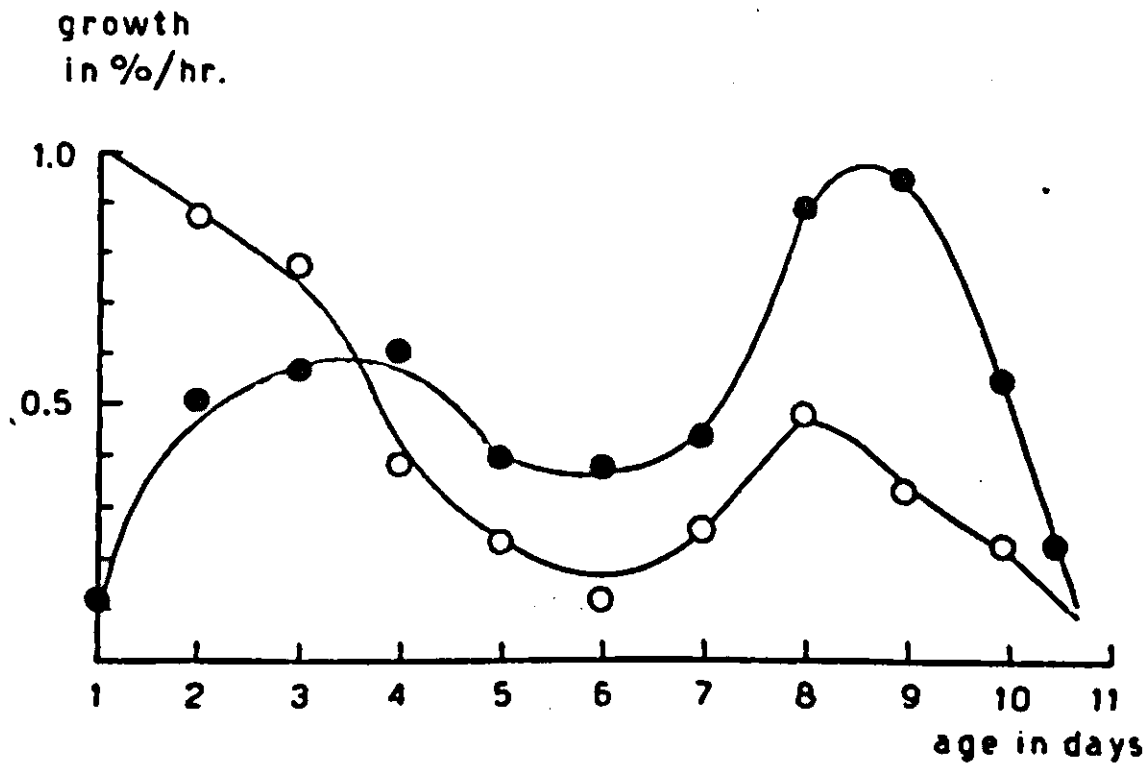


FIG. 10.
Growth of a *Cosmos* internode in % per hour, as a function of age of internode. Open circles: growth rate during the photoperiod; closed circles: growth rate during the dark period. Measurements of 5–18–5–'53.

change from the light to darkness and back, the length of the internodes was measured and this was continued until the internodes had matured. At the same time measurements of average cell length of epidermis cells were made by counting the number of cells per view field on various points of the internode.

In figs. 9 and 10 growth, in μ/hour and in %/hour respectively, has been plotted against the age of the internode. The growth rates during the light period and in darkness have been plotted separately. It will be seen that these growth rates are quite different. Growth during the light period is strongest in the very young internode, after which it decreases with time to reach a minimum when the internode is six days old. The growth rate during the night is very low in the young internode, but increases above that in the light after 3–4 days. From the cell counts it could be concluded that hardly any cell elongation occurred up to about the fifth day so that growth up to that time has to be ascribed to cell division. After the 6th day the cell division was negligible and practically all growth could be explained by the increase in cell length that occurred in part of the internode.

An example of the results of cell counts is given in Table 15. The measurements recorded in this Table have been obtained from plants that had been under supplementary (10 + 8 hours) light treatment for 35 days. Both the young 5 mm internodes and the mature internodes had been formed during this treatment. The results show that the elongation in infrared is due only to greater cell elongation. The absence of a difference in cell length in the 5 mm internodes shows that during the cell division stage there is no effect of the supplementary light. This observation, moreover, explains the results of some preliminary experiments in which it was tried to measure differences in growth rate of young internodes in red and infrared supplementary irradiation. It was not possible

TABLE 15

Average length of epidermis cells in young (3 days) and old (12 days) internodes of *Cosmos bipinnatus*, as affected by supplementary red and infrared irradiation, at an intensity of 1000 ergs/cm²/sec, in a 10 + 8 hours treatment.

Wave length region of supplementary light	Mature internodes		Young internodes	
	Average length of epidermis cells	Length of internode in mm	Average length of epidermis cells	Length of internode in mm
Red	228 ± 3 μ	38	55 ± 3 μ	5.0
Infrared	377 ± 2 μ	69	52 ± 4 μ	5.0

to find significant differences, but this must have been due to the fact, that the young internodes still were in the cell division stage.

6. DISCUSSION

The data, presented in the second section of this chapter indicate that in non-cruciferous plants which are sensitive to day length, the photoperiodic reaction is most sensitive to radiation of wave lengths from 520–700 mμ. This general conclusion seems justified because the small number of plants used in the present investigation gave results that are merely a further confirmation of the results obtained in many earlier investigations. The picture given above is in complete accordance with the action spectra for initiation of flower primordia in *Xanthium saccharatum*, *Hordeum vulgare*, *Hyoscyamus niger*, *Soja max*, as obtained by BORTHWICK *et al.*, and with results obtained by FUNKE (21), KATUNSKY (30), KLESHNIN (34), SCHAPELLE (60), WITHROW *et al.* (83) and others. This similarity in wave length dependence in a large number of plant species leads to the assumption of a very widely spread mechanism that may even be present in all plants.

The formative reactions described in sections 3, 4 and 5 occur independent of day length or of the initiation of reproductive development, as was demonstrated clearly in the experiments with lettuce and with the day length insensitive tomato plants. These formative reactions manifest themselves primarily as excessive cell elongation under the influence of blue and infrared supplementary irradiation, as was demonstrated for *Cosmos* internodes in section 5. The wave length dependence of this formative effect is more or less complementary to that of the photoperiodic reaction in non-cruciferous plants. Moreover, it coincides with the wave length dependence of promotion of flowering in *Cruciferae*. This correlation, combined with the indications obtained for antagonism between blue and infrared wave length regions and the middle part of the spectrum (65, 78), strongly suggests that all these reactions are governed by the same photoreceptive mechanism, consisting of two pigments. This photoreceptive mechanism then should be very wide spread also, if not of universal occurrence in higher plants. However, only the photoreceptive part has been shown to occur in a large number of plants; the reactions brought about in different species are quite different, so that the final reaction might not be of a simple nature. Of course it is very well possible, and even probable, that the pigments involved control only one, or a few reactions, producing or regulating the production of one or a few substances. It is very tempting to assume that growth substances play an important rôle in this mechanism, as the formative reactions bear a great resemblance to effects normally attributed to the action of growth substances; a further discussion will be given in Chapter VIII.

CHAPTER VI

ANTAGONISM BETWEEN EFFECTS OF THE WAVE LENGTH REGION 520–700 mμ, AND THOSE OF 400–500 AND 700–900 mμ

1. INTRODUCTION

In the course of the experiments reported in the foregoing chapters many indications were obtained that an antagonism exists between the action of blue

or infrared radiation on the one hand, and that of the region 520–700 mμ on the other hand. Most of these indications came from differences in either formative, or, in *Cruciferae*, in photoperiodic effects of supplementary white and blue light. Since in most of the treatments with blue and infrared, the total energy was far greater than the amount actually required for maximal response, and further, since $\frac{1}{4}$ to $\frac{1}{3}$ of the energy in the white supplementary light was emitted in the blue wave length region, the absence of elongation in the white controls strongly suggested that the remainder of the spectrum, emitted by the daylight type fluorescent tubes used for white supplementary irradiation, had an antagonistic effect (65, 78).

In *Brassica Rapa* var., white supplementary light given within a short day was not effective in the promotion of flowering, while blue and infrared supplementary irradiation resulted in a distinct promotion of flowering.

To establish this reaction beyond doubt, however, and in order to obtain more information about it, a number of experiments were performed, in which the action of a known mixture of antagonistic wave length regions was studied.

Before these experiments could be designed, it was necessary to study the energy requirements and the time relations of the formative reactions involved in the antagonism. Experiments with *Cosmos* had already shown that a reduction in the supplementary light period to 2 hours per night, at an intensity of 1000 ergs/cm^2/sec, did not result in any reduction in internode elongation in the blue and infrared. Nevertheless, an attempt was made to see how far this period could be further reduced. *Cosmos* plants were given supplementary light of blue, green, red and infrared wave length regions, at an intensity of 1000 ergs/cm^2/sec, for 8 hours, 1 hour, and 10 minutes, immediately after a daily 10 hour period of white light of high intensity. From Table 16, in which the results of this experiment are presented, it is evident that a reduction of the supplementary light period from 8 hours to 1 hour, did not cause a commensurate reduction in elongation. Supplementary irradiation for only 10 minutes per day had little or no effect, so short a period was, thus, below the limit of energy or duration required for a measurable response.

In another series of experiments, the intensity dependence of the formative reaction was determined. *Cosmos* plants received 8 hours of supplementary irradiation, subsequent to a daily 10 hour period in white light of high intensity. The supplementary light was given at intensities ranging from about 30 ergs/

TABLE 16

Effect of length of supplementary light period on elongation of *Cosmos* internodes in blue and infrared supplementary light. Intensity of supplementary irradiation: 1000 ergs/cm^2/sec. Measurements of 22–11–'52, after 19 days of treatment.

Wave length region of supplementary light	Length of second internode in mm		
	8 hours supplementary light	1 hour supplementary light	10 min. supplementary light
Blue	58.2	50.0	30.5
Green	32.5	29.8	33.5
Red	27.6	28.0	28.5
Infrared	72.5	52.0	46.2
White	36.0	–	–
Dark	37.2	–	–

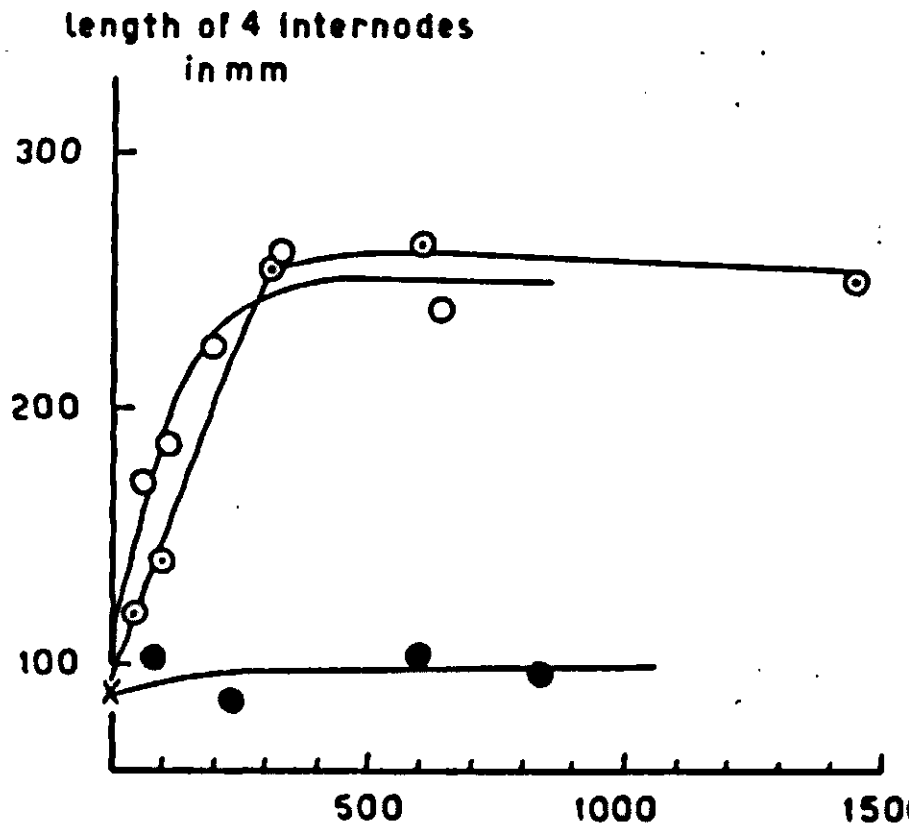


FIG. 11.
Internode elongation in *Cosmos*, as dependent on intensity of supplementary irradiation in blue, green and infrared wave length regions (○ : infrared; ⊙ : blue; ● : green). Measurements of 18–3–'53, after 16 days of treatment.

cm²/sec to 1500 ergs/cm²/sec. The treatment started on 2–3–'53. Fig. 11 shows the results obtained from measurements of the second internode, which had grown out during the 16 days treatment. Under the conditions of these experiments the reaction causing internode elongation seems to have been light saturated at very low intensities. Elongation does not increase with light intensity in blue and infrared at intensities above about 200 ergs/cm²/sec. Light saturation occurred in infrared at a slightly lower intensity than in the blue supplementary irradiation. In the control plants, under a range of intensities of green light, internode length remained unaffected by light intensity.

The results of these experiments were the basis for further experiments, in which the antagonistic wave length regions were given either consecutively of simultaneously.

2. EXPERIMENTS WITH CONSECUTIVE APPLICATION OF ANTAGONISTIC WAVE LENGTH REGIONS

As has been shown in the experiments described in the preceding section, the elongation of *Cosmos* internodes does not appreciably diminish when the supplementary irradiation period is reduced from 8 hours daily to 1 hour, if the intensity remains at 1000 ergs/cm²/sec. It has also been found that a large part of the elongation due to blue and infrared supplementary irradiation in a treatment of 10 + 8 hours occurs in the 6-hour dark period (Chapter V, section 5). From this it was concluded that a treatment in which 4 hours of blue or infrared supplementary irradiation was followed by 4 hours green or red light should demonstrate the antagonism envisaged above.

Young *Cosmos* plants were given treatments consisting of a daily period of 10 hours in strong white light, followed by 8 hours supplementary irradiation. Some of the plants received 8 hours of blue, green, red or infrared supplementary light, while others were given 4 hours blue followed by 4 hours green, 4 hours infrared followed by red, or the reversed sequence. After 18 days, when the second internode, formed since the beginning of the treatment, had completed its elongation, its length was measured. The results of these measurements are presented in Table 17.

These results show a complete inhibition of the excess elongation in blue and infrared supplementary light when green or red supplementary light is given

TABLE 17

Length of the second internode in *Cosmos bipinnatus* CAV., as affected by supplementary light of various spectral regions given for 8 hours per day, in sequences of 4 hours of an elongation promoting radiation, and 4 hours of an elongation inhibiting radiation, as well as the reversed sequence. Measurements of 22–11–'52. Intensity of supplementary light 1000 ergs/cm²/sec.

Wave length region of supplementary light	Average length of second internode, in mm
Blue	90.0
Green	35.2
Red	28.0
Infrared	75.0
Blue–Green	31.0
Infrared–Red	35.0
Green–Blue	51.5
Red–Infrared	57.5
White	36.0
Dark	37.2

after blue or infrared; the length of the internodes is practically the same as in the dark and white controls, and in the plants receiving only red or green radiation. However, when either blue or infrared supplementary light follows the red or green radiation, a marked elongation occurs. It follows that the effect of the last of a sequence of spectral regions dominates, and there is mutual antagonism between red or green, and blue or infrared radiation. Attention is drawn to the significantly smaller elongation in blue and infrared when these irradiations are preceded by red and green light, then when they are given alone.

As was discussed in the introduction to this chapter, this cannot be due to the shorter irradiation period in the first case, but it must be ascribed to the shorter dark period following the blue or infrared supplementary irradiation, so that the time during which the blue infrared reaction can take place, is reduced. An after-effect of this kind does not seem to operate when the sequence of wave length regions is reversed: there is no indication of an after-effect of either blue or infrared when this is followed by either green or red radiation. This behaviour can be understood if it is assumed that at the end of the white light period, the equilibrium is in the red position. Apparently, the change from this red position takes some time at the intensities used, so that blue or infrared supplementary irradiation is not immediately very effective. Red supplementary light, on the contrary, only has to keep the equilibrium in the red position.

3. EXPERIMENTS WITH SIMULTANEOUS APPLICATION OF ANTAGONISTIC WAVE LENGTH REGIONS

After the experiments described in the introduction to this chapter, in which the intensity dependence of the elongating effect of blue and infrared supplementary light was determined, it was possible to design experiments with simultaneous application of antagonistic wave length regions, without objections arising from uncertainties concerning the intensity dependence of the reaction.

TABLE 18

Absolute and relative intensities of red and infrared wave length regions in different mixtures used in experiments with simultaneous application of antagonistic wave length regions. Experiments of 5–13–1–'53 and 26–1 to 13–2–'53.

Plant Position	Intensity in erg/cm²/sec			Percentage of Infrared radiation
	red	infrared	total	
I	2110	800	2910	27
II	1180	840	2020	42
III	640	1370	2010	68
IV	260	1960	2220	86
V	210	2550	2760	92
VI	165	2400	2565	94

Cosmos plants received 8 hours supplementary light per day. When wave length regions were mixed, space limitations in the equipment made it necessary to give supplementary light of one wave length region from above, whereas the light of the second wave length region reached the plants from the sides. Two light units were mounted relatively far apart, so that the percentages of the two wave length regions in the mixture could be varied within wide limits by placing the plants in different positions with respect to the light sources. The blue and infrared radiations were given from above, while in the case of the mixed wave length regions, red radiation was given from the side. Red light was obtained by combination of the same lamps and filters as used in the supplementary light equipment, described in Chapter III, section 3. The total light intensity and the percentages of both spectral regions were calculated from measurements of the light from both units separately.

In the first experiment, the effect of various mixtures of red and infrared on internode elongation of *Cosmos* was determined. The mixed wave length regions were given for 8 hours per day, supplementary to a 10 hour day in white light of high intensity. Table 18 gives the relative and absolute intensities of red and

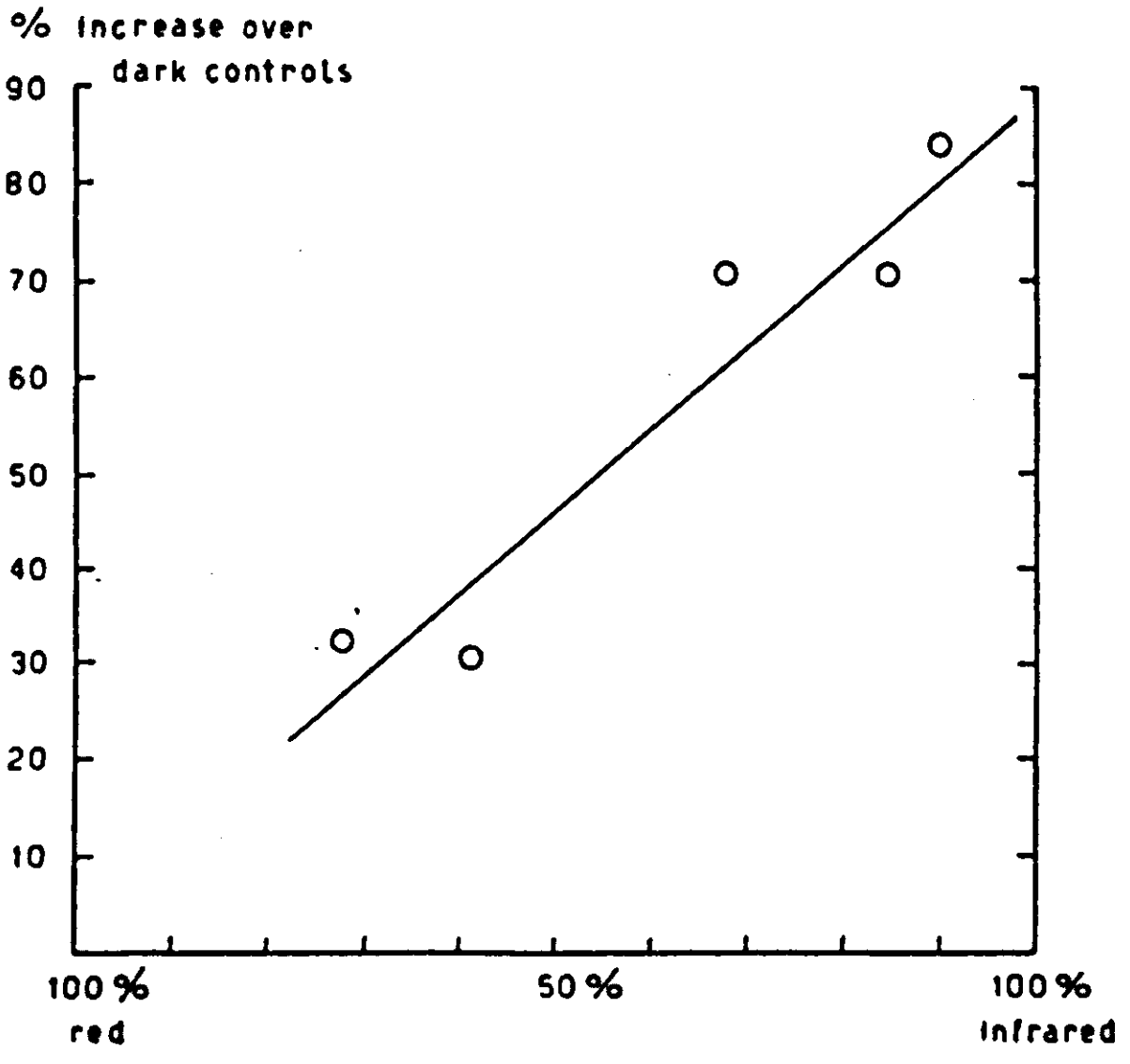


FIG. 12.
Effects of mixtures of red and infrared supplementary irradiation on elongation of *Cosmos* internodes, in a 10 + 8 hours treatment. Measurements of 13–1–'53.

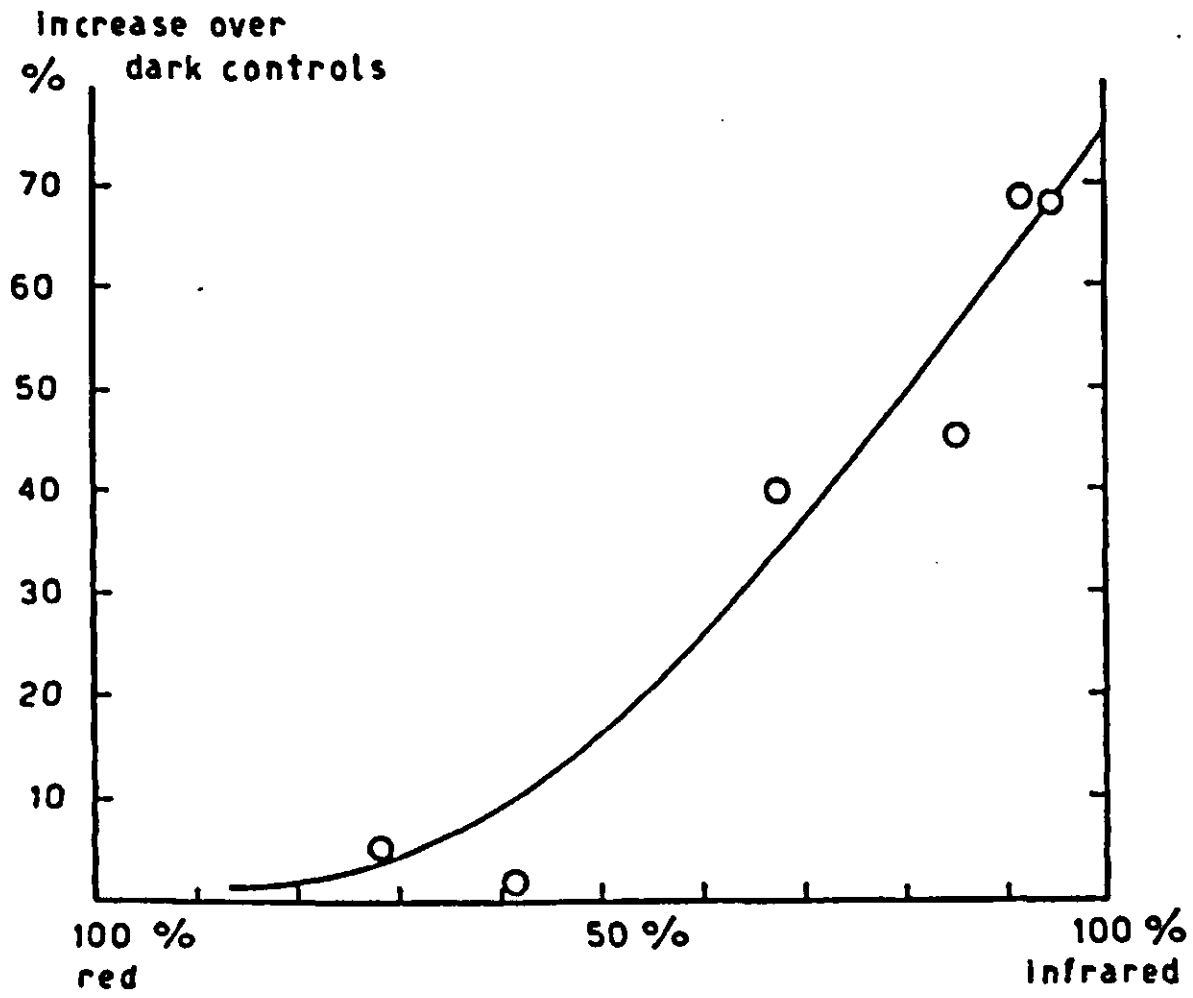


FIG. 13.
Effects of mixtures of red and infrared supplementary irradiation on elongation of *Cosmos* internodes, in a 10 + 8 hours treatment. Measurements of 13–2–'53.

infrared radiation in the mixtures. The experiments was started on 5–1–'53, and measurements of the internodes were made on 13–1–'53, after 8 days of treatment. In Fig. 12, the elongation of the first internode over that in the dark controls has been plotted against the percentage of infrared radiation in the supplementary light.

In a repetition of this experiment, with exactly the same light treatment, similar results were obtained. This experiment was started on 26–1–'53, and measurements of the second internode were made on 13–2–'53 after 18 days of treatment. The results have been plotted in Fig. 13.

On 2–3–'53 another experiment with *Cosmos* was started; in this experiment mixtures of blue and red, and of infrared and red were given as supplementary irradiation in a 10 + 8 hours treatment. In Table 19 the percentage energy in the blue and red, and in infrared and red, as well as the absolute intensities in these wave length regions are given for the different plant positions.

The results obtained from measurements of the second internode, after 11 days, have been plotted in Fig. 14. From Figs. 12, 13 and 14 it is evident that addition of a suitable amount of red light to light of the wave length regions that are active in the formative reactions annuls the formative action. This annulment is roughly proportional to the relative intensity of red radiation. It

TABLE 19

Absolute energy of antagonistic wave length regions, and the percentages in different mixtures, used in the supplementary light in a 10 + 8 hours treatment. Experiments of 2–3–'53.

Plant Position	Red-blue mixtures				Red-infrared mixtures			
	Energy in ergs/cm²/sec			% red	Energy in ergs/cm²/sec			% infrared
	red	blue	total		red	infrared	total	
I	42	1445	1487	97	119	640	759	84
II	165	600	765	79	51	323	373	86
III	254	300	554	55	144	187	331	56
IV	765	90	855	10	255	110	365	30
V	1062	36	1098	6	400	68	468	15

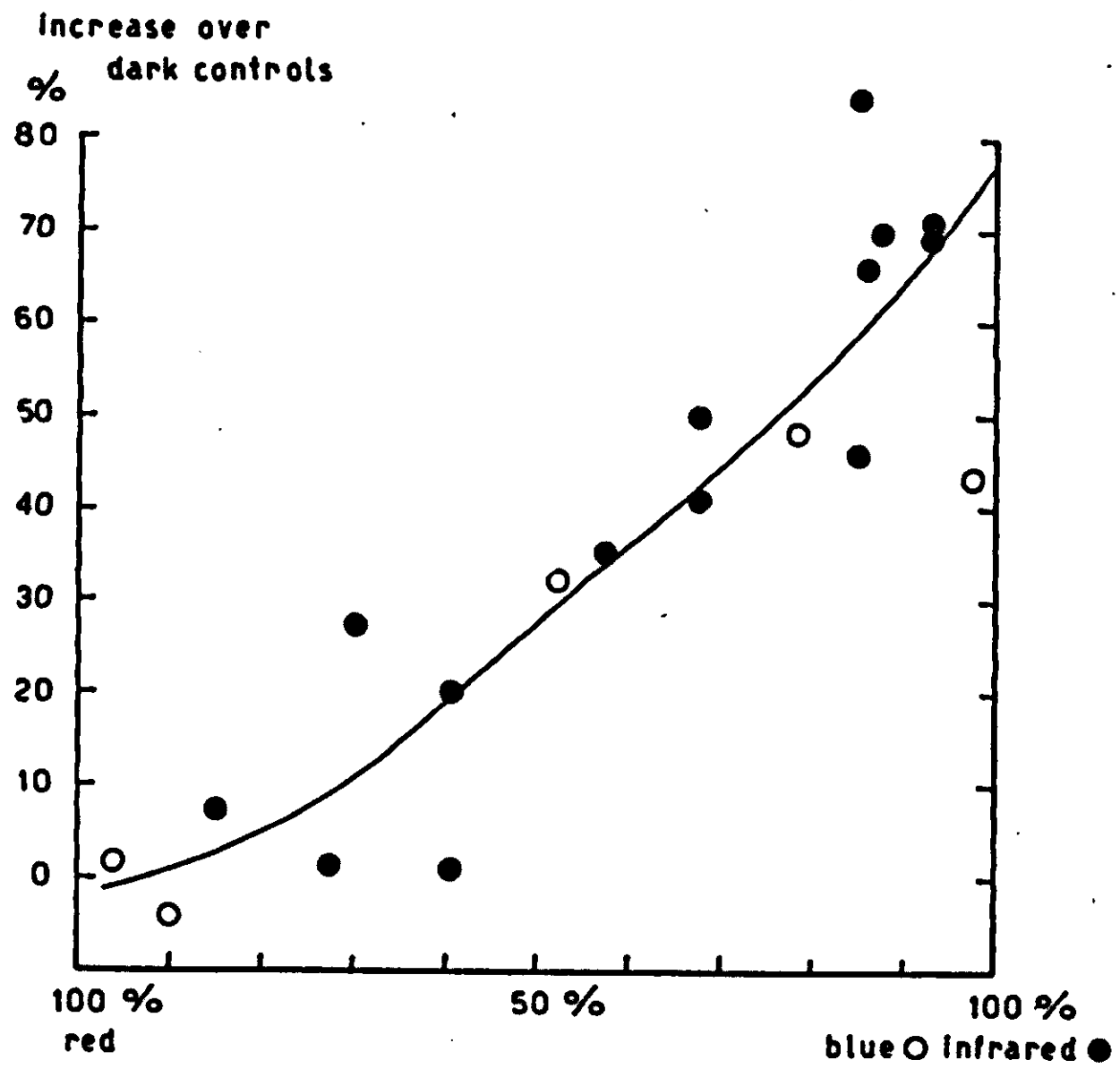


FIG. 14.
Effects of mixtures of red and infrared (●), and of red and blue (○) supplementary irradiation on elongation of *Cosmos* internodes, in a 10 + 8 hours treatment. Measurements of 20–3–'53.

should be remarked that the decrease in elongation is not correlated with the decrease in absolute intensity of blue or infrared radiation associated with a lower blue or infrared percentage in these experiments. The results mentioned in the introduction to this chapter in which the intensity dependence was determined, compared with the absolute intensity values given in Tables 18 and 19, show that in nearly all cases the light intensity was above the saturation value necessary for maximal response. As could be expected more or less, the antagonism in the formative reaction is manifest both in treatments with consecutive and simultaneous irradiation with red and infrared, or red and blue. It can be concluded that both spectral regions influence a reaction governing the elongation. This reaction is reversible: red and infrared have an opposite action, and seem to shift an equilibrium, as shown in the mixed light experiments. The equilibrium could be a photochemical one, or it could be an equilibrium between two products of two photochemical reactions. At the end of the daily 10 hour period in white light of high intensity the equilibrium of this reaction is close to the "red" end, which is demonstrated by the fact that red supplementary light only causes a small reduction in internode elongation as compared with the dark controls. Infrared or blue supplementary irradiation shifts the equilibrium to the "infrared" position, resulting in extra elongation. From the temperature dependence of the internode elongation it can be concluded that in darkness a similar shift occurs, which is enhanced by high temperatures.

4. DISCUSSION

The evidence presented in the foregoing sections substantiates the remarks on antagonism by WASSINK *et al.* (78) and STOLWIJK (65) that were based on evidence of a more indirect nature. Since then, BORTHWICK *et al.* (9) have presented evidence for the existence of an antagonism of the same nature as outlined above in the process governing light sensitive seed germination in a

variety of *Lactuca sativa*. Somewhat later, BORTHWICK *et al.* (8) published the results of experiments in which they showed that their action spectra for the photoperiodic reaction had to be extended with a negative effect of near infrared wave length regions, for the short day plant *Xanthium*. Since this publication, a number of reactions have been described in which this red-infrared antagonism showed up. In 1951, MONTFORT and ROSENSTOCK (43), and ROSENSTOCK (58) published the results of an investigation on the effect of light on respiration of different plant organs, and which indicated a differential effect of different wave length regions, but they concluded that red light was inactive, and did not stimulate respiration, although to the present author the assumption of an antagonism would seem more reasonable. Since then, LEOPOLD and GUERNSEY (40) did some crucial experiments and proved the antagonism of red and infrared wave length regions in the light stimulation of respiration. However, in all this work no mention is made of an antagonism between blue and red wave length regions. BORTHWICK *et al.* (8, 9) state that the action of radiation of the wave length region 400–500 mμ is not yet clear. Their results indicate that it can act both ways, in the infrared and in the red direction of the equilibrium. EVENARI (13), however, denies the existence of an inhibiting effect of blue light on germination of lettuce seed, and states that the infrared transmission of the filters used has not always been taken into account. The basic work of FLINT (15), and FLINT and MCALISTER (16) indicated that blue also had an inhibitory effect on germination of lettuce seed, while these authors were fully aware of the difficulties arising from the high activity of infrared in inhibition of seed germination.

VAN DER VEEN and DAAMS have found an antagonism in the action of low intensities of blue and red light on growth of hypocotyl sections of *Helianthus* seedlings (personal communication). The antagonism between red and infrared and between blue and red was already observed in (65) and (78), in several formative reactions, and has been proved beyond doubt in the experiments described in this chapter. EVENARI's general criticism with respect to infrared transmission of blue filter glasses does not apply here, because the light sources used do not emit an appreciable fraction of radiation in the near infrared wave length region, and moreover, the energy relations in blue and infrared are rather similar, whereas the near infrared content of the blue light is less than 1% (*cf.* Fig. 2B).

From our experiments we would like to conclude that there is a photoreceptive pigment which absorbs both blue and infrared radiation as in no case it has been possible to distinguish between the effects of the two wave length regions. In different plants the effects varied, but we never found them qualitatively different, at least with relatively low intensities of supplementary light.

BORTHWICK *et al.* have already envisaged the possibility that also the pigment associated with the "red" reaction shows absorption in the blue region of the spectrum (9). If so, this might decrease or even reverse the "infrared" action of blue radiation, dependent on the ratios of the concentrations of the two pigments, or their substrates.

CHAPTER VII

GROWTH AND DEVELOPMENT OF PLANTS IN HIGH INTENSITY LIGHT OF NARROW SPECTRAL REGIONS

1. INTRODUCTION

The application of supplementary light of different wave length regions at low intensities, after a short day in white light of a much higher intensity, has been shown to reveal certain morphogenetic influences of light in plants (Chapters IV, V, VI). A serious disadvantage, however, is the difficulty of evaluating the effect of the short day in white light of high intensity, and its possible interference with the effect of the supplementary light. Moreover, the intensities in the various wave length bands of the natural day light are much higher than those that can be used in supplementary light treatments, and it is probable that a change in intensity of a specific wave length region modifies its effect. In order to study the effect of narrow wave length regions at higher intensities, a special equipment was designed in our laboratory. A description of this equipment and some results obtained with it have been given in a preliminary paper (79). Since then, more cabinets have been added, and improvements have been made (*cf.* Chapter III, and [73a]).

Our knowledge of the morphogenetic influences of light at high intensities on plants is not nearly as extensive as that concerning the effects of low light intensities. This, no doubt, is due to experimental difficulties associated with this type of experiment. The isolation of narrow wave length bands from a continuous spectrum at sufficiently high intensity, and on a sufficiently large area for cultivating plants over long periods is a difficult task, especially since other environmental factors (temperature, humidity) must be controlled as well. In most cases, concessions have to be made to spectral purity by using broad wave length regions, or to constancy of conditions such as light intensity, temperature, etc. In most earlier experiments the near infrared was included in the spectral regions used, because of the high infrared transmission of the filters and the relatively high intensity of near infrared in the emission of light sources with a continuous spectrum. The experiments described by POPP (50) who used sunlight and greenhouses covered by coloured glasses provide an example of this type. This author only used the relatively sharp short wave length cut-off of ordinary glass filters, and found that the absence of the blue spectral region resulted in a strong elongation of the plants.

During the last two decades gas discharge lamps and fluorescent tubes have become available which enabled a considerable improvement in spectral purity and intensity of the coloured light.

ROODENBURG (56), and ÅBERG (1) used mercury, neon and sodium gas dischargelamps as light sources for the cultivation of plants under "monochromatic" light. KLESHNIN (35) and VAN DER VEEN (70) describe experiments in which fluorescent tubes with single phosphor coatings were used. These authors, however, did not filter the coloured light obtained in this way, so that an appreciable contamination by light from the exciting mercury arc was present. In the equipment used in the experiments described in this paper, the addition of suitable filter glasses removed this contamination.

TABLE 20

Stem length of *Lactuca sativa* L., as affected by wave length of light when grown in "monochromatic" light, given for 16 hours daily. Averages of 6 experiments; stem length expressed in % of the stem length of the white controls.

Wave length region	Relative stem length
Violet	95 [1])
Blue	102
Green	263
Yellow	288
Red	195
White	100

[1]) in one experiment only.

2. EFFECTS OF LIGHT OF NARROW SPECTRAL REGIONS ON GROWTH OF PLANTS

Since the initial experiments, the light equipment has been improved and extended to its present form. Many of the earlier experiments were of an introductory nature, and will not be dealt with here in great detail. The later experiments with *Cosmos bipinnatus* and *Solanum Lycopersicum* were made with the equipment in its present form, and the data from these experiments will be treated at relatively greater length. As has been described in Chapter III, the wave length bands used have the following maxima: violet 400 mμ, blue 460 mμ, green 540 mμ, yellow 589 mμ, red 660 mμ.

Lactuca sativa L., the varieties "Meikoningin" and "Attractie" have been used in a number of experiments, to study the effect of wave length on stem growth. This species did not show a very uniform response of the plants in a single experiment so that the results of 6 experiments have been averaged and presented in Table 20. The results summarized in this Table indicate that absence of short wave length radiation results in excessive elongation; it seems that in yellow light the stem elongation is even greater than in red light.

Apart from the effect on stem elongation other effects occurred. In Table 21 it is shown that the number of leaves and the leaf shape in *Lactuca sativa* var. "Meikoningin", was also affected by a of 34 days' treatment with monochromatic light.

From the results mentioned above, it appears that light of restricted spectral regions with the exclusion of other light, and applied over long periods, has a

TABLE 21

Stem length, number of leaves, and average leaf dimensions in *Lactuca sativa* var. "Meikoningin", as affected by different spectral regions in a 34 days' treatment. Observations of 25–3–'52. Averages of 8 plants per treatment.

Spectral region	Stem length im mm	Average length of mature leaves in mm	Average width of mature leaves in mm	Total number of leaves per plant	Ratio $\frac{\text{Leaf length}}{\text{Leaf width}}$
Violet	4.5	110	63	9	2.75
Green	10.2	121	44	10.2	2.78
Yellow	23.0	133	53	10.2	2.50
Red	15.5	130	69	10.8	1.90
White	4.8	95	61	10.2	1.55

TABLE 22

Petiole length, average leaf diameter, number of leaves and leaf area of a *Fragaria* hybrid, as affected by culture in light of various spectral regions. Averages of 6 plants. A: Observations of 27–10–'51, after 52 days of treatment. B: Observations of 29–11–'51, after 75 days of treatment.

Spectral region	Average petiole length, mm	Average leaf diameter, mm	Number of leaves	Leaf area (relative units)
A Blue . . .	87	57	5.5	41
Green . .	95	69	8.6	89
Yellow . .	99	71	9.5	102
Red . . .	108	71	10.25	122
White . .	62	60	8.5	72
B Blue . . .	86	59	7.0	56
Green . .	98	66	11.0	114
Yellow . .	119	77	11.0	155
Red . . .	128	70	13.0	152
White . .	74	61.8	11.0	98

marked formative effect on lettuce plants. The effects, contained in the data of Tables 20 and 21 resemble very strongly those of supplementary light, however, with a different wave length dependence (78). In both cases the abnormalities in the vegetative condition comprise stem elongation and elongation of the leaves. In the supplementary light experiments, however, this elongation was brought about by blue and infrared supplementary irradiation, while in the experiments described above, it is the absence of blue and violet light which causes excessive elongation.

Another species, used in the earlier experiments was a *Fragaria* hybrid, var. Madame Moutot. The light intensity in this experiment was 52.000 ergs/sec/cm^2 ø sphere, the temperature was 22 °C during the 16-hour photoperiod, and 15 °C during the dark period. The treatment started on 5–9–'51. Measurements were made on 27–10–'51, after 52 days, and on 29–11–'51, after 75 days of treatment. The results of these measurements are given in Table 22.

In none of the treatments in this experiment, stem formation could be induced in *Fragaria*, as was possible in *Lactuca*. Table 22 shows that petiole length in all spectral regions was found to be greater than in white light which, as such, so far is not explained. Comparing the separate spectral regions, it is obvious that especially yellow and red cause excessive petiole elongation. Growth was most satisfactory in yellow and red light, the leaf area is clearly biggest in these spectral regions. The plants in blue light had hard leaves, as if they were dry, and growth was very slow.

An experiment with *Iberis coronaria* var. Empress, as representative of the *Cruciferae* demonstrated that this plant reacted in much the same way. The experiment started on 21–4–'52, 15 days after sowing. The light intensity was 56.000 ergs/sec/cm^2 ø sphere, the temperature during the 16-hour photoperiod was 20 °C, and 15–17 °C during the dark period. After 38 days of treatment the plants were measured. Table 23 shows the data obtained. In *Iberis*, too, the absence of the 400–500 mμ region results in a marked increase in stem length, whereas flowering is somewhat retarded and the flowers are smaller. It is possible, however, that this is due to competition for photosynthates between elongation

TABLE 23

Stem length and degree of flowering in *Iberis coronaria* var. Empress as affected by culture under various spectral regions with the exclusion of white light. Observations of 29–5–'52, after 38 days of treatment. Partly taken from (79).

Spectral region	Stem length, mm	Degree of flowering
Violet	292	10.0
Green	362	10.0
Yellow	565	9.25
Red	530	9.0
White	299	10.0

and flowering, more than to a direct effect of the absence of the blue wave length region.

Also *Cosmos bipinnatus*, in a number of experiments, showed a reaction very similar to that of *Lactuca*, *Frageria* and *Iberis*. In one of the earlier experiments two different treatments were given, beginning on 25-7-'51. The plants had been transplanted on 16-7-'51, 5 days after sowing. At the start of the treatment the plants had already developed one internode. The photoperiod temperature was 23 °C, that of the dark period, 18 °C. The spectral regions were given at an intensity of 52.000 ergs/sec/cm² ø sphere. One lot of the plants received 16 hours coloured light daily, and 8 hours of darkness; another lot received 10 hours of white light of high intensity, followed by 8 hours supplementary light of various spectral regions, also at high intensity. The plants were measured after 13, and 28 days of treatment. Some of the results are presented in Fig. 15 and in Table 24. Another experiment was started on 16-12-'51, with plants still in the cotyledon stage. In this experiment the blue light cabinet was replaced by a violet one. The temperature during the 16-hour photoperiod in which various spectral regions were given, was 20 °C, the dark period temperature was 15 °C. On 18-2-'52, *i.e.*

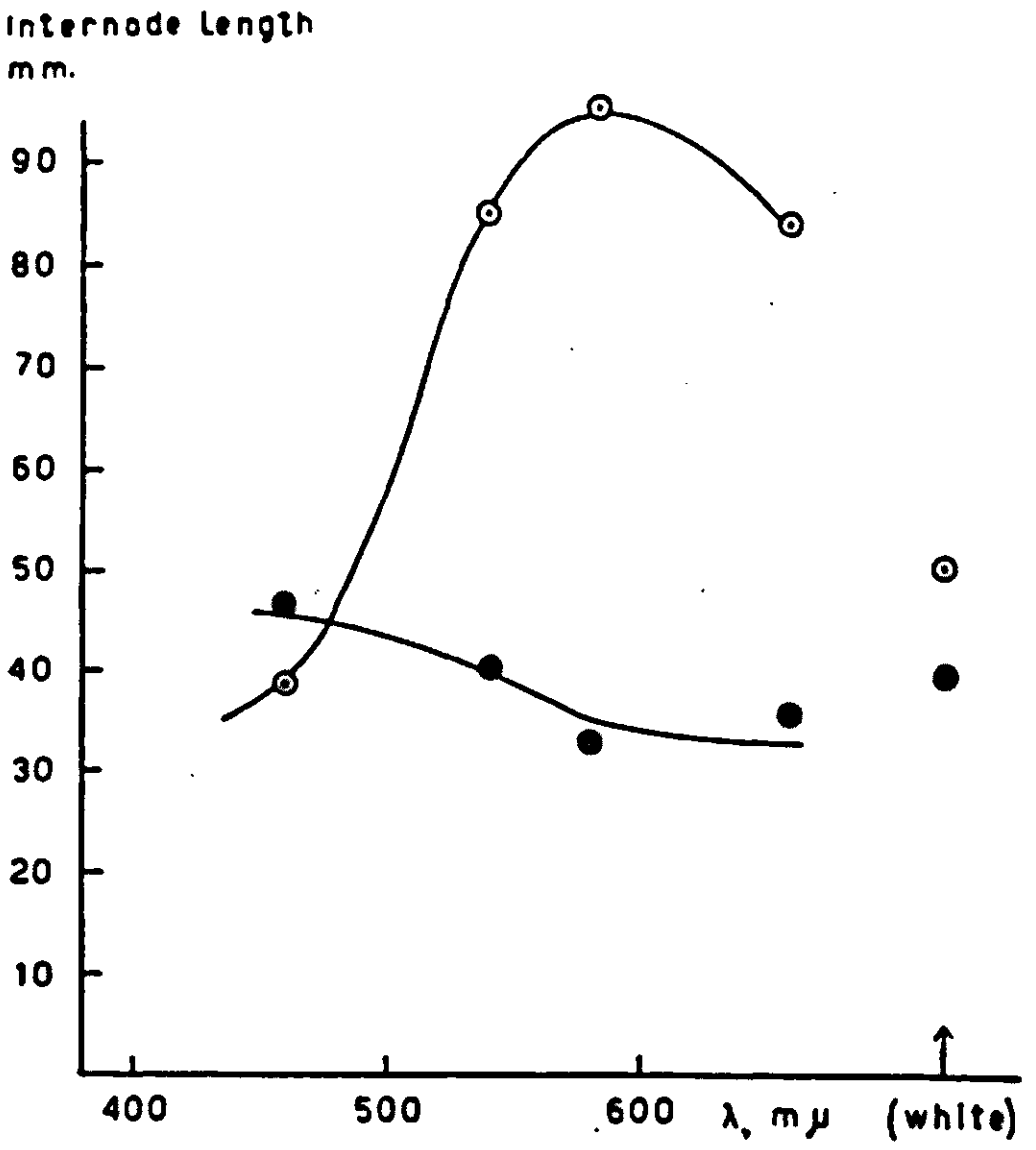


FIG. 15.
Internode length of *Cosmos bipinnatus* as affected by daily 16-hour photoperiods in coloured light (⊙), and by 10-hour photoperiods in white light supplemented with light of various spectral regions at high intensity during 8 hours daily (●).

TABLE 24

Internode length in *Cosmos bipinnatus*, in mm, as affected by various spectral regions, given either for 16 hours per day, or supplementary to a 10-hour day in white light. Observations of 22–8–'51, after 28 days of treatment.

Spectral region	16 hours						10 + 8 hours						
	I	II	III	IV	V	VI	I	II	III	IV	V	VI	VII
Blue	91	97.5	77.5	65	38	31	87.5	91.0	54	47	49	25	15.5
Green	68	80.5	73	71.5	85	81	61	51.5	41.5	40	51	42	19
Yellow	80	82	84	90	97	73	71.5	56.5	39	32	23	22	20
Red	70	71.5	86	76	83	81	64	65	42.5	35	36	25	21
White	79.5	65	63	51.5	50	52.5	55.0	48.5	41	38.5	44	21	18.5

after 64 days of treatment, the plants were measured, and dry weight determinations made. The data obtained are presented in Table 25. In an experiment which was essentially a repetition of the previous one, the conditions were identical. It started on 17–3–'52 and measurements were made on 22–4–'52, after 36 days of treatment. The results are given in Table 26.

TABLE 25

Internode length of *Cosmos bipinnatus*, in mm, number of internodes, and dry weight, in mg per plant, after 64 days exposure to various spectral regions. Observations of 18–2–'52. Partly taken from (79).

Spectral region	Internode no.								Dry weight in mg/plant	Number of internodes
	I	II	III	IV	V	VI	VII	VIII		
Violet	23.0	34.0	37.5	45.0	35.0	25.0	12.0	–	259	7.25
Green	62	63	62	62	62	42	9	–	744	7.0
Yellow	64.5	81	78.5	99	99.5	82.5	49.5	–	517	7.5
Red	69.5	73.0	87.0	86.5	96.5	81.0	41.0	17	1144	7.75
White	28.0	52.5	45.0	44.5	55.0	44.0	43.0	–	710	7.00

From the data obtained in the experiments with *Cosmos*, it is clear that this plant also reacts with a strong elongation to the absence of the blue and especially violet components in the various spectral regions, as compared with the white controls. The position and the dimensions of the leaves were not strongly affected by the wave length of the light. The data show that dry weight increases

TABLE 26

Internode length and dry weight of *Cosmos*, as affected by 36 days of culture in light of various spectral regions. Observations of 22–4–'52.

Spectral region	Internode length in mm				Dry weight in mg/plant
	1	2	3	4	
Violet	47.5	40	27.5	–	359
Green	79	78	53	27	707
Yellow	70	60	57	23	999
Red	90.5	74.5	57	29	921
White	39	44	43.5	18.5	–

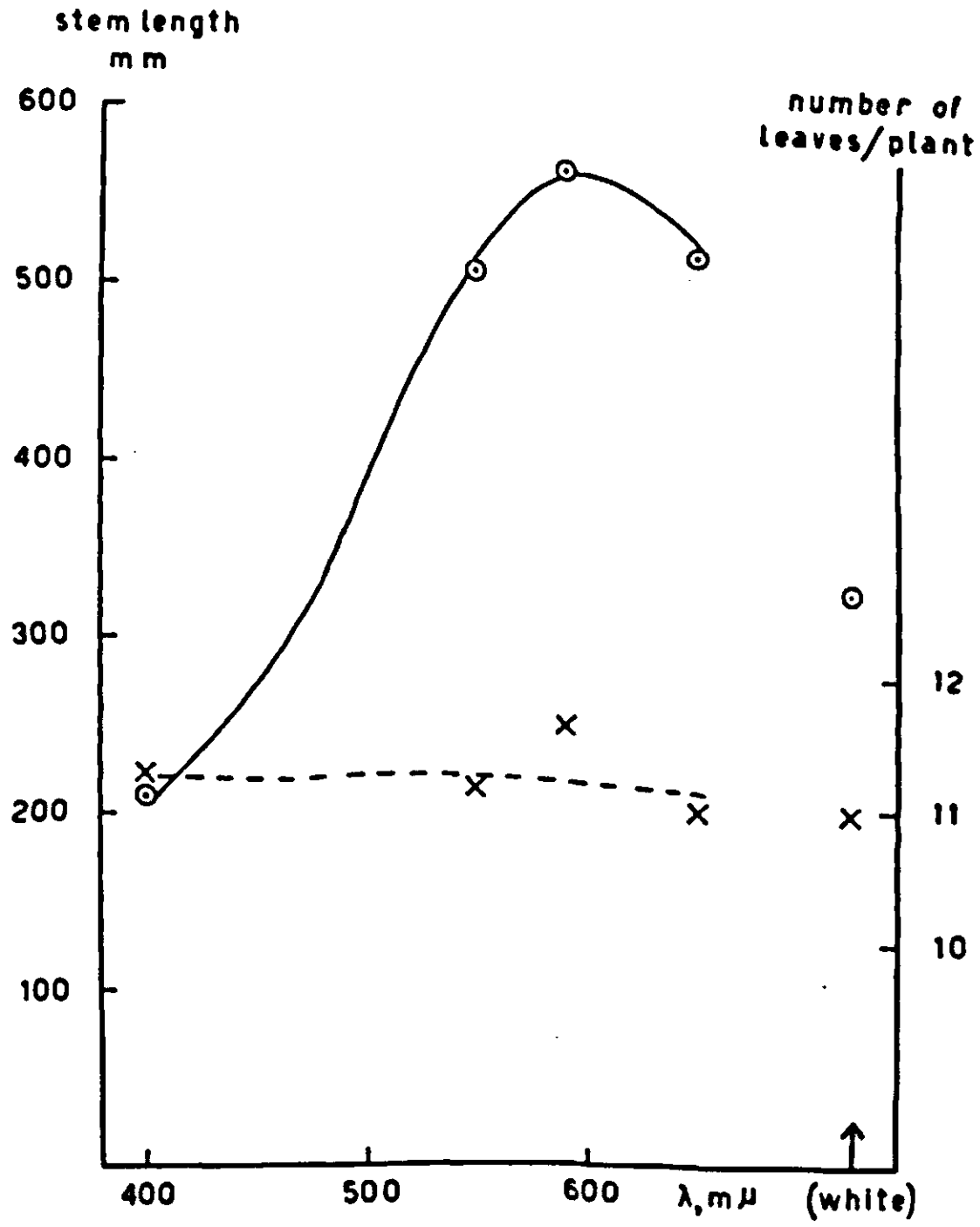


FIG. 16.
Effects of various spectral regions at high intensity on stem elongation (⊙) and number of leaves per plant (×) in *Solanum Lycopersicum* var. Vetomold 121. After 30 days of treatment with 16-hour photoperiods. Measurements of 2–6–'52.

with increasing wave length. However, the dry weight in the violet is smaller than could be explained on the basis of the energy content of the quanta. Dry weight in violet is only about one third of that in the red, whereas theoretically about 60 % should be expected. It is impossible to decide from the data whether

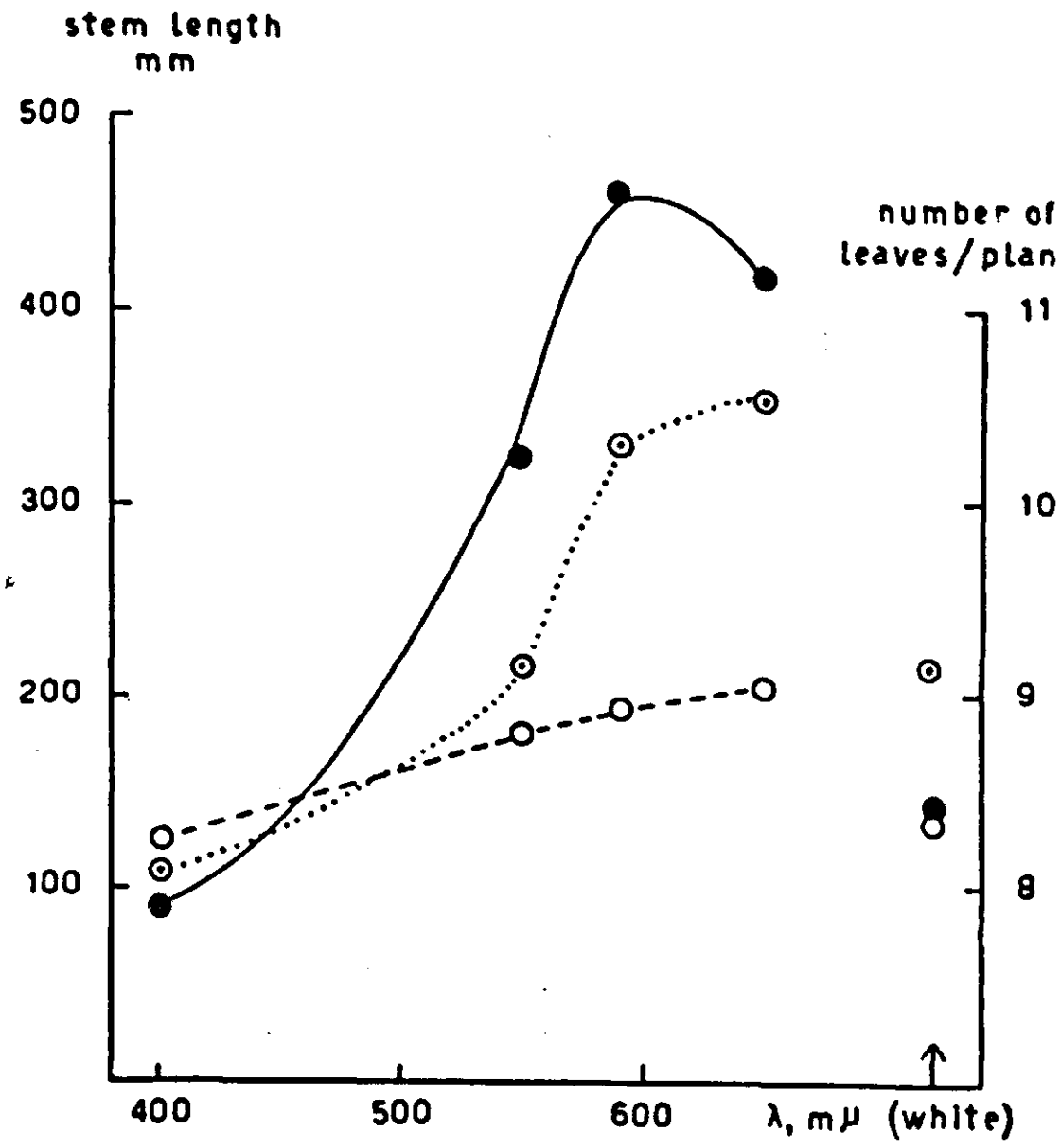


FIG. 17.
Effects of various spectral regions at high intensity on stem length (●), number of leaves (⊙), and leaf length (○) in *Solanum Lycopersicum* var. Vetomold 121. Photoperiod of 16 hours at 20 °C. Dark period: 17 °C. Measurements of 29–10–'53.

this is due to decreased photosynthetic quantum efficiency in the violet region, or whether it is in indirect effect, due to decreased leaf area; both possibilities could be responsible for the smaller leaf area that was consistently found.

Solanum Lycopersicum L. var. Vetomold 121 showed even more pronounced reactions; some more extensive experiments were made with this plant. In the first experiment the young plants, sown on 11–4–'52, were transplanted on 23–4–'52; the treatment started on 3–5–'52. The light intensity was 56.000 ergs/sec/cm² ø sphere, the temperature was 20 °C during the 16-hour photoperiod, and 15–17 °C during the dark period. On 2–6–'52, after 30 days of treatment, the plants were measured. Some of the data obtained are presented in Fig. 16. Apart from the very strong elongation in the absence of violet light demonstrated in this figure, some other phenomena were observed which were studied in greater detail in subsequent experiments. The leaves were abnormally flat in plants growing in violet light, whereas the plants in green, yellow and red light had leaves highly abnormal in other respects. The leaflets were curled and pendulous, the petioles of the young leaves formed a small acute angle with the stem, while the petioles of the older leaves formed an obtuse angle with the stem. The appearance of the plants was, therefore, very abnormal.

On 28–9–'53, a second experiment with tomato was started. The light intensity was 35.000 ergs/sec/cm² ø sphere. The temperature was maintained at 20 °C during the 16-hour light period, and at 17 °C during the dark period. The plants were measured after 22 and 31 days of treatment, on 20–10–'53 and 29–10–'53 respectively. Table 27 and Fig. 17 and 18 show the results obtained.

From Table 27 and Fig. 17 it can be seen that the absence of violet light results in a very big increase in stem length, because more and longer internodes are formed. Internodes number 6 and 7 were, of course, present in the violet plants, but they were so small that no reasonably accurate measurements could be made without damaging the plants. The leaf numbers showed much less variation than stem length. There is a difference in the angles of old and young leaves on one plant. This difference is small in the violet plants, but in the plants in yellow or red light, the young leaves emerge from the stem at angles $< 40°$, whereas the older leaves turn downward at angles $> 90°$. The epinasty of the leaflets consists of two types of curling. The midribs of the leaflets bend downward towards the tip, and moreover each leaf blade bends downward in a direction perpendicular to that of the midrib.

The dry weight data, shown in Fig. 18, indicate that the dry weight increases

TABLE 27

Internode length, stem length, number of leaves, angle between stem and petiole of 3rd leaf, and length of 3rd leaf of *Solanum Lycopersicum* var. Vetomold 121, as affected by wave length of light given for 16 hours daily, at an intensity of 35.000 ergs/sec/cm² ø sphere. Observations of 20–10–'53, after 22 days of treatment. Averages of 8 plants.

Spectral region	Internode length in mm							Stem length in mm	Number of leaves per plant	Angle of 3rd leaf	Length of 3rd leaf, in mm
	I	II	III	IV	V	VI	VII				
Violet	17.2	10.6	9.4	10.4	5.7	–	–	53	6.95	80 °	110.6
Green	25.4	29.3	46.3	54.3	31.7	17.3	7.3	212	7.25	46 °	155.9
Yellow	27.1	35.1	49.7	55.4	55.4	36.3	18.9	290	7.60	50 °	182.1
Red	28.2	24.6	38.9	45.9	49.7	27.0	12.0	230	7.45	51 °	174.6
White	19.4	14.0	15.1	15.8	13.1	8.0	3.0	87	7.25	62 °	132.6

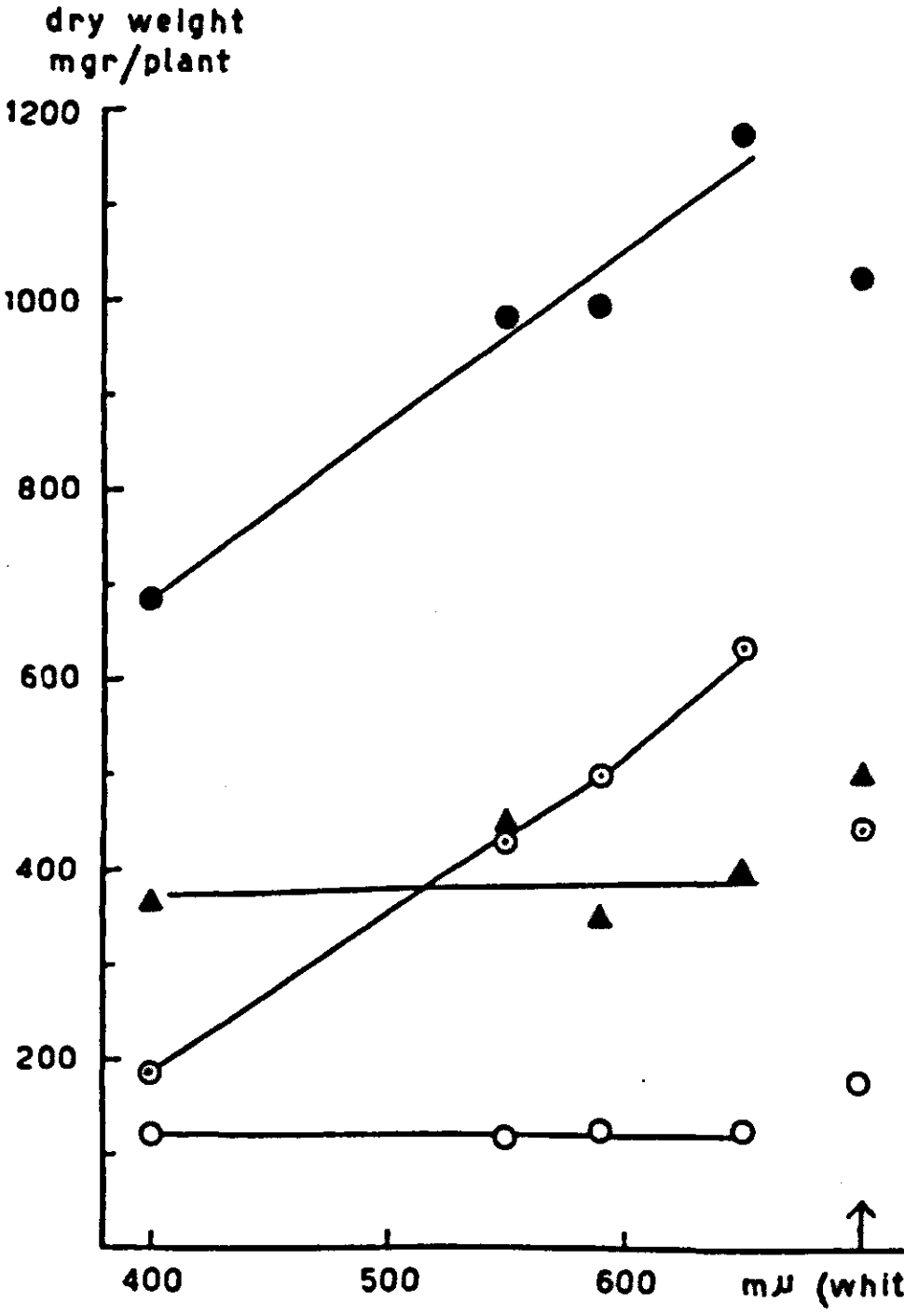


FIG. 18.
Effects of various spectral regions at high intensity on dry weight and dry weight distribution in *Solanum Lycopersicum* var. Vetomold 121. Total dry weight (●);dry weight of stem (⊙), of leaves (▲), and of roots (○). Measurements of 29–10–'53.

with wave length of the light. It is remarkable that the slope corresponds exactly to the theoretical values determined by the numbers of quanta in equal energies of the different wave length regions. However, this straightforward explanation must be regarded with the utmost caution, since it is by no means certain that photosynthesis is the only process determining dry weight increase in these experiments. All dry matter arises from photosynthesis, but the destination of the dry matter is largely determined by other light and temperature sensitive processes. These processes govern, e.g., the expansion of the photosynthetic apparatus and, thus, future increase in dry matter. As is shown by Fig. 18 the dry matter increase at longer wave lengths is practically only in the stem, whereas the leaves are not significantly affected. On this basis, it would seem probable that energy efficiency in photosynthesis indeed is the main factor responsible for the differences in dry weight in tomato in this experiment. This would indicate that quantum efficiency in photosynthesis in tomato is independent of wave length between 400 and 660 mμ, so that HOOVER's curve (28) of the spectral dependence of photosynthesis in wheat does not seem to hold under the conditions of the present experiments. Of course the curves obtained by HOOVER are essentially absorption curves of chlorophyll. The minimum in the absorption in yellow and green decreases in importance as the chlorophyll concentration or the number of layers increases. A very good treatment of this question is found, *e.g.*, in a paper by RABIDEAU *et al.* (53).

It was also of some interest to see whether the leaf pigments are affected by the wave length region in which the plants had been growing. Absorption spectra were measured of methanol extracts of leaves from different spectral regions. In no case significant differences ascribable to shifts in relative concen-

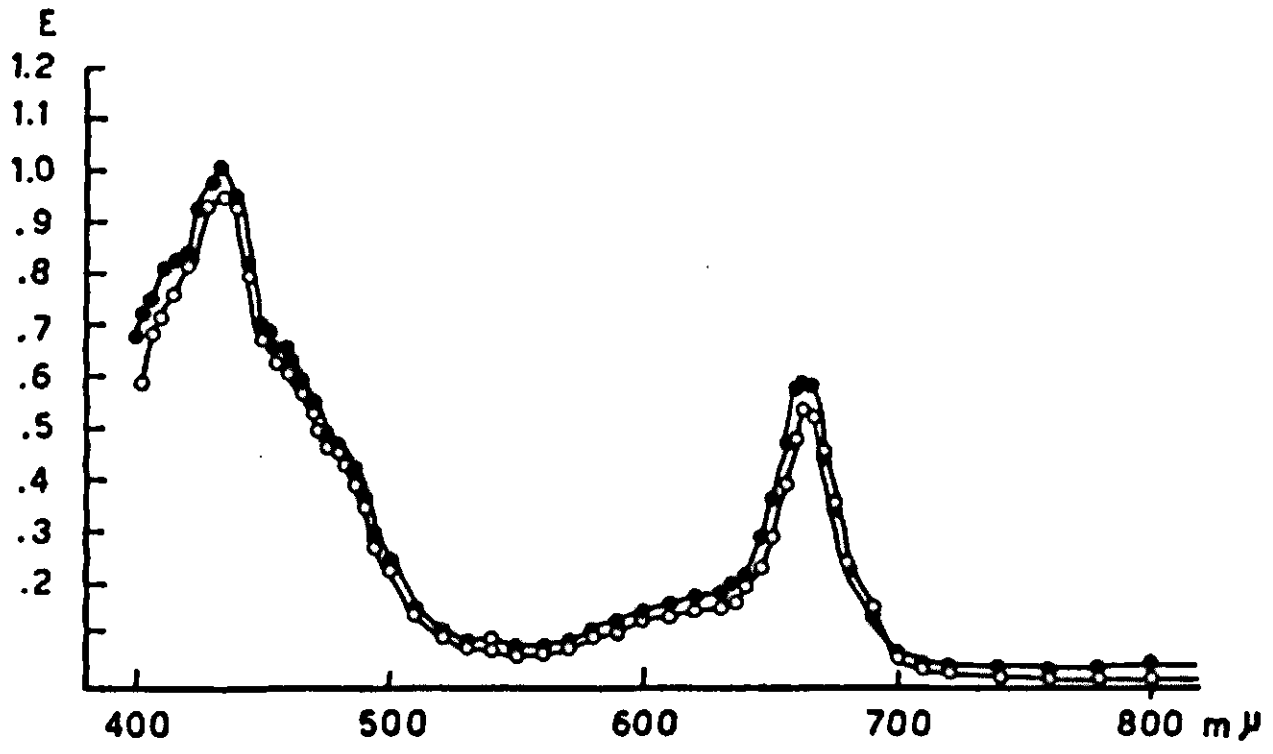


FIG. 19.
Effects of various spectral regions at high intensity on leaf pigments in *Solanum Lycopersicum*. Absorption spectra of methanol extracts, from 5th leaf of plants, grown in red (●), and violet (⊙) light. Measurements of 11–12–'53.

tration of the main pigments could be detected. Even the absolute amounts of pigment from comparable leaves of plants in, *e.g.*, violet and red were the same. In Fig. 19 absorption spectra of extracts from the fifth leaf of both red and violet plants, measured on 29–10–'53, are given. The deviations are not larger than those usually found between leaves of the same plant. It is not impossible, however, that relatively large changes occur in pigments that are present in smaller concentrations, and which escape detection when the measurement is not preceded by chromatographical separation. This has not been attempted so far. As found so far, however, it seems certain that the main pigments are not affected.

In an experiment with tomato that started on 4–11–'53, two new cabinets were added, *viz.*, a blue one, and one in which the energy in the red between 620 and 700 mμ was the same as in the red cabinet, but a very large amount of infrared was added by using 40 W incandescent lamps as light sources. The energy in the visible was 35.000 ergs/sec/cm² ø sphere; in the red + infrared cabinet about 20 times as much infrared between 700 and 2500 mμ was added. The temperature during the 16-hour photoperiod was kept at 20 °C, the dark period temperature was 15 °C. After 36 days, on 10–12–'53, the plants were measured. The data obtained are given in Table 28. From a comparison of the plants in violet (400 mμ) and blue (460 mμ) light, it is evident that the spectral region around 460 mμ is not nearly so efficient in the inhibition of elongation, as the violet, around 400 mμ. The elongation in blue is somewhere halfway

TABLE 28
Internode length, stem length, number of leaves, and leaf area in tomato plants as affected by 36 days of exposure to light of various wave length regions. Observations of 10–12–'53.

Spectral region	Internode length, in mm									Number of leaves	Stem length mm	Leaf area cm²/pl.
	1	2	3	4	5	6	7	8	9			
Violet	13.5	11.9	10.7	6.0	17.7	10.0	8.0	4.3	–	8.25	102	500
Blue	27.2	26.0	27.1	29.0	45.5	49.0	30.7	12.7	7.1	8.75	259	425
Green	20.6	32.4	26.0	69.5	62.3	40.3	27.9	16.0	4.0	9.0	369	475
Yellow . . .	24.5	37.0	46.0	19.0	75.5	61.6	73.3	78.0	60.0	10.6	561	520
Red	24.6	31.4	31.1	24.7	56.8	65.0	56.3	62.7	44.6	10.6	405	660
Red + infrared	42.0	51.7	55.0	33,1	60.5	52.1	11.8	–	–	7.6	320	76
White	18.5	18.6	16.0	11.6	31.0	32.4	22.0	16.5	8.5	8.75	189	640

between that in violet and green; the epinastic abnormalities of the plants in red, yellow and green light, however, are absent in the plants grown in blue light (*cf.* Plate 2). The reaction of the plants in red + infrared is different from those in red, although the amount of photosynthetically active light energy is the same in both cases. It would seem that the addition of a large amount of infrared has a very unfavourable effect on growth of tomato. It is improbable that the photosynthetic efficiency of the red light is reduced by the addition of infrared, but, as has been stated before, in this type of experiment a second factor, *i.e.*, the expansion of the photosynthetic apparatus plays an important rôle. From the leaf area data in Table 28 it is evident that the leaf area in the plants in red + infrared is much smaller than in the other plants. This is also reflected in the total dry weight data obtained in this experiment; red + infrared gave 253 mg per plant, as compared with 938 mg in violet and 1990 mg in red. The internode length data of the first internode reveal that elongation was much more pronounced in red + infrared than in red: 42 mm and 24.6 mm respectively for the first internode. This indicates that elongation in red + infrared is potentially much stronger than in red, but that evidently in this process too much of the photosynthates is used for stem elongation, resulting in poor leaf growth, thus decreasing the future growth of the plant. After a few leaves have been formed, the plant seems to reach a kind of equilibrium between leaf growth and stem growth, as is demonstrated in the younger internodes. This equilibrium, however, results in poor growth of the plant, because leaf growth is slower, and the leaves moreover are smaller (Table 28).

3. RELATION BETWEEN THE EFFECTS OF COLOURED LIGHT OF HIGH INTENSITY AND THOSE OF SUPPLEMENTARY COLOURED LIGHT OF LOW INTENSITY

The formative effects of various spectral regions given at high intensity under exclusion of white light, or supplementary to a short day in white light, at lower intensities, have several features in common. The elongation occurring in plants grown in the absence of light of wave lengths < 500 mμ is very similar to that found in plants receiving blue or infrared supplementary irradiation. Although the requirements in intensity and wave length are different, the aspect of the plants is similar, with the exception that in most plants the effects of red, yellow and green light of high intensity under the exclusion of white light are more pronounced than those obtained by giving the plants supplementary irradiation with blue or infrared wave length regions (*cf.* the experiments with tomato, in which leaf epinasty was much less pronounced in the supplementary light treatment).

In order to investigate whether a relation between these two light reactions could be established, some experiments with *Cosmos* were made, in which the plants received 10 hours of coloured light of high intensity, followed by 8 hours supplementary irradiation with various spectral regions at low intensity. The first experiment started on 17–6–'52. The plants received 10 hours of light of various spectral regions at an intensity of 52.000 ergs/sec/cm^2 ø sphere, followed by 8 hours supplementary irradiation with light of various spectral regions at an intensity of 1000 ergs/cm^2/sec, and 6 hours of darkness daily. The temperature during the main light period was 23 °C, and 20 °C during the supplementary light period and during the dark period. The supplementary irradiation started on 30–6–'52. After 21 days of supplementary irradiation, the first internode

TABLE 29

Internode length of *Cosmos bipinnatus*, in mm, as affected by various combinations of spectral regions at high and low intensities. Measurements of 20–7–'52, after 21 days of supplementary irradiation.

Spectral region of supplementary light / Spectral region in main light period	Blue	Green	Red	Infrared	White	Dark	Sums
Violet	35	18	18	37.5	18.5	42	169
Green	66.5	43	43	39	43	32	276.5
Red	77	41	66	111	46	47.5	388.5
White	81	27	38	80	29	40	275
Sums	259.5	129	165	267.5	136.5	161.5	

grown out since the start of the supplementary light treatment was measured. The mean values are given in Table 29.

On 23–7–'52, a second experiment was started under the same conditions. Measurements of the fourth internode were made on 14–8–'52, 15 days after the start of the supplementary irradiation. The results are presented in Table 30. The observations compiled in Table 29 and 30 show variations which are bigger than usual. This may be due to two reasons: in the first place, because of space limitations in the light cabinets, only 3 plants per treatment could be used. Secondly, due to the relatively great distance between the two light equipments, the plants were more vulnerable to damage during the (twice) daily handling. Notwithstanding these circumstances, the data obtained strongly indicate that both elongating influences can occur independently and at the same time. This is seen most clearly in the summations given in both Tables. It can be concluded that neither by growing *Cosmos* in red light only, nor by giving infrared supplementary irradiation, the limit of potential elongation is reached, since in both cases, elongation is enhanced by a combination of the two. This also means that under the conditions of these experiments the available photosynthates do not limit the increase in length.

TABLE 30

Internode length of *Cosmos*, in mm, as affected by various combinations of spectral regions at high and low intensities. Measurements of 14–8–'52, after 15 days of supplementary irradiation.

Spectral region of supplementary light / Spectral region of main light period	Blue	Green	Red	Infrared	White	Dark	Sums
Violet	60	63	45	44	46	71	329
Green	102	42	57	73	62	58	393
Red	96	48	65	89	68	64	430
White	67	45	56	88	51	51	358
Sums	325	198	223	294	227	244	

4. THE EFFECT OF LIGHT OF VARIOUS WAVE LENGTH REGIONS ON THE AUXIN CONTENT OF TOMATO PLANTS

As the effects arising from a monochromatic irradiation with green, yellow or red light strongly suggest relations to auxin content, it was attempted to establish a relationship between the formative effects and the extractable auxin content. After some preliminary experiments it was decided to adopt the cress root technique developed by MOEWUS (42), with some modifications. In the preliminary experiments it was found that only cress seeds from some sources and from some years gave entirely reliable results. In the experiments described here, seed of the 1953 crop was used.

The procedure was as follows: From a large number of *Lepidium* seeds the discoloured and damaged seeds are discarded. The remaining seeds were germinated in 9 cm Petri dishes with three layers of filter paper, wetted with 5 ml distilled water. About one hundred seeds were put at regular distances into each culturing dish. This, as well as all subsequent handling was done in red light (neon discharge tube with red filter), as it was found that the seeds became light sensitive very soon after wetting. The culture dishes were then placed in a dark thermostate, at 27 °C. After about 22–24 hours, the longest roots are about 8–10 mm long. At about that time the seedlings, having roots of 5 ± 0.5 mm, are taken out with a pair of paper forceps, so as not to damage the delicate roots. The selected seedlings from each culturing dish are transferred into three test dishes, two of which contain the same dilution of the solution to be tested, whereas the third one contains distilled water. The dishes are provided with three layers of filter paper, wetted with 5 ml of solution or distilled water. After the transfer of the seedlings, the test dishes remain in the dark thermostate for another 17.5 hours. After this period the roots are measured to the nearest

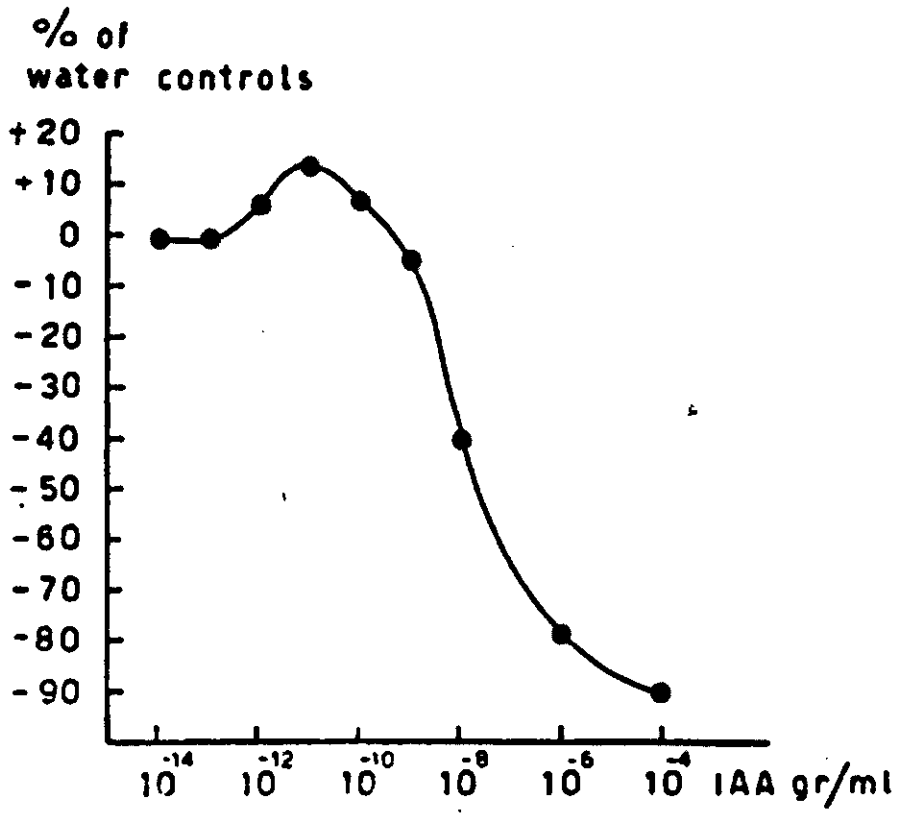


FIG. 20.
Standard curve for IAA, in the cress root test.

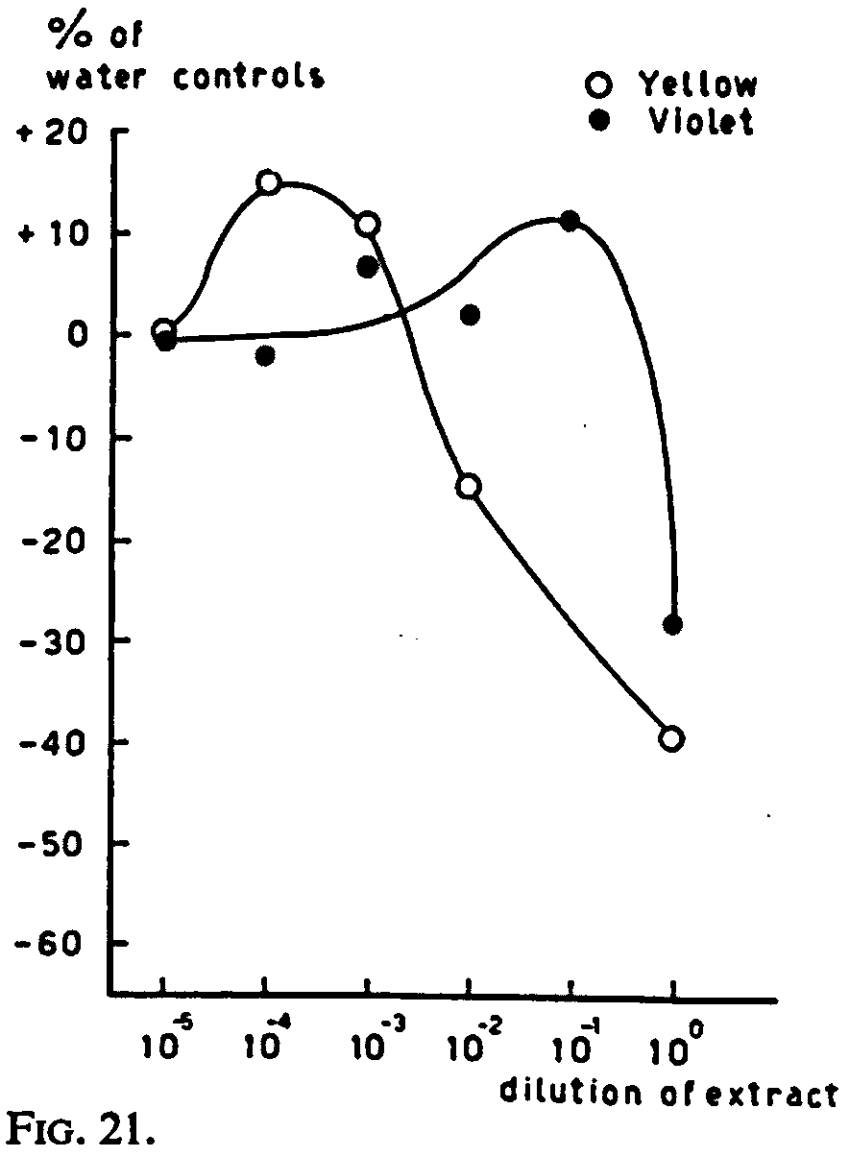


FIG. 21.
Representative curves of cress root tests of water extracts from tomato plants, grown in yellow, or in violet light. Exp. of 8–12–'53.

millimeter. The water controls should then have reached a length of about 22 mm. With auxin solutions of different concentrations a curve of root growth promotion or inhibition is obtained as presented in Fig. 20. It was necessary to have a water control to each dilution, as in some cases a particular culturing dish would produce seedlings in which the root growth was abnormal, and this would produce an uncertainty in the final curve, which can now be distinguished as unessential from the comparison with the accompanying water control. The standard curves published by MOEWUS could be reproduced rather accurately as is shown in Fig. 20. The extraction procedure is largely based on the extensive work of TERPSTRA (66) on extraction of auxins.

Tops of tomato plants (5 cm long), from which the leaves had been removed, were cut into pieces of about 0.5 cm long and extracted in distilled water in the dark, at a temperature of 3 °C, during 24 hours. According to the data of TERPSTRA (66), this procedure would be best to avoid enzymatic destruction of auxin, as well as enzymatic liberation of auxin from precursors.

The extract prepared in this way was diluted up to 1 in 10^5 or 10^6, and 5 ml of each dilution were applied to two test dishes. The extract as well as the dilutions were prepared in subdued red light.

The result of a representative experiment is given in Fig. 21. From this figure it can be concluded that the 5 cm tip of a plant grown in violet light contained about 0.01 μg IAA, or the equivalent of that, and that of a plant grown in yellow light the equivalent of about 0.1 μg. In MOEWUS' original technique the sharp drop in the violet curve would indicate a rather large amount of growth inhibitors. However, since many other substances have the same effect, and the extra inhibition occurs in a high concentration of the original extract, this can hardly be regarded as significant at this moment. It must also be added that the absolute amount of growth substance extracted varied somewhat, but the difference in auxin content of the plants in violet, and in yellow or red light, always remained in the same direction.

5. DISCUSSION

The experiments described in this chapter have demonstrated formative reactions that, although of a nature similar to that of the reactions occurring in supplementary irradiation, require different spectral regions and different intensities. The method used does not generally allow statements about the efficiency of photosynthesis, unless it is certain that leaf area is not affected by the various treatments. With this restriction in mind it can be said that in tomato photosynthesis and total dry weight accumulation are independent of wave length, between 400 and 700 mμ. In other plants, such as strawberry and *Cosmos*, the dry weight accumulation in violet and blue is less than would be expected from a comparison with, *e.g.*, the red plants, even when based on equal numbers of quanta. In none of the plants the marked drop in photosynthesis in green and yellow light, as recorded by HOOVER (28) for wheat, has been found.

The morphogenetic action of the light can be characterized as follows: The absence of the wave length region 400 to 500 mμ results in a strong elongation of stems, petioles and in some cases, of leaves. Cell counts showed that cell elongation was a much more important factor than cell division. The growth pattern in this case is more complicated than in experiments with supplementary light, owing to transitional stages, and requires a more detailed analysis. It is

remarkable that the wave length region around 400 mμ is much more effective in inhibition of elongation, than the region around 460 mμ. Both spectral regions show strong phototropic activity, while the greatest phototropic activity in etiolated material has been found between 450 and 500 mμ. In some preliminary experiments with this equipment, 460 mμ was phototropically more active than 400 mμ, as was found with *Arachis*, tomato and potato. It would seem that for maximum effectiveness in internode suppression and in phototropism different wave length regions are required. In view of the effects obtained, especially with tomato, it is very probable that auxins act as intermediate agents between light reaction and internode growth. GALSTON *et al.* (24) demonstrated that in etiolated peas auxin destruction by light was most effective at wave lengths around 460 mμ. From the results with blue and violet wave length regions, it must be concluded that this is not valid for green plants, but that under these conditions either by way of screening pigments, or because another pigment shows greater activity, maximum effectiveness is displaced to shorter wave lengths.

On the whole, the present results, bearing on the morphogenetic action of various wave length regions, are in accordance with results of previous investigations. SCHANZ (59) and POPP (50) used day light, from which various parts in the short wave length regions were absorbed by glass filters. Although SCHANZ did not measure his light intensities, and POPP used an illumination meter calibrated in foot candles, a procedure yielding very unsatisfactory light measurements, both authors concluded that absence of short wave length regions resulted in elongation of the plants.

Some more recent investigations with gas discharge lamps and selective-emission fluorescent tubes under better controlled conditions yielded similar results. ÅBERG (1), using a high pressure mercury lamp as light source in the violet-blue region, concluded that for tomato this region did not suppress elongation. Since his light measurements and energy equalization appear to be quite satisfactory, it must be assumed that the light of the yellow and green mercury lines which he did not remove, was responsible for this negative result. MOSHKOV (44) found a decrease in stem elongation in *Perilla* grown in blue-violet and red-orange light, as compared with plants in yellow-green light. He used the violet-blue, and yellow-green mercury lines, and incandescent lamps with a red filter for the orange-red region. The only assumption that could be made so far is that the appreciable infrared content of the orange-red light is responsible for the reduction in stem length and dry weight of the plants grown in this region.

In the present investigation a detrimental effect of such an infrared addition to red radiation was observed. This is another indication, that somehow the red, green and yellow wave length regions still inhibit the elongation to some extent, whereas the infrared stimulates elongation to an amount that is beyond the plant's resources, under the conditions of the present experiments.

CHAPTER VIII

GENERAL DISCUSSION

The effects obtained with supplementary light of various wave length regions, as described in Chapters IV, V and VI, indicate that in a large number of plants a photo-receptive system is present, consisting of two pigments. These two

pigments are essentially the same in all plants investigated, since the spectral dependence of the reactions initiated by light absorption in these pigments is identical, as far as can be determined with the present equipment. This equipment provides six well-defined wave length regions between 400 and 900 mμ. It has been demonstrated that various formative effects are brought about by supplementary light of wave lengths between 400 and 500 mμ, and between 700 and 900 mμ. Although the ultimate effects in different plant species are different, an excessive elongation of some part of the plant is always found. Not only this similarity, but especially the identicals pectral dependence, and the reversal of the reaction in blue and infrared by red, green, or yellow irradiation strongly suggests a common mechanism.

Since the first indications of an antagonism were obtained in this laboratory (WASSINK *et al.* [78]), which were extended to more species in a subsequent paper by the present author (STOLWIJK [65]), BORTHWICK *et al.* (9) confirmed and extended FLINT's (15) earlier observations on a reversible photoreaction governing light sensitive seed germination. Several other reactions of the same type have since been described. LEOPOLD and GUERNSEY (40) described experiments in which light induced changes of the respiratory rate in photoperiodically sensitive plants were regulated by a reversible photoreaction of the same type; LIVERMAN and BONNER demonstrated a similar reversibility in light-dependent growth stimulation of *Avena* coleoptile sections (41).

These examples will suffice to show that probably a similar mechanism is present in most, if not all plants. In two cases (BORTHWICK *et al.* [7], STOLWIJK [65]), it has been reported that attempts to detect the pigments involved, failed. BORTHWICK *et al.* could set an upper limit of 0.001% to the absorption of the red-absorbing pigment in etiolated albino barley seedlings, and the present author found a similar limit of less than 0.1% characteristic absorption in the infrared in concentrated extracts of pea seedlings.

These findings do not give much hope for spectrographic detection, unless a suitable method for concentrating the plant extracts becomes available, or a material is found in which the pigments occur in higher concentrations. The isolation of the pigments and the measurement of their absorption spectra would be very helpful in the explanation of some controversies that seem to arise, especially concerning the effect of blue light. In the present paper evidence has been presented that the effects of blue light are not due to infrared contamination, as was suggested by EVENARI *et al.* (13) for experiments on seed germination. It will be attempted to explain why in experiments by EVENARI (13) and BORTHWICK *et al.* (9), who used a technique different from ours, the effect of blue light can be much less evident or even opposite.

Isolation of the pigments involved in the reversible photoreaction, and measurement of the absorption spectra might show that the pigment associated with the red light effect also absorbs in the blue region of the spectrum, and possibly its absorption extends to part of the near infrared. The result of irradiation with blue light in this case would depend on the relative concentrations and absorptions of the two pigments. When most of the quanta are absorbed by the red-absorbing pigment, the reaction will proceed into the direction induced by red light. If, on the other hand, the infrared-blue absorbing pigment receives more quanta, the reaction will proceed into the "infrared" direction. This concept is in complete accordance with the theoretical considerations proposed by BORTHWICK *et al.*, and by EVENARI and STEIN (14).

The arguments that BORTHWICK *et al.* (9)[1]) offer in favour of the concept of a purely photochemical equilibrium arise from the energy relations, and from light reversal experiments. These authors found a linear relation between log energy and effect, in control of internode inhibition of etiolated barley and in seed germination experiments with *Lactuca sativa*. Further arguments were found in the fact that if for some reason the energy required for a certain effect decreased in the red region, the energy required in the infrared to reverse this effect increases by about the same factor. BORTHWICK *et al.* also showed that the reaction can be reversed a number of times, even in very short periods. Assuming a purely photochemical equilibrium, they built up an attractive hypothesis which fits their results very well. Their concept, however, implies that the equilibrium reached when both pigments absorb light at a certain wave length, must be independent of the intensity of the radiation, as only the speed with which it is reached is affected. Some of the experiments described in the present paper show that the effect of blue light can be reversed by a large increase in intensity, as described in experiments on internode elongation of *Cosmos* in Chapter VII. The action spectra measured by BORTHWICK *et al.* (6, 47, 49) also contain indications of a reversal in the blue near 480 mμ. In these action spectra, the energy required for a given reaction is very high at 480 mμ and drops sharply towards both longer and shorter wave lengths. A comparison of the action spectra for photoperiodic control of floral initiation in *Xanthium* and soy bean (given in [47]) reveals that, in general, soybean needs less light energy at all wave lengths than *Xanthium*. However, the ratio of energies required in those two species is different at different wave lengths. An even greater difference appears when the action spectra for photoperiodic reaction are compared with the spectrum of internode inhibition in etiolated, potentially green and albino barley seedlings (7). Especially in the region 470 to 480 mμ these differences were very large. It is very important to note that a relative increase in the energy required in the blue is always correlated with a displacement of the long wave length limit to shorter wave lengths. This means that a relative increase in the energy required for a certain effect in the 480 mμ region thus is generally accompanied by a similar increase near 700 mμ. This phenomenon is evident in the action spectra for barley, cocklebur, soybean and *Hyoscyamus*. In view of the action of blue and infrared that is antagonistic to that of red radiation, an effect of these regions as indicated in the action spectra of BORTHWICK *et al.*, in which only "red" activity was recorded, means that the original antagonistic action of blue and infrared has been reversed by increase of the intensity. This reversal by increased intensity cannot be explained on the basis of a purely photochemical equilibrium. In a photochemical equilibrium only the rate at which it is established will be increased by increasing the intensity.

We want to suggest, therefore, that two pigment systems are present in constant concentrations, their ratio varying from species to species. One pigment, associated with the action of blue and infrared, has absorption peaks around 750 and 480 mμ. The other pigment, associated with the action of red light has an absorption peak around 650 mμ, and some absorption throughout the region 400–700 mμ, and possibly beyond that. Each pigment is engaged in a light

[1]) The paper by BORTHWICK *et al.* (Bot. Gaz. **115**, 205–225 [1954]), in which they present a more detailed account of their views was at our disposal only after this manuscript went to press.

reaction. This light reaction is preceded and followed by dark reactions and thus constitutes a link in a reaction chain. The two chains have one or more links in common, *e.g.*, by bifurcation of the reaction chain, or by a cyclic process, as suggested in the morphogenetic photocycle of LIVERMAN and BONNER (41). Above a certain light intensity the supply of substrates for the photoreactions, or the subsequent dark reactions may limit the rate of the photoreaction. If it is then assumed that the blue-infrared reaction is limited by dark reactions more readily than the red reaction, a sufficiently large increase in intensity at wave lengths that are absorbed by both pigments will result in an ultimate dominance of the red effect. In a given case, the effect of blue light of wave lengths around 480 mμ will depend on the number of quanta absorbed by both pigments, which is a function of concentration and specific absorption coefficient. Which way the reaction moves is determined by concentration and absorption, up to the point where the light intensity is increased above the saturation value of one of the light reactions. If the most efficiently absorbing pigment is the first to become light saturated, a reversal of the effect will occur provided the ratio between the saturation values of the two light reactions is higher than the ratio of the effective absorptions. This hypothesis could explain the flattening of the red maximum in the action spectra of BORTHWICK *et al.* as being due either to low concentration of the blue-infrared pigment, or to a low light saturation level of the blue-infrared photoreaction, or to both reasons. It also could explain the reversal of the effect of blue light with increasing light intensities that was found in experiments described in Chapter VII. In cases that the blue-infrared pigment occurs in a concentration so low that the effective absorption at 480 mμ is lower than that of the red absorbing system, no effect of blue other than that produced by the red absorbing system will be found. This might explain some cases in which the "infrared" effect of blue is not, or only partly found, as, *e.g.*, in seed germination.

It must be mentioned that a possible alternative to the hypothesis given above is the assumption that the effect of blue light is sensitized by a pigment different from the red-infrared system. This concept has been considered critically and it has to be rejected on the following grounds:

a. The blue effect can be reversed by red light in exactly the same way (*cf.* Chapter VI) as that of infrared.

b. From the action spectra of BORTHWICK *et al.* it can be seen that a relative increase in energy required in the blue is generally accompanied by a similar increase in energy required in the region around 700 mμ, where, according to the above hypothesis, the same situation would occur.

The finding of BORTHWICK *et al.* (8, 9) that the energies required for both processes change in opposite directions, when they change for some reason, such as ageing, could also be explained on the basis of a thermal displacement of the equilibrium between two substrates present in limiting concentrations, while the concentrations of the pigments remain constant.

LIVERMAN and BONNER (41) suggested that the substrates of the photoreactions consist of an auxin-non-receptive precursor and an auxin-acceptor complex. Red light is supposed to activate the non-receptive precursor, so that an auxin-receptor is formed that combines with auxin, to form an auxin-acceptor complex that is active in plant responses. Either a temperature sensitive dark reaction or infrared radiation can destroy the auxin-acceptor complex, thus reducing the effective auxin concentration in the plant. The experiments on

which this theory is based were made with coleoptile sections of dark grown *Avena* seedlings. On the basis of this theory, these authors were able to connect coleoptile growth in *Avena*, light dependent seed germination, and photoperiodism.

Although their concept of this morphogenetic photocycle is an attractive working hypothesis, some of the experimental results presented in the foregoing chapters do not fit in very well. In their discussion on the photoperiodic reaction they assume that the auxin level determines flowering in long and short day plants. However, flowering in most plants, when grown in "monochromatic" light, is hardly affected by the wave length of the light, whereas the extractable auxin shows large variations, involving a factor 10 or more between plants in violet and yellow light (see Chapter VII).

On the other hand, auxin determinations by *Avena* tests of extracts from leaves of plants receiving supplementary light of low intensity [1]) failed to show a consistent difference in auxin content in plants receiving red or infrared supplementary light respectively. Notwithstanding this, the same plants showed a big difference in their flowering reaction. Of course, it is conceivable that extracted auxin does not give a correct idea of the auxin activity in the plant, and possible fluctuations in the sensitivity of the plant to auxin can also obscure the picture. However, then it is hard to understand that auxin, applied to the leaves, can have an effect (68, 37) that does not arise when the auxin level in the plant is affected from outside by monochromatic blue or violet irradiation.

The effects of different wave length regions on internode elongation of plants grown in "monochromatic" light can hardly be explained by a non-specific destructive action of short wave length radiation, although probably processes as described by GALSTON *et al.* (24) play an important rôle. FORTANIER (17), in experiments with *Arachis hypogea* could demonstrate that the addition of a relatively small amount of violet-blue light very effectively reduced the closing of leaves of plants growing in red light, indicating that the lack of blue light is a very important factor in the abnormalities produced by "monochromatic" red light. There are, however, indications that stem elongation and also epinasty of the leaves of tomato plants growing in red or yellow light are not just due to lack of blue light. The stem elongation in tomato and other plants growing in yellow light is always stronger than in red light, indicating a positive effect of yellow light in this respect. The same difference is noticed in leaf epinasty. This can not be explained in terms of lack of blue light, since blue light is equally absent in both wave length regions. The effect of a combination of red and infrared provides an even stronger argument. The addition of infrared stimulates extension growth even stronger than red light alone. This again indicated that the absence or presence of blue light is not the only factor determining extension growth in light grown plants.

The evidence, presented in Chapter VII, suggests that light of different wave length regions, when given at high intensities and under the exclusion of white light, somehow affects the auxin level, in contrast to the effect of coloured light of low intensity, given supplementary to a short day in white light, which may influence the activity of auxin already present in the plants. The results of the growth substance tests described in Chapter VII show that the extractable auxin content is dependent on the wave length of the "monochromatic" light

[1]) The author wishes to thank Dr W. TERPSTRA, then of the Botanical Laboratory Utrecht, for carrying out these growth substance determinations.

in which the plants grow. It can not yet be decided whether this is the result of enhanced auxin synthesis in the longer wave lengths, or of destruction in blue light as described by GALSTON *et al.* (24). As in the present experiments photosynthesis appears to be largely independent of wave length, the described effect of light on auxin content must be placed somewhere in the chain of reactions between photosynthesis and the photoreaction involving the red and blue-infrared pigment systems which possibly regulates auxin activity. The grounds for the assumption that the effect of low intensity supplementary light is mediated by auxin activity are found in the fact that no correlated variations in extractable auxin content are found, as well as in the results obtained by LIVERMAN and BONNER (41) and LANG and LIVERMAN (37). However, it is realized that, *e.g.*, in photoperiodism, as well as in some other processes, the picture must be more complicated than the above hypothesis suggests.

SUMMARY

Experiments are described in which a number of plants were exposed to light of various well defined spectral regions. The light of these spectral regions was given at low or high intensities, supplementary to a short day in white light, or at high intensity, as exclusive source of light. Special attention was paid to photoperiodic and formative responses of the plants to the wave lengths of supplementary light, and to formative responses to exclusively "monochromatic" light.

In photoperiodically sensitive plants, two types of wave length dependence of the photoperiodic reaction were found:

a. Cruciferous plants *e.g. Brassica Rapa* f. *oleifera* subf. *annua, Sinapis alba, Iberis coronaria*, were found sensitive to the blue and infrared wave length regions, and showed no response to the region between 520 and 700 mμ.

b. Other plants, *e.g. Cosmos bipinnatus* and *Spinacia oleracea* were sensitive to the region between 520 and 700 mμ, and showed little or no response to the blue and infrared wave length regions, which is in accordance with the action spectra for other plants as determined by previous workers.

c. In day neutral plants (*Lactuca sativa, Solanum Lycopersicum*) flowering was not affected by wave length of supplementary light.

Experiments with *Cruciferae* show that blue and infrared supplementary light, even when given within a short day which was found insufficient for flowering in white light, still promote elongation, and also flowering. From this, it can be concluded that the region 520–700 mμ inhibits the promotion of flowering due to the blue component of white light.

All species show strong formative responses to supplementary irradiation of the blue and infrared wave length regions, consisting of excessive elongation of some part, *e.g.*, internodes, leaves or petioles. It was shown that in *Cosmos* internodes this elongation must be attributed to cell elongation only and that it occurs mainly during the supplementary light period and the subsequent dark period. Supplementary light has no effect on cell division.

The elongation caused by blue or infrared supplementary light can be inhibited by simultaneous or subsequent exposure to red or green supplementary light. A preceding exposure to green or red does not prevent excessive elongation caused by subsequently given blue or infrared radiation.

The elongating influence of supplementary blue light depends on the inten-

sity: below about 300 ergs/cm²/sec, elongation is proportional to light intensity; it was shown to be independent of intensity between 300 and 1500 ergs/cm²/sec. When the intensity of blue supplementary light is increased to 15.000 ergs/cm²/sec, or higher, its elongating influence again becomes smaller.

Plants grown in light of restricted spectral regions with the exclusion of white light also reveal very strong formative effects. In red, yellow and green light excessive elongation of stems, petioles and leaves occurs, as well as epinastic curvatures of the leaves. The plants in blue light are comparable to the white controls while plants in violet light are shorter than the white controls. This was shown in experiments with *Cosmos bipinnatus*, *Lactuca sativa*, *Solanum Lycopersicum*, *Iberis coronaria*, and a *Fragaria* hybrid. In experiments with tomato it was shown that dry weight production was not specifically affected by the wave length of the light. The number of leaves, leaf area, and the leaf pigments in tomato were also shown to be nearly unaffected by the wave length of the light. Growth substance determinations in extracts from tomato plants grown in yellow and violet light showed that tips of tomato plants in yellow light contain more auxin than those in violet light, so that strong elongation was correlated with a high content of extractable auxin.

If red light was given together with a large amount of infrared this addition of infrared seems to have a detrimental effect: the elongation growth was stimulated, but the number of leaves, the leaf area, and the chlorophyll content as well as the final dry weight were very much reduced. When plants, grown in coloured light of high intensity were exposed to supplementary coloured light of low intensity, the effects of these two treatments were superimposed, indicating that they can occur independently or combined.

The results are discussed, and it is suggested that the "monochromatic" light of high intensity regulates the auxin level, whereas the supplementary light might affect the activity of, or the sensitivity to auxin. The nature of the photoreceptive mechanism and the antagonism of the low intensity reaction are discussed.

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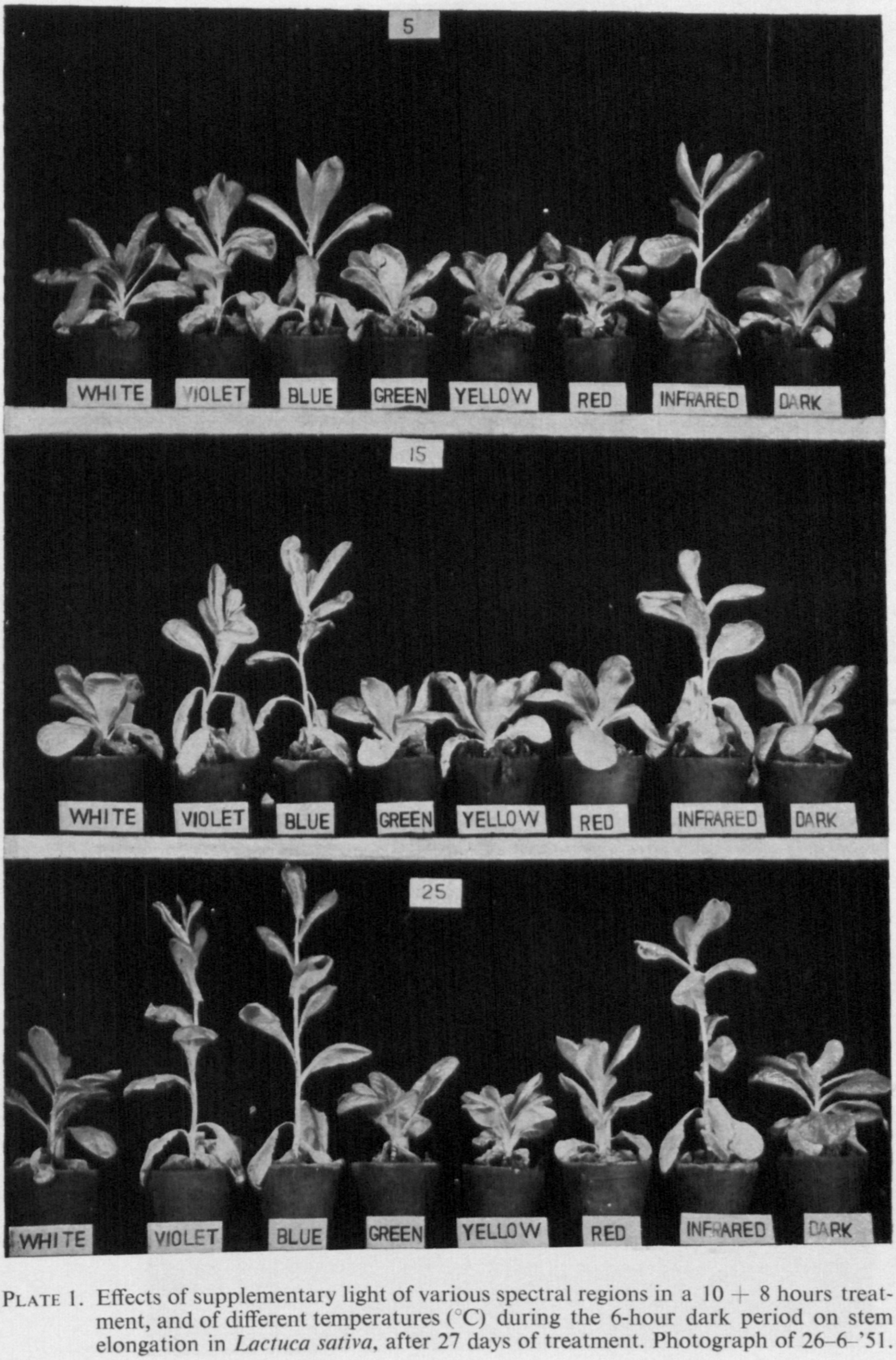

PLATE 1. Effects of supplementary light of various spectral regions in a 10 + 8 hours treatment, and of different temperatures (°C) during the 6-hour dark period on stem elongation in *Lactuca sativa*, after 27 days of treatment. Photograph of 26–6–'51.

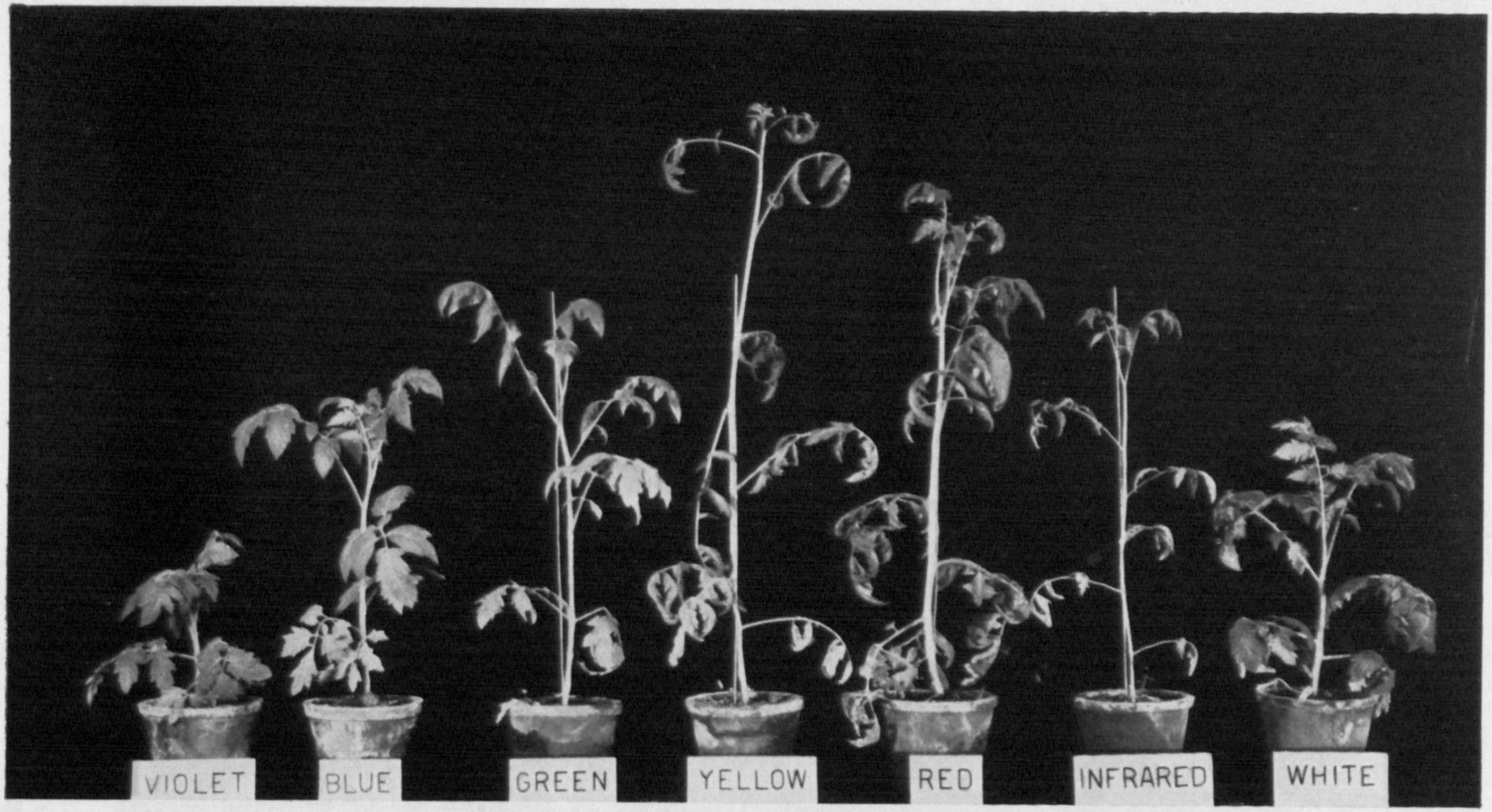

PLATE 2. *Solanum Lycopersicum* var. Vetomold 121, grown in light of various spectral regions. Photoperiod: 16 hours, at 20 °C. Dark period temperature: 15 °C. After 36 days of treatment. Photograph of 10–12–'53. "Infrared" = red + infrared.