A PRELIMINARY STUDY OF PHOTOPERIODIC AND FORMATIVE PROCESSES IN RELATION TO METABOLISM, WITH SPECIAL REFERENCE TO THE EFFECT OF NIGHT TEMPERATURE

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Dit proefschrift met stellingen van

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landbouwkundig ingenieur, geboren te Leeuwarden, 8 Mei 1924, is goedgekeurd door de promotor Dr E. C. Wassink, hoogleraar in de physiologie der planten.

De Rector Magnificus der Landbouwhogeschool,
W. J. Dewez

Wageningen, 10 Mei 1954

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS IR W. J. DEWEZ,
HOOGLERAAR IN DE LANDBOUWPLANTENTEELT,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
DER LANDBOUWHOGESCHOOL TE WAGENINGEN
OP DONDERDAG 3 JUNI 1954 TE 15 UUR

DOOR

SIBERGINA WAGENAAR

H. VEENMAN & ZONEN – WAGENINGEN – 1954

De inhoud van dit proefschrift verschijnt tevens in de Mededelingen der Landbouwhogeschool te Wageningen/Nederland 54 (2) 1954

STELLINGEN

I

Voor de bloemaanleg is het energetisch aspect van de stofwisseling van ondergeschikte betekenis.

II

Wanneer *Spinacia oleracea* var. Nobel midden in een veertien urige nacht gedurende korte tijd wordt belicht, bestaat er een optimum energie waarbij de belichting een grootste effect heeft.

Dit proefschrift

III

Tijdens een korte belichting midden in een veertien urige nacht is in *Spinacia oleracea* var. Nobel een donkerreactie nauw gekoppeld aan de lichtreactie.

Dit proefschrift

IV

De term „groei" kan slechts worden gebruikt indien tevens wordt aangegeven welk aspect van dit gecompliceerde verschijnsel in beschouwing wordt genomen.

BURSTRÖM, H., Physiol. Plant. **4** 685–692, 1953

V

De door WENT gebruikte methode ter bepaling van concentraties van wortelvormende stoffen geeft een onvolledig beeld van de situatie.

WENT, F. W., Verh. Akad. Wet. Amsterd. **37** 445–455, 1934

VI

Wanneer photosynthese metingen worden gecorrigeerd voor ademhaling, dient er rekening mee gehouden te worden, dat de ademhaling door belichting kan worden beinvloed.

ROSENSTOCK, G., Planta **40** 70–92, 1951

VII

Bij de verklaring van de bloei van lange dag planten in het tropische gebergte hield BÜNNING onvoldoende rekening met vernalisatie.

BÜNNING, E., Biol. Zentralbl. **67** 3–7, 1948

VIII

Het optreden van Fe-gebrek bij verschillende planten hangt meer samen met een gestoorde stofwisseling dan met moeilijkheden in de ijzer opname.

BIDDULPH, O., Plant Physiol. **28** 576–594, 1953

Diss. S. Wagenaar (Wageningen, 1954)

IX

De bepaling van concentraties van tabaksmozaiekvirus met behulp van de methode van TAKAHASHI en ISHII geeft een onvolledig beeld van de aanwezige hoeveelheid smetstof.

TAKAHASHI, W. N. en M. ISHII, Amer. J. Bot. **40**, 81–84, 1953

X

Voor de vermeerdering van cacao verdient in vele gevallen oculeren aanbeveling boven stekken.

XI

Het onderzoeken van en het worstelen met de werkelijkheid krijgt eerst haar rechte zin wanneer die werkelijkheid door de onderzoeker beschouwd wordt als een openbaring van God.

J. LEVER, Het Creationisme, inaugurele rede Amst. 1952
V. J. KONINGSBERGER, Scientia amabilis, diesrede Utrecht 1953

XII

De emancipatie der vrouw heeft zich in het wetenschappelijk milieu in Nederland nog slechts ten dele voltrokken.

Aan de nagedachtenis van mijn Vader
Aan mijn Moeder

VOORWOORD

Het is mij een genoegen om bij het verschijnen van dit proefschrift mijn dank uit te spreken aan allen, die hebben meegewerkt aan mijn wetenschappelijke opleiding.

In de eerste plaats gaat mijn dank uit naar U, hooggeleerde WASSINK, hooggeachte promotor, voor de gelegenheid, die U mij geboden hebt om onder Uw leiding kennis te maken met het wetenschappelijk onderzoek en de wijze waarop U mij dit moeilijke onderdeel van onze bezigheden hebt doen waarderen. Uw advies bij de keuze van het onderwerp voor dit proefschrift, Uw voortdurende belangstelling tijdens het onderzoek en de waardevolle suggesties, die U mij gaf, zijn mij tot grote steun geweest. Ik ben U erkentelijk voor Uw niet verflauwde interesse ook tijdens de definitieve vormgeving van het manuscript.

U, zeergeleerde mevrouw WASSINK dank ik voor de prettige wijze, waarop ook U steeds van Uw belangstelling hebt blijk gegeven.

Van U, hooggeleerde COOLHAAS en SCHUFFELEN ontving ik speciaal tijdens mijn ingenieursstudie mijn landbouwkundige opleiding, waarvoor ik U zeer erkentelijk ben.

De collega's van het laboratorium voor plantenphysiologisch onderzoek dank ik voor hun voortdurende belangstelling en hulp bij het onderzoek. Inzonderheid ben ik dank verschuldigd aan Mr S. D. RICHARDSON, M.A., B.Sc. voor de correctie van de Engelse tekst en voor zijn waardevolle suggesties. Ook gaat mijn dank uit naar Mej. Ir A. C. WIERSEMA en de heer J. A. D. ZEEVAART die mij tijdens hun studie behulpzaam zijn geweest bij de uitvoering van verschillende proeven. Het technische personeel, dat mij steeds op prettige en accurate wijze heeft geholpen ben ik eveneens dank verschuldigd.

De organisatie voor Zuiver Wetenschappelijk Onderzoek ben ik erkentelijk, dat zij mij in de gelegenheid heeft gesteld dit proefschrift te bewerken.

CONTENTS

CHAPTER I

INTRODUCTION, MATERIALS AND METHODS

1. STATEMENT OF THE PROBLEM AND SCOPE OF THE INVESTIGATION

In the last few decades, many attempts have been made to explain the nature of flowering in plants. In spite of ingenious and extensive work, however, many of the fundamental physiological problems involved, remain unsolved. As early as 1918, KLEBS outlined a concept of flowering. The results of extensive experiments with *Sempervivum funkii* led him to the hypothesis that flowering is determined by the balance of organic to inorganic material in the plant. Thus, a high concentration of carbohydrate relative to nitrogen (i.e. a high C/N ratio) will stimulate flowering, while the opposite condition, or a predominance of dissimilatory processes, will tend to maintain the plant in the vegetative state. The basic requirement for flowering as visualised by KLEBS may, therefore, be described as a "high energy potential". Shortly after this publication came the well known one of GARNER and ALLARD (1920) in which they demonstrated conclusively that, for a large number of species, daylength is the controlling factor in flowering. Thus, plants like soya, cocklebur and Maryland mammoth tobacco will only flower under short days, while spinach, *Hyoscyamus* and lettuce will only flower under long days.

The paramount importance of daylength in determining the onset of the flowering condition cannot be disputed; and the rôle of metabolism must be regarded as less primary than was supposed by KLEBS. Nevertheless, it is self evident that metabolism must play a rôle in flowering, albeit a subordinate one. Flowering requires energy, just as any other developmental or growth process and it might be expected, therefore, that the influence of photoperiod is exercised through its control of the energy balance within the plant. The question of whether or not this is so, has never been satisfactorily cleared up and, in view of certain unsatisfactory features in previous work, a further investigation seemed desirable. The present study was undertaken, therefore, in an attempt to elucidate the relation between photoperiodism and metabolism.

It is well known that metabolic processes are markedly affected by temperature; and it seemed feasible to make use of this fact in attacking the present problem. It was argued that if the temperature relations of flower induction and related morphogenetic processes could be worked out and compared with those of known metabolic processes, interactions between the two sets of phenomena might in this way be emphasized. A number of experiments was performed in which the temperature was varied during a long illumination period at low intensity. Since, under these conditions, processes other than flowering may be influenced and interfere with the effect of temperature on flowering, this effect was also studied during a brief period of flower-inducing illumination in the middle of the night. Apart from these observations, a number of other metabolic activities was investigated. Increase in dry weight, starch hydrolysis, and respiration in relation to temperature and conditions of illumination were studied, and related to flower initiation.

2. INTRODUCTION TO THE LITERATURE

In view of the extensive reviews of work on the physiology of flowering by LANG (1952) and GREGORY (1948), detailed treatment here is not proposed. This section will be confined to a discussion of the more important papers which bear on the relation between photoperiodism and metabolism and, in particular, of those investigations which prompted the present one. The literature relating to the particular problems investigated experimentally, is briefly discussed at the beginning of each relevant chapter.

The investigations initiated by KLEBS were continued by KRAUS and KRAYBILL (1918). They emphasized that in addition to the relative concentrations of carbohydrate and nitrogen and the supply of moisture, other factors are probably effective in determining the vegetative and the reproductive stage of a plant. This remark has largely been overlooked, and, up to now, experiments based on the work of these two students of KLEBS, have been undertaken either to prove or disprove that the C/N ratio determines flowering. In the greater part of these experiments, however, no data are given about the time of flower initiation, flowering or fruitset.

When differences are found in C/N ratio between flowering and non-flowering plants, it cannot be concluded that these differences are the cause of flowering. It is not surprising that not all experiments agree. POTTER and PHILIPS (1930) found more insoluble nitrogenous compounds in flowering apple spurs than in non-flowering ones, so that the C/N ratio was rather low in the former. Ringing, however, increased this ratio and favoured flowering. Shaded and defoliated

plants did not flower, although their relative carbohydrate content was lowered by these treatments. NIGHTINGALE (1927) could not always find better flowering accompanied by higher C/N ratios. TINCKER (1928) examined the influence of daylength on C/N ratio. N-concentration was hardly influenced by the treatments, and differences in C/N ratios were due almost entirely to differences in C-concentration. This author also demonstrated that the part of the plant from which the samples are taken is important. Thus, young leaves behave differently from old leaves.

There are indications that differences in C/N ratios are a consequence rather than the cause of flower initiation. BIDDULPH (1935) examined N-compounds during the 7 day period necessary for flower initiation in *Cosmos*. During induction, glutathion showed a daily fluctuation in concentration. No differences in other metabolites were found until the 4th day, when the concentration of reducing sugars increased somewhat. In plants with flower initials he found somewhat more reducing sugars and less amides than in plants without flower initials. MURNEEK (1937) also found differences in C- and N-concentrations after flower initiation. HURD–KARRER, and DICKSON (1934), PURVIS (1934), POLSTER (1938) and DUPÉRON (1953) measured C- and N-concentrations in vernalised plants. Before flowers were initiated no differences between vernalised and unvernalised plants were found. In all the experiments mentioned, the question arose as to whether special attention should be given to soluble and insoluble C- and N-compounds separately, or to special substances.

According to VON DENFFER (1940) and CAJLACHJAN (1945) it is possible to change the critical daylength of some plants by growing them with different N-concentrations in the medium. Several long-day plants flower sooner when the nitrogen concentration decreases, while several short-day plants flower sooner when the nitrogen concentration increases. Other long- and short-day plants are N-neutral. VON DENFFER mentions this as a possible explanation of the confusion which exists in literature concerning this subject.

One may conclude from this work that to connect flower initiation only with special C/N ratios is a premature and an unjustified simplification.

Before a balanced judgement can be formed more information is required concerning the rôle of other metabolic processes in flowering and, in particular, concerning the interaction daylength and metabolism.

3. MATERIALS AND METHODS

Most experiments were carried out with spinach (*Spinacia oleracea* L., var. Nobel), while some studies were also made with *Hyoscyamus niger* L., var. *pallidus*, and *Brassica Rapa*, f. *oleifera* L., subf. *annua*. The spinach seed was obtained partly from Dr A. F. SCHOOREL of the Central Institute for Agricultural Research, and partly from Ir J. SNEEP of the Institute for Horticultural Plantbreeding. The *Hyoscyamus* seed was obtained from Dr A. LANG, Pasadena. The *Brassica* was selected from plants already used in other experiments in this Laboratory (WASSINK, SLUYSMANS and STOLWIJK [1950]).

For the experiments with spinach and *Brassica*, plants were grown in the greenhouse for about one month; when the second pair of leaves had appeared they were brought indoors. In the greenhouse, the plants were given a 10 hour day in order to ensure that flower initiation did not take place. During winter, the light intensity in the greenhouse was increased with the aid of a high pres-

sure mercury lamp, yielding a light intensity of at least 16000 erg/cm²/sec. Nevertheless, the plants grew only slowly and the experiment had to be continued for a longer time than if they were grown in the greenhouse during summer. *Hyoscyamus* plants had to stay in the greenhouse for 2–3 months in order to develop sufficiently for starting experiments. All plants used were carefully matched.

During the experimental period the plants received a basic 10 hour day under sets of 8 "daylight" fluorescent tubes at an intensity of 22000 erg/cm²/sec in a controlled temperature room. During winter the day temperature in this room was 20 °C; during summer, the temperature could only be kept constant at 23° C. For the rest of the 24 hour cycle the plants were brought to various rooms kept at temperatures which could be maintained to within 0,5 °. For supplementary irradiation of long duration, low light intensities were used, in order to ensure that no photosynthetic activity influenced photoperiodic induction. "Daylight" fluorescent tubes were used for this irradiation. For brief periods of irradiation in the middle of the dark period high light intensities were applied. Various light sources were used, as described in the various experiments. Different temperatures during darkness were applied in thermostats, constant to within 0,2 °.

Light intensities were measured at the surface of the upper leaves, with a light-meter constructed in this laboratory, and consisting of a selenium barrier layer photo-cell connected with a 50 μ A meter, and provided with filters allowing measurement over a wide range of intensities. This meter follows the cosine law since it is provided with a slightly convex opaline glass that eliminates deviation arising from reflections at acute angles of incidence. The photo-cell had been calibrated in energy values for every light source used.

Throughout this report the word "night" is meant to cover whole of the 24 hour cycle other than when basic illumination was given. Thus, even when supplementary illumination of low intensity was given for 14 hours, this period is still regarded as "night".

CHAPTER II

THE INFLUENCE OF TEMPERATURE DURING DARKNESS AND DURING SUPPLEMENTARY IRRADIATION ON FLOWERING AND VARIOUS FORMATIVE PROCESSES IN SPINACH, *HYOSCYAMUS* AND *BRASSICA*

1. INTRODUCTION

The influence of temperature on flowering is rather complicated. During the day, the influence is small both for long-day and short-day plants (PARKER and BORTHWICK [1939], HAMNER and BONNER [1938], and LANG and MELCHERS [1943]). SNYDER (1940), however, found a pronounced effect of temperature during the photoperiod when its duration was near the lower critical value; under his experimental conditions this was 2 hours. The influence of temperature during the dark period is more pronounced. Low temperature can change the critical daylength by some hours (LONG [1939]), or change the minimum number of inductive cycles required for flower initiation (HAMNER and BONNER

[1938]). For *Xanthium*, the optimum night temperature is 65 °–75 °F. In *Hyoscyamus*, the influence of night temperature varies with the daylength. At the critical daylength low temperature favours flowering, while at a daylength greater than 14 hours, high night temperatures bring about early flowering, (LANG and MELCHERS [1943]).

The experiments described in this chapter concern the influence of night temperature on flowering, and on special formative processes in some long-day plants grown at different daylengths.

2. INTRODUCTORY EXPERIMENTS CONCERNING THE INFLUENCE OF SUPPLEMENTARY IRRADIATION OF DIFFERENT INTENSITIES ON FLOWERING OF SPINACH

Referring to the general description in chapter I we may add that the plants, after their preliminary cultivation in the greenhouse were transferred to the experimental set-up with a basic irradiation of 10 hours per day, at an intensity of 22000 erg/cm²/sec. This illumination was provided by fluorescent light ("daylight" type); the day temperature was 20 °C. Supplementary irradiation was given for 14 hours per 24 hour cycle at an intensity of 42, 85, 125, 170, and 250 erg/cm²/sec. with „daylight" fluorescent tubes at a temperature of 13°. Control plants received 14 hours of darkness at 13 °C. Other plants received 14 hours of darkness at 2°, 9°, 20° and 27 °C in the same run. For each treatment 4–5 plants were used.

TABLE 1

Leaf number in spinach after different durations of treatment. Basic irradiation for 10 hours at 20 °C. Supplementary irradiation at 13° at different intensities

intensity of supplementary irradiation (erg/cm²/sec.)	0	42	85	125	170	250
after 14 days of treatment .	8.8	8.75	8.75	9.25	10.0	10.5
after 20 days of treatment . . .	11.0	11.3	13.0	13.5	15.25	16.25

Fourteen days after the start of the experiment the leaves were counted (Table 1). Every macroscopically visible leaf was included in these leaf counts. In the plants receiving 4 hour supplementary irradiation at intensities of 42 and 85 erg/cm²/sec the number of leaves was the same as in the dark controls; plants receiving supplementary light at intensities of 125 erg/cm²/sec or higher, showed an increase in leaf number with increasing light intensity. After 20 days the plants receiving supplementary light at an intensity of 85 erg/cm²/sec also showed an increase in leaf number, as compared with the dark controls. Table 2 shows that by this time stem elongation had started. Plants with elongated stems were found to have microscopically visible flower initials within a few days. Thus, under these conditions, stem elongation can be taken as an indication that the plant has started flower initiation.

TABLE 2

Stemlength (cm) in spinach after different durations of treatment. Same experiment as in Table 1

intensity of supplementary irradiation (erg/cm²/sec)	20 days of treatment	39 days of treatment	45 days of treatment
42	0.25	5.33	7.5
85	0.55	7.85	12.5
125	0.83	10.85	14.25
170	2.0	21.8	25.0
250	2.1	28.0	28.3

In spinach the inflorescence primordium is surrounded by a number of small leaves. Although no macroscopically visible flower buds were observed after 20 days of treatment, it is probable that the greater leaf number found at higher intensities of supplementary light was correlated with the initiation of floral primordia. However, as the number of plants that could be used in these experiments did not permit the sacrifice of a sufficiently large number for microscopical dissection at different intervals, the flowering responses had to be measured by macroscopical characteristics, so that this assumption could not be substantiated.

TABLE 3

Number of days before flower buds and flower formation in spinach. Same experiment as in Table 1 and 2

intensity of supplementary irradiation (erg/cm²/sec)	42	85	125	170	250
flowerbuds	29	26	26	25	23
flowers	42	38	35	34	30

Table 3 gives the number of days required in the different treatments for development of flower buds and flowers. In the case of flower buds the macroscopic visibility was taken as the criterion, and the ripening of the pollen or visibility of stigma was used in the case of flowering. At supplementary light intensities of 125 erg/cm²/sec and lower, the plants showed large individual differences; the data, therefore indicate the time when 50% of the plants reached this stage of development. At higher intensities the individual plants developed flower buds and flowers on practically the same day for any given treatment. The development of flower buds and flowers was accelerated by increases in intensity of supplementary light.

TABLE 4

Influence of the temperature during 14 hour dark periods per daily cycle on leaf number and leaf area after 40 days of treatment, and on stemlength after 70 days of treatment in spinach. Experiment run parallel to that of Table 1

temperature	2°	9°	13°	20°	27°
number of leaves	12.0	14.0	16.0	17.5	20.0
leaf area (cm²)	55.6	57.5	104.7	164.7	105.7
stemlength (cm)	0.7	2.0	2.0	2.4	5.0

Table 4 shows data concerning plants that have been under short day treatment at various night temperatures. In this experiment the leaf area per plants was determined by measuring the length and width of the leaves, the leaf being almost elliptical. Leaf areas were greatest at 20 °, although more leaves were present at 27 °. After 70 days of treatment most of the plants had small stems, though no flower initials could be found after dissection. Stemlength increased with increasing temperature.

3. INVESTIGATION INTO THE MOST SUITABLE DAYLENGTH FOR EXPERIMENTS ON THE INFLUENCE OF TEMPERATURE ON FLOWERING OF SPINACH

Plants were given daylengths of 10, 12, 14, 16, and 18 hours. The first 10 hours of light were, in every case, at an intensity of 22000 erg/cm²/sec and a temperature of 23 °C. Supplementary

irradiation was given at 20° at an intensity of 340 erg/cm²/sec. The temperatures during the night were 2°, 5°, 9°, 13°, 20°, and 27 °C.

At a daylength of 12 hours the plants formed no flower buds during the period of the experiment. After 50 days the growing points were examined under a 40-fold magnification and it appeared that, at all temperatures, one out of 4 plants had initiated flowers. Under these conditions, thus, a daylength of 12 hours acts largely as a short day.

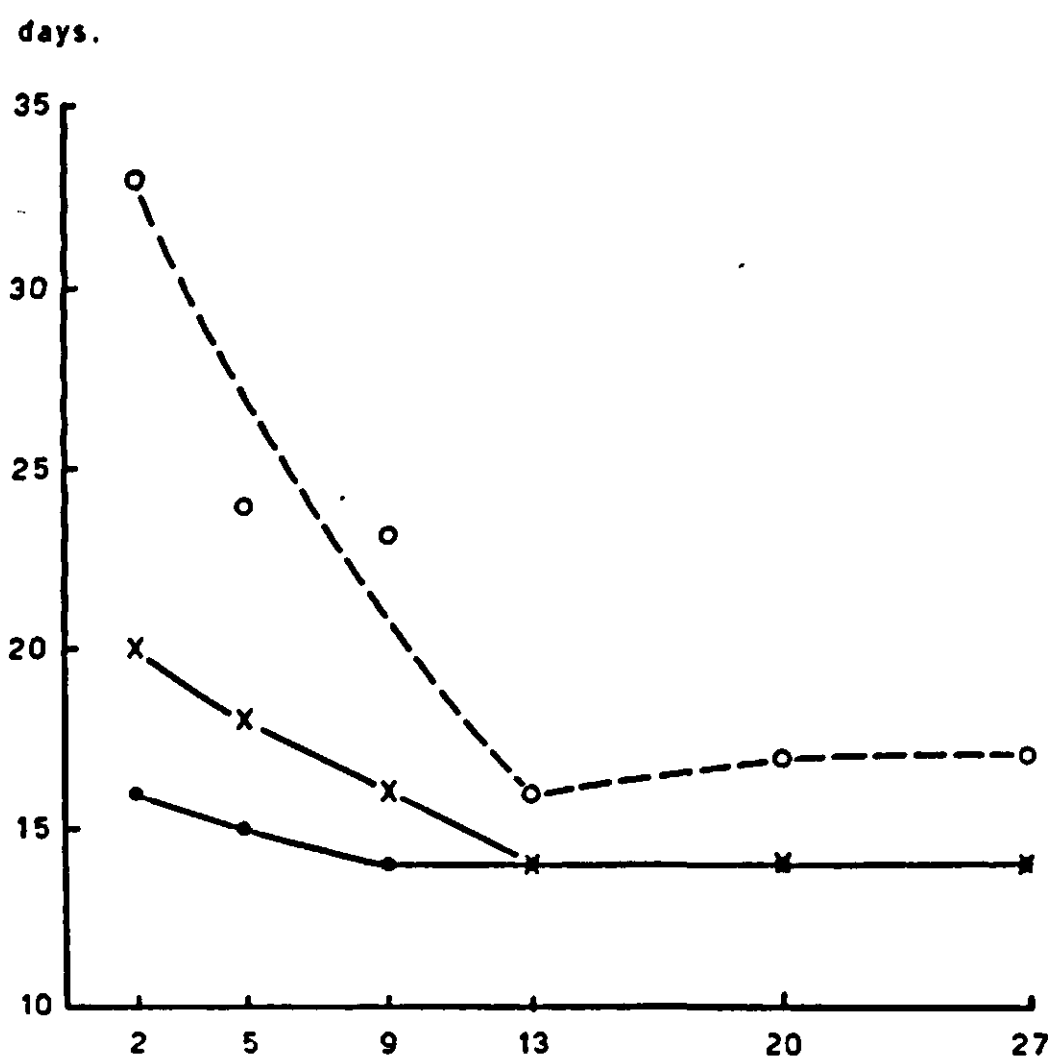


FIG. 1.
Number of days (ordinate) up to flower bud formation in spinach. Basic irradiation 10 hours at 20 °C. Supplementary irradiation for 4 hours (o---o), 6 hours (x——x) or 8 hours (•——•). Various temperatures applied during darkness (abscissa).

In longer days all plants initiated flowers. Figure 1 gives the number of days before flower buds were visible. At daylengths of 16 hours or longer, flower bud formation was little affected by night temperatures over 13 °C. At lower temperatures the flower buds were formed a few days later. At a daylength of 14 hours the first flower buds were found at a temperature of 13 °. At the higher temperatures they appeared somewhat later; the difference, however, was small, but was found to be consistent in many experiments.

The delay in flower bud formation due to low temperature was considerably greater at a daylength of 14 hours than at one of 16 or 18 hours. In later experiments, therefore, a daylength of 14 hours was chosen as most suitable for studying the effect of night temperature.

4. THE INFLUENCE OF NIGHT TEMPERATURE ON FLOWERING AND VARIOUS FORMATIVE PROCESSES IN SPINACH AT DAYLENGTHS OF 10, 14 AND 24 HOURS

All plants received the 10 hour basic irradiation period at an intensity of 22000 erg/cm²/sec, from "daylight" fluorescent tubes, at 20 °C. The experiment was performed twice, the first time with supplementary light at an intensity of 170 erg/cm²/sec and the second time at 420 erg/cm²/sec. The supplementary irradiation was applied for 14 hours per day, in order to examine the influence of a series of temperatures, *viz.*, 2°, 5°, 9°, 13°, 20°, and 27°C. Other plants were exposed to these various temperatures during a 14 hour dark period, in order to investigate its influence under non-flowering conditions. Finally plants received these temperatures both during a 4 hour supplementary irradiation and during a dark period of 10 hours. In the first experiment, 5 plants were used per treatment and 4 in the second.

In both experiments, at a total daylength of 24 hours, stems first appeared in plants at a temperature of 20 ° during the supplementary light period. Flower

FIG. 2.

Number of days (ordinate) up to stem elongation (•——•), visible flower buds (x——x) and flowering (o---o), in spinach. Temperature during 10 hours basic irradiation 20 °C. During the rest of the 24 hours cycle the plants were illuminated at an intensity of 170 erg/cm²/sec at various temperatures (abscissa).

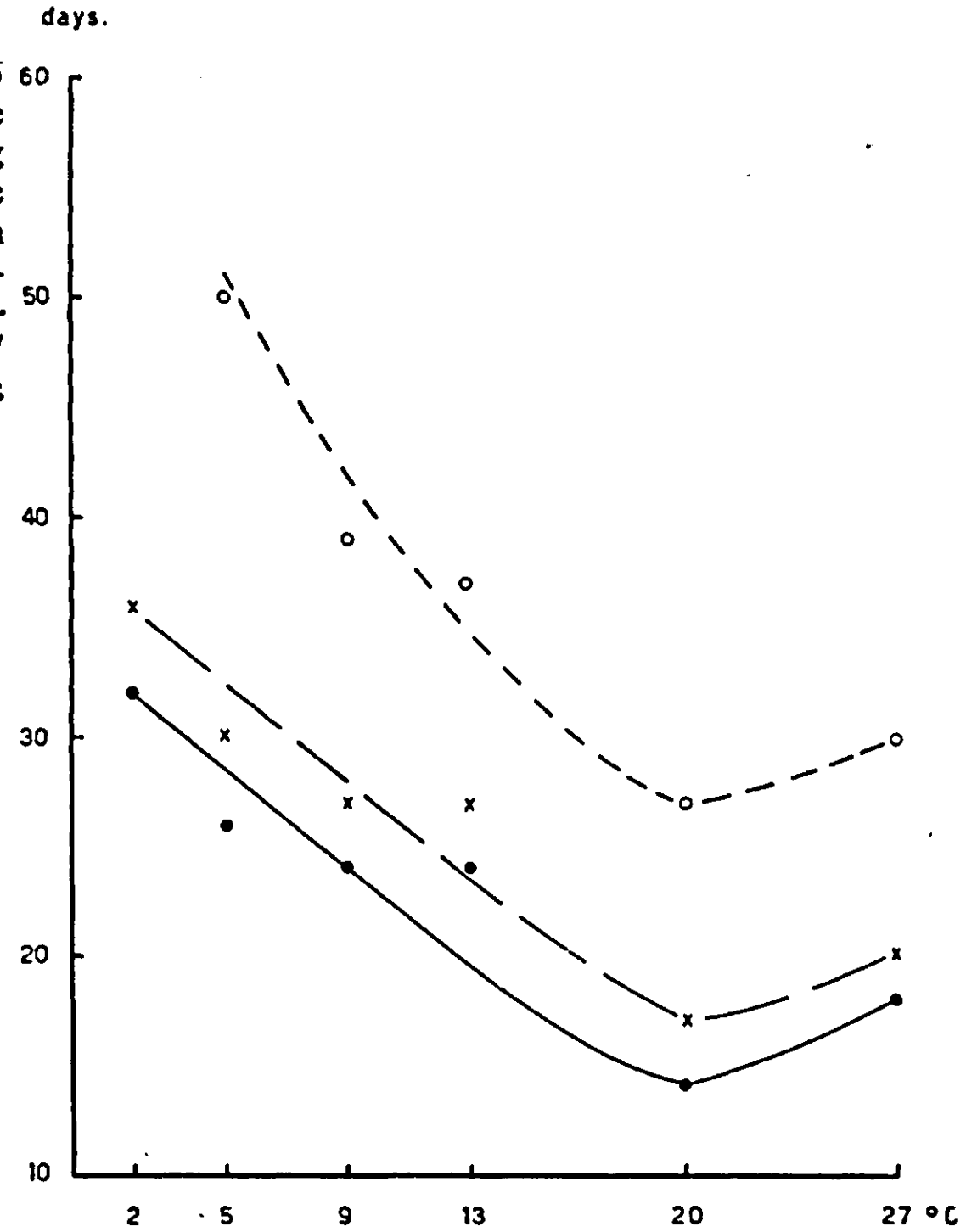
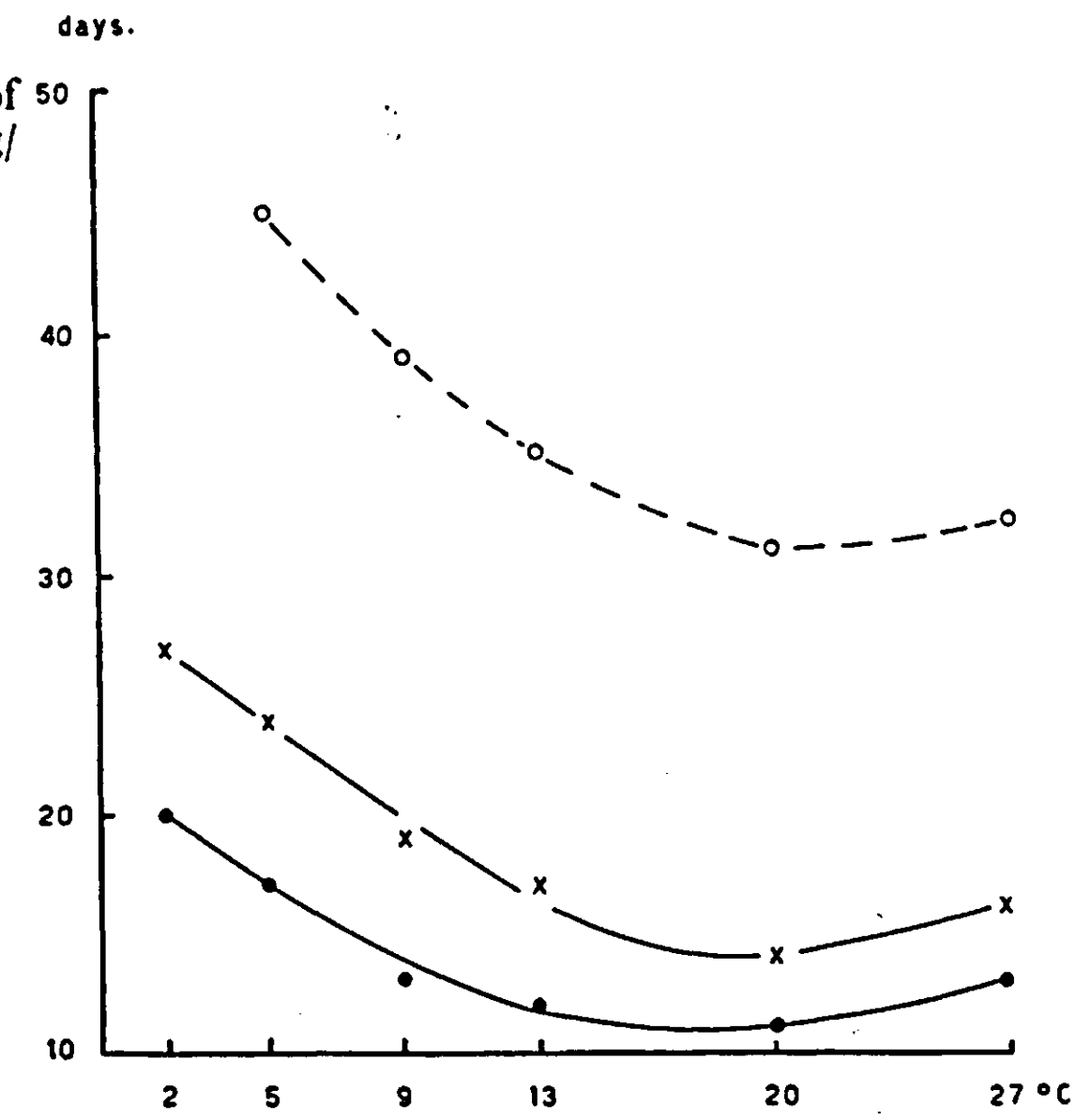


FIG. 3.

Legend as for fig. 2. Intensity of supplementary irradiation 420 erg/cm²/sec.



buds and flowers also appeared earliest at this temperature, as can be seen from figures 2 and 3. At the lowest light intensity (figure 2), the difference in flowering due to temperature treatment was greater than it was at the higher intensity (figure 3). As already found by CAJLACHJAN (1945) and LANG and MELCHERS (1943) the influence of environmental factors other than daylength is greatest when the photoperiod is close to its critical value.

In the first experiment, plants in a total daylength of 14 hours had no visible flower buds after 50 days of treatment. The growing points, therefore, were examined at 40-fold magnification. It appeared that at 9 °, 13 ° and 20 ° all plants had flower-initials, while at the other temperatures, only 3 out of 5 plants were reproductive. In the second experiment the light intensity of the supplementary irradiation was increased because it seemed feasible that differences in flowering would then show up in a sufficiently definite way to be distinguished macroscopically (see p. 52). In this experiment, flower buds were first visible in the plants exposed to 13 °, a little later in the plants at 20 ° and 27 °C, and much later in those plants that had been at 2 °, 5 °, and 9 ° during the period of supplementary irradiation and during the subsequent dark period. These experiments suggest that the optimum temperature for the formation of flower buds at a daylength of 14 hours is a little lower than at a daylength of 24 hours (figure 4).

TABLE 5

Leaf number in spinach after 14 days of treatments. Basic irradiation for 10 hours, at 20 °C. Supplementary irradiation for 0, 4 or 14 hours, respectively, at intensities of 170 erg/cm²/sec in experiment I and 420 erg/cm²/sec in experiment II. Temperatures as indicated in the Table were applied during supplementary irradiation, and during darkness

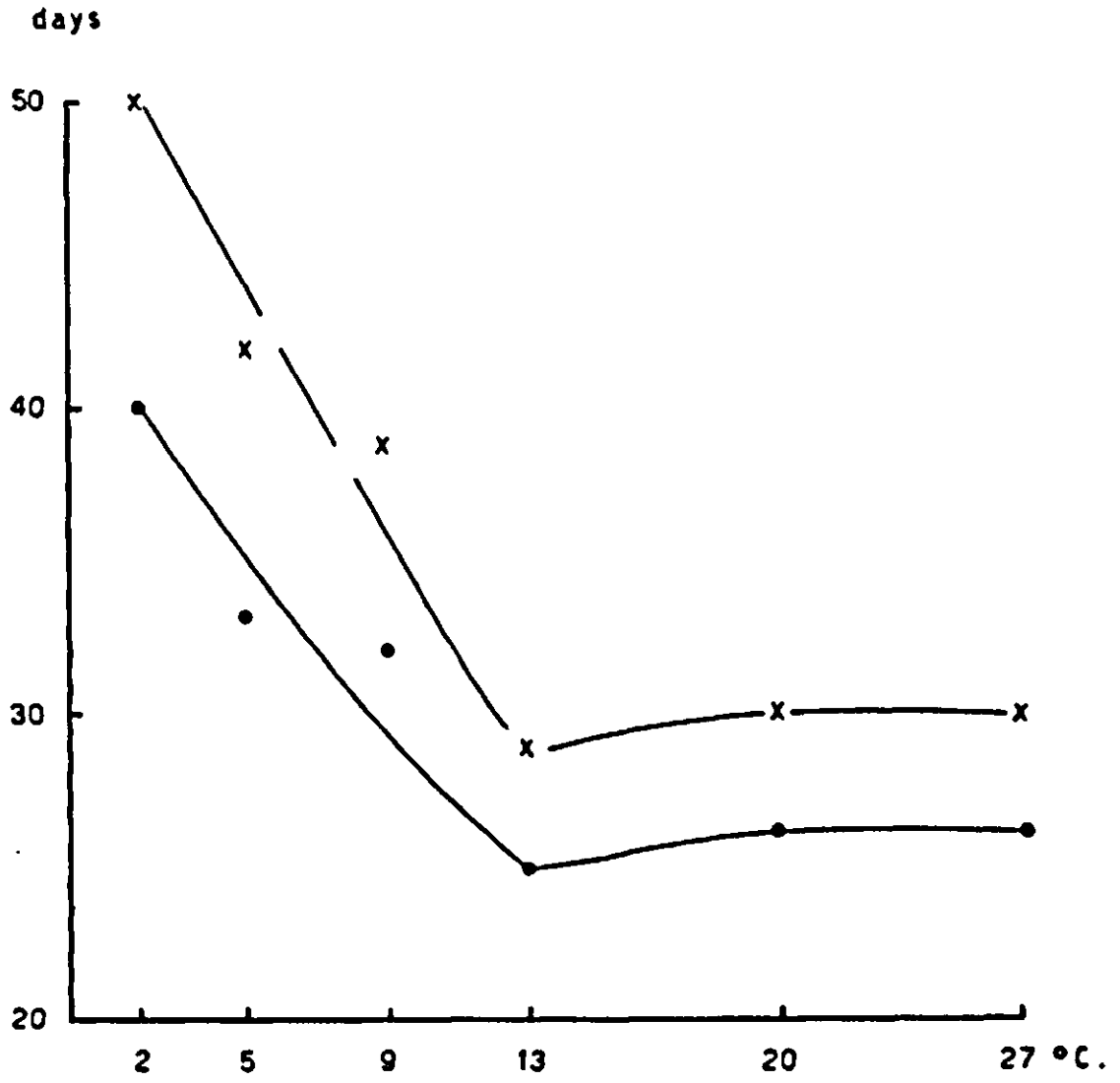
	experiment I			experiment II		
total daylength / temperature	10h.	14h.	24h.	10h.	14h.	24h.
2°	7.6	8.6	8.2	5.8	6.0	6.0
5°	8.8	8.6	8.4	6.0	5.8	7.0
9°	9.2	9.8	9.6	6.0	6.5	7.2
13°	9.8	10.0	9.6	6.5	6.0	8.3
20°	9.6	10.6	12.4	6.5	6.8	8.5
27°	10.8	11.2	12.8	8.8	8.2	9.0

After 14 days of treatment the leaf number was determined. The data are given in Table 5. In all cases the number of leaves increased with increasing temperature. There was only a slight influence of daylength on the number of leaves.

The leaf areas were measured in plants at daylengths of 10 and 14 hours (figures 5 and 6). The lower 2 leaves had developed in the greenhouse and, therefore, were almost of the same size in all plants; the third leaf was the first formed during the experiment. Thus, the differences in leaf area due to differences in treatment are greater than those actually shown in the figures. In the second experiment, the oldest 3 leaves at a daylength of 14 hours were not measured, because some of them had died. The leaf area was greater at a daylength of 14 hours than at one of 10 hours, and in both experiments the greatest leaf area was found at a temperature of 13 °, independent of daylength. Since leaf

FIG. 4.

Number of days (ordinate) up to stems elongated (•——•) and flower buds visible (x—x) in spinach. Temperature during the 10hours basic irradiation 20 °C.
Various temperatures during the rest of the 24 hour cycle (abscissa). For the first 4 hours after basic illumination, the plants were illuminated at an intensity of 420 erg/cm²/sec.

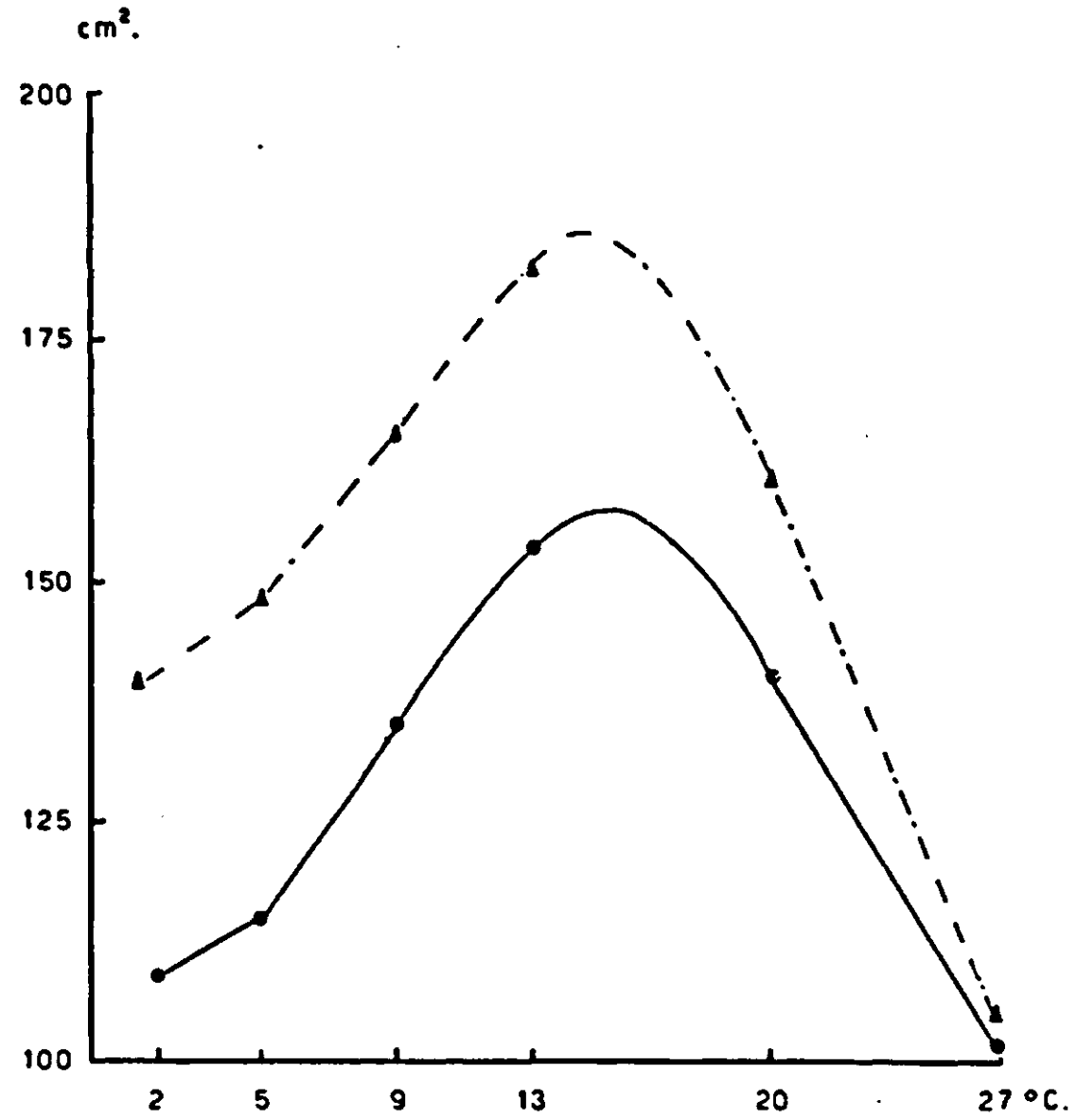


area in 13 ° and 20 ° were never found to differ very much, it follows that the optimum temperature for leaf development is somewhere between 13 ° and 20 °.

Plants receiving a high temperature had a yellowish colour when compared to those at lower temperatures. Chlorophyll content was, therefore, measured, by extracting 5 cm² of leaves of the same physiological age with 10 cc. of 80% ethanol. The percentage transmission of light at a wavelength of 6500 Å was measured. The chorophyll content was indeed lower at higher temperatures. At a daylength of 14 hours less chlorophyll was found than at a daylength of 10 hours (Table 6).

FIG. 5.

Leaf area per plant (ordinate) in spinach after 37 days of treatments. Basic irradiation for 10 hours at 20 °C. Supplementary irradiation for 0 (•——•), or 4 hours (▲ -·-·- ▲) respectively, at an intensity of 170 erg/cm²/sec. Temperatures (abscissa) applied during supplementary irradiation and during darkness.



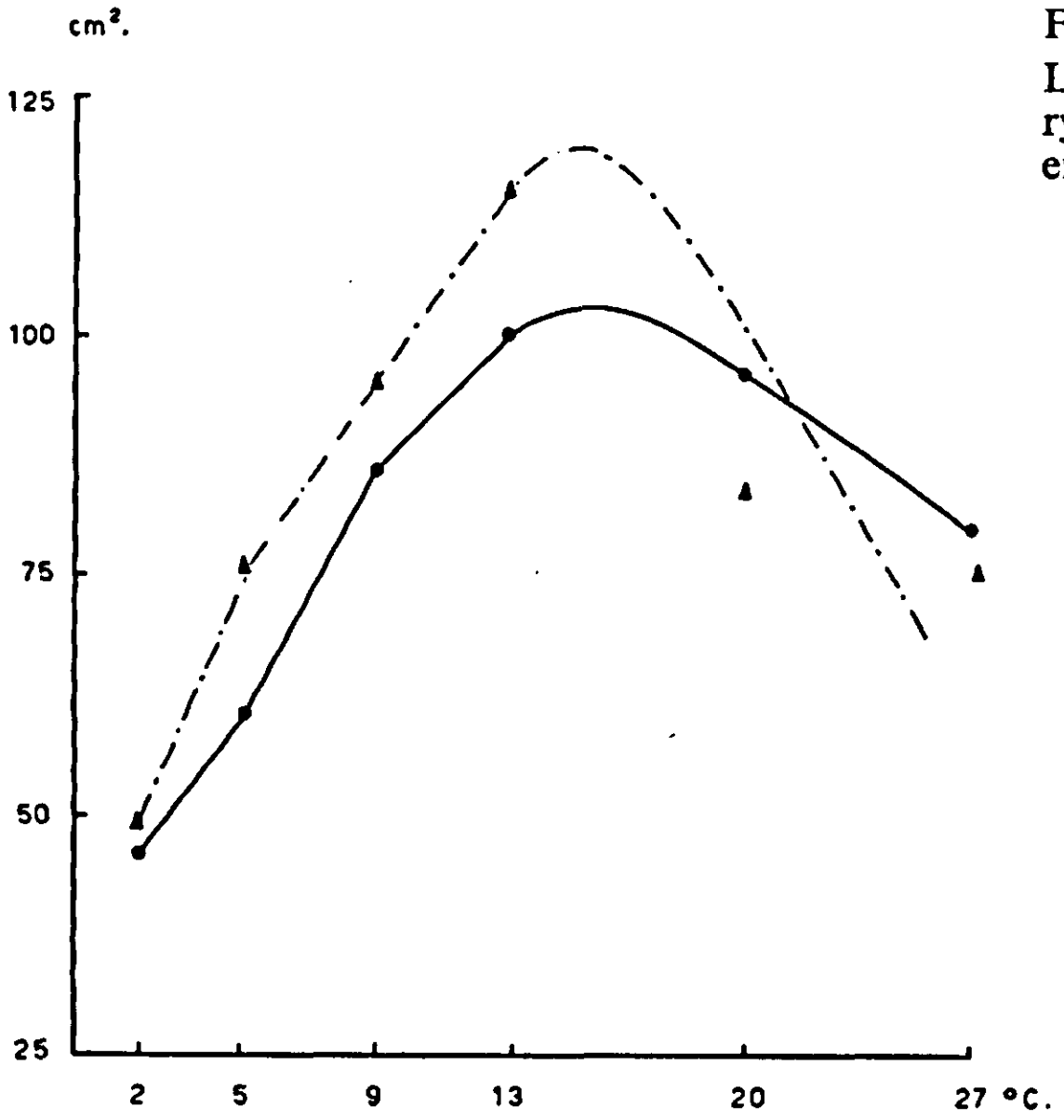


FIG. 6.
Legend as for figure 5. Supplementary irradiation at an intensity of 420 erg/cm²/sec.

TABLE 6

Transmission values of comparable chlorophyll extracts in spinach after 47 days of treatment. Same plants as in Table 5. Experiment II

daylength	2°	5°	9°	13°	20°	27°
10 hours	34.5	39.5	–	43.0	45.5	50.5
14 hours	48.0	38.8	50.5	51.5	49.5	59.0

From the experiments discussed in this section it can be concluded that at a daylength of 24 hours, 20 ° is most effective in promoting flowering, while at a daylength of 14 hours, 13 °C is most effective. Leaf area seemed to be greatest somewhere between 13 ° and 20 °C, while the largest number of leaves was produced at 27 °. Chlorophyll content decreased with increasing temperature.

5. THE INFLUENCE OF NIGHT TEMPERATURE ON FLOWERING AND VARIOUS FORMATIVE PROCESSES IN *HYOSCYAMUS* AT DAYLENGTHS OF 10, 14 AND 24 HOURS

The plants were sown in the greenhouse and brought indoors in August. They had formed 16–17 leaves when the experiment started. The plants were treated in a way similar to that in the spinach experiment. They were illuminated for 10 hours per day at an intensity of 20000 erg/cm²/sec and 23° C. For the remainder of the 24 hour cycle they were exposed to the various temperatures, *viz.*, 2°, 13°, 20° and 27°C. One lot of plants remained in darkness during the 14 hour period, another lot was illuminated for only 4 hours at an intensity of 525 erg/cm²/sec, while a third lot received 525 erg/cm²/sec for 14 hours. Five plants were used for each treatment. The lot with continuous irradiation started with 12 plants per treatment, of which at least 5 were kept for 84 days, the others were dissected at various times during the experiment, in order to examine the growing points.

At a total daylength of 14 hours only some plants formed flower initials. After 30 days treatment one plant at 27 ° and one at 20 ° were dissected and examined under 40-fold magnification. The growing points were not strictly

vegetative; they rose slightly and broadened somewhat so that they were comparable to the stage *c* of LANG and MELCHERS (1943). The leaf numbers were 40 and 44 respectively. After 50 days, one plant at 20 ° and one at 27 °C were examined. Both plants were at stage *c*, and had 53 leaves. After 84 days of treatment the experiment was terminated and the 3 remaining plants at 20 ° and 27 ° were dissected. At 27 ° all plants were at stage *c*, while the average number of leaves formed was 55.7. At 20 ° one of the plants had flower initials. The other plants were at stage *c*. The mean leaf number was 44.0. With regard to the lower temperatures, neither at 13 ° nor at 2 ° had plants initiated flowers. Under the conditions of the experiment, a daylength of 14 hours is near the critical value. It could not be decided whether plants would have flowered earlier at 27 ° or at 20 °. If the plants would flower at all at 13 °, they would have been later than at the higher temperatures.

We will now discuss the plants that received continuous irradiation (i.e. 10 hours high light intensity followed by 14 hours low light intensity at various temperatures). It was found that at 2 ° one plant had started to initiate flowers after 84 days of treatment. At the higher temperatures the plants were dissected after different times. At 13 °, two plants were dissected after 30 and 40 days respectively. Both plants had reached stage *c*. After 50 days two plants each had two flower initials, while a third initial in the course of was development. After 60 days one plant was examined and found to have 4 flower initials. After 84 days the remaining plants were dissected. Two of them were at stage *c*, two other plants had a small ring at the base of the growing point, on which later stamens developed; they had reached stage *e*; the last plant had flower initials. The plants at stage *e* had stems of 0,75 cm. The mean leaf number after 84 days of treatment was 58.8. At 20 ° plants were dissected after 15 and 18 days of treatment. Both growing points rose a little, and were at stage *b*. After 22 days, 2 plants were at stage *c*. After 29 days two plants were dissected, one of the plants had 3 flower initials, with a stem of 1.5 cm, the other plant was at stage *c*, with a stem of 0.5 cm. After 36 days, one plant was examined and found to have 3 flower initials and a stem of 0.5 cm. It is very difficult, in *Hyoscyamus*, to see exactly whether the plant has started to elongate or not. So the date at which elongation was observed to have started is not an exact measure of the stage of development, it is only an indication of flower initiation. With this in mind the start of stem elongation was used as a measure for flower initiation. The average number of days before the plants started to elongate was 24.7 at 20 °. At 27 °, two plants were dissected after 12 days treatments. One plant had reached stage *c*, another had 3 flower initials. The stem of the latter plant was 0.5 cm. After 15 days, out of two plants that were dissected one also had 3 flower initials, but a stem of 1,5 cm. Another plant was still at stage *c*. After 18 days one plant had a stem of 0.5 cm and was at stage *c*. Another plant had 5 flower initials and a stem of 1 cm. The average time after which the plants started to elongate was 14.3 days.

Another measure of the influence of the external conditions on flower initiation is the number of leaves formed during the experiment before the appearance of the first flower bud. As can be seen from figure 7, this number increased with decreasing temperature. The leaf number given for 13 °, is probably somewhat too low, because the count was made when the plants had just started to initiate flowers, and it was difficult to decide whether some of the primordia would give leaves or belonged to the developing flowers.

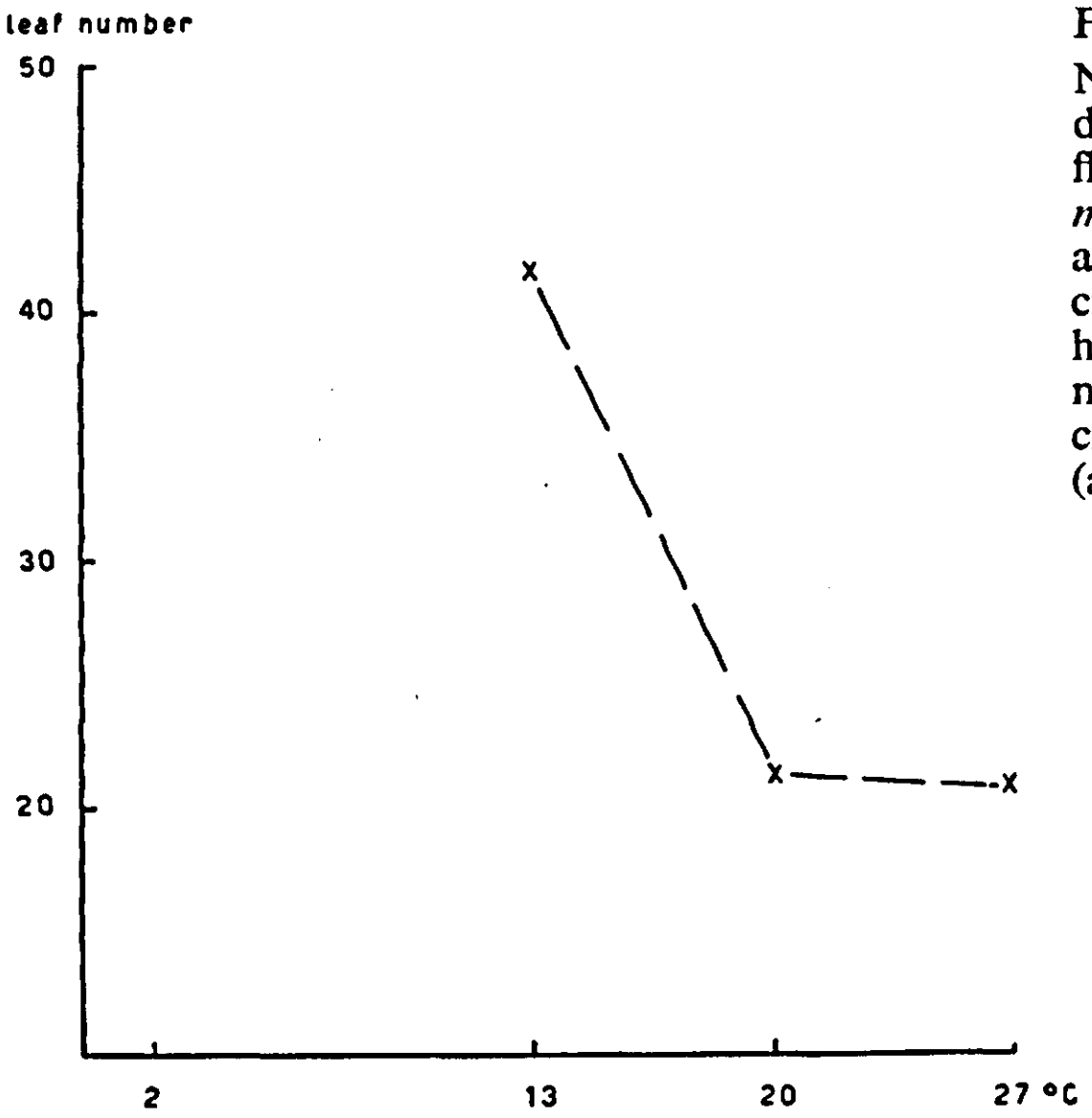


FIG. 7.
Number of leaves (ordinate) formed during the experiment up to first flower buds appeared in *Hyoscyamus*. Basic illumination for 10 hours a day at an intensity of 20000 erg/cm²/sec. During the rest of the 24 hour cycle the plants were illuminated at an intensity of 525 erg/cm²/sec. at various temperatures (abscissa).

In the experiments of LANG and MELCHERS (1943) with *Hyoscyamus*, the critical daylength decreased with decreasing temperature; it lay between 10 and 12 hours. The flowering response increased with temperature when daylengths longer than 14 hours were applied. These authors used incandescent lamps in their experiments, which may result in a shorter critical daylength than when fluorescent tubes are used. This indeed was found by PARKER and BORTHWICK (1953). In our experiments, a daylength of 14 hours appeared to be near the critical value, but no indication was obtained that the plants at 13 ° would initiate flowers earlier than at 20 ° or 27 °C. At the daylength of 24 hours the flowering response increased with increasing temperature. Another difference between the experiments of LANG and MELCHERS and ours is the way in which the day was lengthened; they used high light intensities, while in our experiments days of 10 hours were extended with low intensities.

TABLE 7

Leaf number in Hyoscyamus *following various treatments. Basic illumination 10 hours, at 23 °C. Supplementary illumination for 4 or 14 hours, respectively, at an intensity of 525 erg/cm²/sec. Temperatures as indicated in the Table were applied during the supplementary illumination and during darkness*

duration of treatment	50 days			85 days		
total daylength / temperature	10h.	14h.	24h.	10h.	14h.	24h.
2°	24.0	24.6	25.1	33.8	33.8	35.4
13°	28.4	29.4	30.0	40.6	41.0	40.0
20°	34.6	36.5	36.0	47.3	44.0	47.3
27°	37.0	41.5	54.0	51.4	55.7	–

The total leaf number at the end of the experiment increased with increasing temperature (Table 7). There was almost no influence of daylength. When 40 leaves had been formed at 27 ° and continuous irradiation, flowers were formed while the position of the leaves changed. Before flower initiation it was $\frac{3}{8}$; whereas leaves developing after flower initiation were arranged in two opposite rows. The leaf area of the plants was measured at the end of the experiment. The margin of the leaves is crenate so, the width of the leaves was taken as the average of the inner and outer values. Length and breadth were multiplied. It appeared that 0.7 times the resultant value represented the leaf area. At the end of the experiment most of the plants at 24 hours and 27 ° had lost their vegetative leaves, so that the leaf areas of these plants were not measured. It can be seen from Table 8 that leaf area increased with temperature.

TABLE 8

Leaf area (cm^2) *in* Hyoscyamus. *Same experiment as in Table 7*

total daylength	2°	13°	20°	27°
10 hours	84	131	218	245
14 hours	75	172	208	281
24 hours	63	182	206	–

In this experiment, *Hyoscyamus* reacted slowly. Even after 84 days treatment only one lot flowered (24 hours, 27 °). It may be that the external conditions were not favourable for flowering and stem elongation.

6. THE INFLUENCE OF NIGHT TEMPERATURE ON FLOWERING AND VARIOUS FORMATIVE PROCESSES IN *BRASSICA* AT DAYLENGTHS OF 10, 14 AND 24 HOURS

The plants were treated in the usual way. Basic irradiation was given for 10 hours at an intensity of 22000 erg/cm^2/sec and 20°. Supplementary irradiation was given for 0, 4 and 14 hours at an intensity of 525 erg/cm^2/sec, at 2°, 9°, 13°, 20° and 27 °C. During darkness, the same temperatures were used as during the supplementary irradiation. For each treatment 4 plants were used in the first experiment, and 6 plants in the second one.

In the variety of *Brassica* used, there has been little selection; it is a cross pollinator, so that the individual differences are large and plants with a more of less day-neutral character are often found. All experiments started with seed from plants that, in the greenhouse, had shown to be long-day plants. Selection for long-day forms was continued, so that greater sensivity to long day resulted.

TABLE 9

Number of days before flower buds were visible in Brassica *following various treatments. Basic irradiation 10 hours, at 20°C. Supplementary irradiation for 4 or 14 hours, respectively, at an intensity of 525 erg/ cm^2/sec. Temperatures as indicated in the Table were applied during the supplementary irradiation and during darkness. I first experiment, II second experiment*

	total daylength	2°	9°	13°	20°	27°
I	10 hours . .	44.0	33.5	32.5	28.3	24.5
	14 hours . .	41.0	31.5	26.8	29.8	19.5
	24 hours . .	35.0	31.5	26.0	17.5	17.3
II	10 hours . .	39.8	–	38.7	43.5	35.6
	24 hours	31.0	–	23.3	17.7	13.5

Nevertheless plants in different experiments showed somewhat different sensitivity to daylength. For each experiment seeds of one special plant was used. The plants used in the first experiment showed visible flower buds earlier than those used in the second experiment, as can be seen from Table 9. In the first experiment, the influence of temperature on the formation of flower buds was greater than in the second one. Generally, at higher temperatures the plants formed flower buds in a shorter time.

FIG. 8.
Leaf area (ordinate) in *Brassica* after 45 days of treatment. Basic irradiation for 10 hours at 20 °C. supplementary irradiation for 0 (●——●), 4 (▲-·-·-▲) or 14 hours (■---■) respectively at an intensity of 525 erg/cm²/sec. Temperature (abscissa) applied during supplementary irradiation and during darkness.

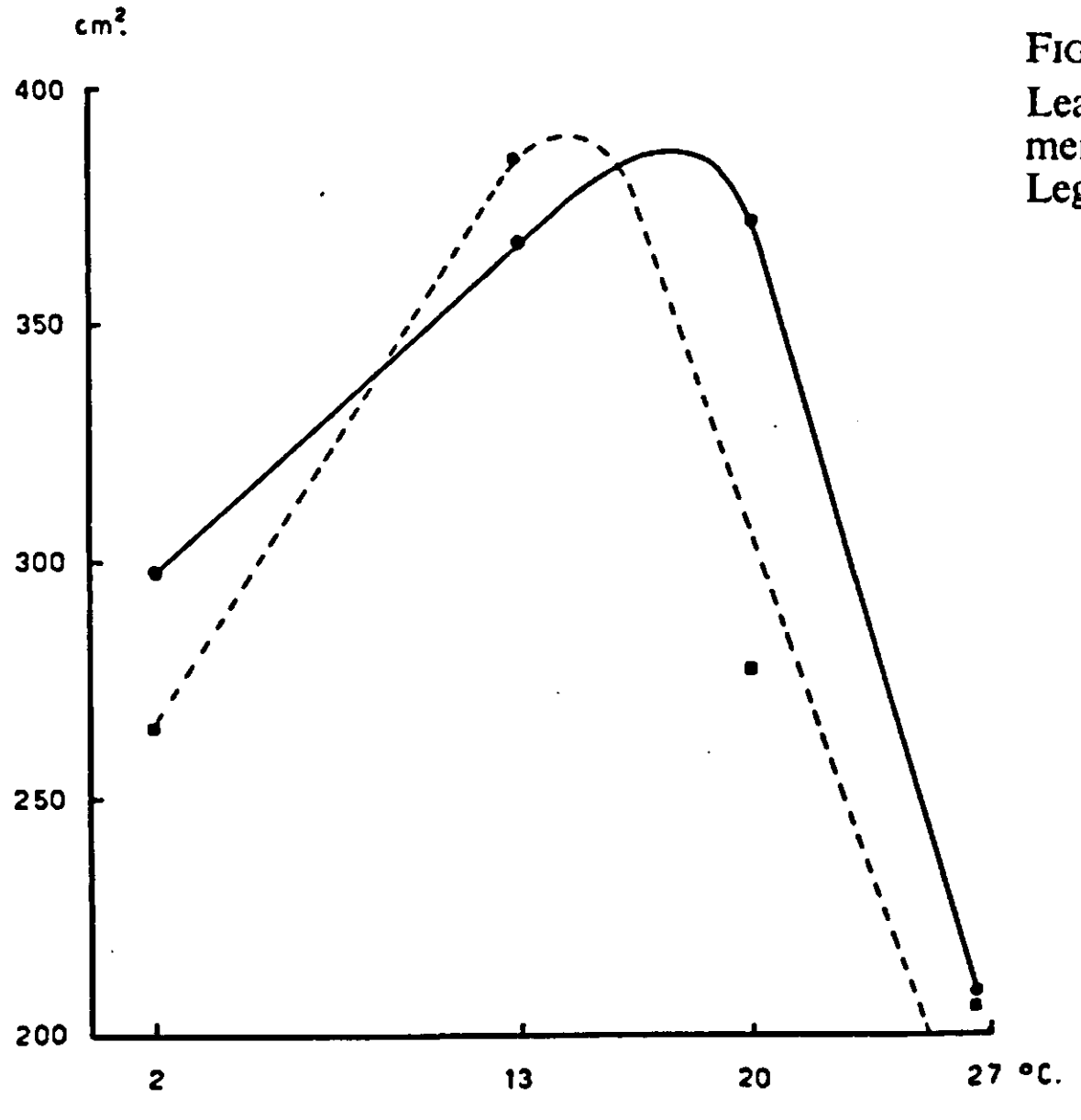


FIG. 9.
Leaf area in *Brassica*. Same treatmen as in Fig. 8 second experiment. Legend as in Fig. 8.

Leaf areas are given in figures 8 and 9. The greatest leaf was somewhere between 13 ° and 20 °C in both experiments. The optimum temperature for leaf growth decreased somewhat with increasing daylength.

Stem lengths of the plants after 45 days of treatment are given in Table 10. It can be seen that, generally, stem length increased with increasing temperature.

TABLE 10.

Stemlength (cm) in Brassica. *Same experiment as for Table 9. I first experiment measurement after 38 days treatment, II second experiment Measurement after 45 days treatment*

	total daylength	2°	9°	13°	20°	27°
I	10 hours . .	0	1.8	4.1	3.5	13.5
	14 hours . .	0	1.9	4.2	3.0	17.0
	24 hours . .	0.3	1.8	3.8	32.0	21.9
II	10 hours . .	1.3	–	1.2	1.3	3.4
	24 hours . .	1.3	–	7.1	13.0	20.1

7. CONCLUSIONS

We have seen, in section 4, that the optimum temperature for flowering in spinach changed with daylength. It was 13° at a total daylength of 14 hours, and 20° at a total daylength of 24 hours (various temperatures being applied during the periods of supplementary irradiation and darkness). Under both daylengths, the plants at 27 ° flowered at the same time or somewhat later than those at 13 ° and those at 20 °. The differences between the various treatments increased when the intensity of the supplementary irradiation decreased, but the time during which the treatment had to be given increased also.

In *Hyoscyamus*, flowering was promoted by high temperature. It appeared, however, that a daylength of 14 hours was too short for flowering under the experimental conditions. But, at the end of the experiment, after 84 days of treatment, some plants at 20 ° and 27 ° had started to initiate flowers; it seems probably that if the treatment had been prolonged, flower initials would have been visible in all plants at 27 °.

Brassica flowered most rapidly at 27 ° at all daylengths.

All three long-day plants behaved differently with respect to temperature. *Hyoscyamus* and *Brassica* ressembled each other in that the flowering response increased with temperature. In spinach, however, the flowering response was promoted by intermediate temperatures.

In *Hyoscyamus* the greatest leaf area was found at 27 °, while in spinach and *Brassica* it lay between 13 ° and 20 °. The leaf number on vegetative plants increased with temperature in both spinach and *Hyoscyamus*. In *Brassica* this number could not be determined since the plants flowered under all daylengths applied.

It seems appropriate to conclude that, since temperature has different effects on leaf number, flowering, and increase in leaf area, it is improbable that these properties are governed by one and the same metabolic process.

The optimum temperature for flowering of spinach was found to be 13 °–20 °; this indicates that at least two processes interact. It is possible that these processes run simultanously and have different temperature coefficients. LANG and MELCHERS (1943) distinguished a promoting process and an inhibition, and

suggested that the latter is inactive at low temperatures. They considered these processes to be metabolic processes, but CLAES (1947) could find no quantitative relation between the influence of low light intensities upon flowering and its influence upon metabolism. It is conceivable that these processes are consecutive and that the temperature requirement varies at different times during development.

In order to obtain further information on this point, experiments were set up in which different temperatures were given at the beginning and at the end of an experiment; these experiments are described in chapter III. Another approach involved irradiation of the plants in the middle of the night at various temperatures. This procedure was followed in experiments to be described in chapter IV; it has the advantage that processes other than flowering will interfere less with the flowering response to temperature.

CHAPTER III

THE INFLUENCE OF INITIAL TREATMENT WITH COLD NIGHTS ON FLOWERING AND VARIOUS FORMATIVE PROCESSES IN SPINACH, *HYOSCYAMUS* AND *BRASSICA*

1. INTRODUCTION

In many long-day plants a cold treatment during or after germination will accelerate flowering (GASSNER 1918). This phenomenon is now called vernalisation. There are indications that photoperiodism and vernalisation are closely related and may even interact. For instance, KLEBS (1918) could not induce *Sempervivum funkii* to flower in a warm greenhouse in winter, thus indicating that this plant needs vernalising before it can flower. When, however, the plants were illuminated continuously in the greenhouse in winter, they flowered. The later the plants were taken indoors, the fewer days of continuous illumination were needed. To a certain degree, therefore, the vernalisation requirement can be replaced by long-day treatment. In winter rye, on the other hand, cold treatment can be replaced by short day treatment (PURVIS [1934]).

HARDER and STÖRMER (1936) vernalised mustard in 10–30 days, and subsequently cultivated the plants under short or long days respectively. In long days, the cold treatment did not speed up flowering, while in short days, it did. According to VON DENFFER (1939) winter barley shows the same characteristic though to a lesser degree. Cold has to be given for more than 20 days in order to bring about a promoting effect upon flowering in short days. PARKER, BORTHWICK and HEINZE (1941) treated plants of the same species for 7 days with low temperatures, but found no influence on flowering. This treatment was of insufficient duration. WELLENSIEK and VERKERK (1950) found that beet when grown under continuous light required less cold than under alternating light and darkness.

LANG and MELCHERS (1943) based their theories of the influence of dissimilation on flowering upon experiments with *Hyoscyamus niger*. Two forms of this plant are known, a biennial form that requires cold treatment, and an annual form which flowers without cold. In the latter, however, flowering can be accelerated by cold treatment (LANG [1941]). All these results taken together,

indicate, that a process comparable to vernalisation accelerates flowering in long-day plants at short-day and low temperature. Under long days, the influence of this process is masked by that of photoperiod, so that high temperature accelerates flowering.

A promoting effect of cold on flowering of spinach was first found by KNOTT (1939). In the experiments, reported in the preceding chapter, spinach showed a different dependence on temperature of flowering at different daylengths. This was also the case with *Hyoscyamus*.

The question now arises as to whether, in these plants, a few cold nights, as well as some cold days in the beginning of the experiment, would modify the effect of daylength. Experiments on this point were carried out with the three long-day species used previously.

2. EXPERIMENTS WITH SPINACH

Cold treatment was applied to plants receiving photoperiods of either 10 or 14 hours. The 10 hour illumination was given by "daylight" fluorescent tubes at high intensity (22000 erg/cm²/sec) at 23°. The 14 hour photoperiod comprised this same basic illumination, supplemented by 4 hours of "daylight" fluorescent tubes at 525 erg/cm²/sec. The cold treatment was started immediately after the basic 10 hour illumination, and continued for the whole night. A comparison of 0 and 5 cold nights at 2° was made. After the cold treatment, the plants received 2°, 13°, 20° and 27° respectively, during darkness and during the 4 hour period of weak irradiation. The experiment was performed twice, each time with 4 plants per treatment.

Throughout this chapter, plants which received no cold treatment (0 nights) will be referred to as "control plants".

When no cold was given, there was little difference in time of elongation between temperatures of 13 °, 20 ° and 27 ° as can be seen from Table 11.

TABLE 11

Number of elongated plants in spinach following various treatments. Basic irradiation given for 10 hours at 23 °C. During the following 14 hours, different temperature treatments were given, consisting of different numbers of nights at 2°, followed by nights at various temperatures. Supplementary irradiation was given during the nights at an intensity of 525 erg/cm²/sec. I = first experiment, II = second experiment. Measured after 27 and 18 days respectively

	I		II	
number of cold nights / night temperature	0	5	0	5
2°	0	0	2	2
13°	4	2	5	3
20°	4	3	4	4
27°	0	0	5	1

At 13 °, when 5 cold nights were given, stem elongation was retarded, at 20 ° flower initiation was somewhat earlier than at 13 ° but almost no difference was found when compared with plants not receiving cold nights. The influence of temperature after 5 cold nights was of the same nature as that found in plants under a daylength of 24 hours (see chapter II section 4). The length of time before flower buds were visible is given in Table 12, where it can be seen that the retardation of flower bud development was proportional to the cold treatment given. Bud development in plants illuminated continuously at 2 ° required 40 days. We can assume, therefore that during 5 cold nights at 2 °, $^5/_{40}$ or $^1/_8$ of

the total formation of flower buds was completed, and that $^7/_8$ remained to be formed at the other temperatures. At 20 °, bud development took 30.7 days; $^7/_8$ of this is 26.8 days; so the total number of days before flower buds appeared at 20 ° should, if the above assumption is correct, be 31.8 days; and this is exactly the number found experimentally. One cannot, of course, attach much value to an estimation based on so few figures, but, as far as it goes, it indicates that any effect of cold treatment on the formation of flower buds does not extends beyond the actual period of treatment.

TABLE 12

Number of days before flower buds became visible. Same experiment as in Table 11, second experiment

number of cold nights / night temperature	0	5
2°	40.5	40.5
13°	30.0	31.3
20°	30.7	31.8
27°	30.0	34.0

The total leaf number was hardly influenced by the cold treatment, as can be seen from Table 13. On this occasion fully unfolded leaves only were counted and this explains why, in some cases, the greatest number of leaves was not, as in previous experiments, always found at the highest temperature.

TABLE 13

Leaf number in spinach. Same experiment as in Table 11. Supplementary irradiation for 0 or 4 hours. Measured after 25 days of treatment in the first experiment and after 17 days in the second

number of cold nights	daylength / night temperature	10	14	10	14
0	2°	10.5	10.3	10.4	11.0
	13°	11.8	14.3	11.8	14.2
	20°	14.5	15.8	11.7	15.2
	27°	13.0	14.8	11.4	14.8
5	2°	10.5	10.3	10.4	11.0
	13°	11.5	15.3	11.7	15.2
	20°	11.3	16.0	11.5	14.2
	27°	15.0	15.0	11.4	14.2

The dependence of leaf area on night temperature was similar to that found previously. The data indicate that, under the conditions of our experiments, the optimum temperature for leaf increase is between 13 ° and 20 ° (Table 14 and 15). Dry weights were taken some days after the leaf area measurements. The plants at 13 ° had the greatest dry weight, except in the first experiment where they were greatest at 20 °. In this experiment relatively high leaf area were also found at 20 ° and this fact may have been responsible for the high dry weight values. It was impossible, with the limited number of plants available, to correlate leaf area and dry weight with any certainty. Nevertheless, both experiments indicate that at the higher temperatures, the dry weight per unit leaf area is less than at the lower ones.

TABLE 14

Leaf area (cm²) and dry weight (mg) in spinach, measured after 34 and 54 days respectively. Experiment as in Table 13 I

number of cold nights	daylength / night temperature	leaf area		dry weight	
		10	14	10	14
0	2°	80	93	750	728
	13°	127	157	850	903
	20°	260	203	1273	1173
	27°	94	106	458	540
5	2°	80	93	750	728
	13°	111	152	910	1050
	20°	149	127	720	997
	27°	120	117	650	420

TABLE 15

Leaf area (cm²) and dry weight (mg) in spinach, measured after 34 and 40 days respectively. Same experiment as in Table 13 II

number of cold nights	daylength / night temperature	leaf area		dry weight	
		10	14	10	14
0	2°	100	137	552	628
	13°	124	238	616	760
	20°	148	169	480	587
	27°	133	114	391	374
5	2°	100	137	552	628
	13°	158	216	658	766
	20°	171	202	608	643
	27°	130	145	478	490

Stemlength was virtually uninfluenced by the cold nights, as can be seen from Table 16.

TABLE 16

Stemlength (mm) in spinach. Same experiment as in Table 11. Measurement after 54 days in the first experiment and 17 days in the second one

	I		II	
number of cold nights / night temperature	0	5	0	5
2°	13	13	2	2
13°	68	29	45	42
20°	148	172	42	30
27°	66	80	41	37

From these experiments, it can be concluded that the cold treatment had hardly any influence on flower bud formation, leaf area, dry weight or stem-length. Initiation of flowers was slightly influenced by the cold nights and appeared to be more advanced at 20 ° when compared with 13 °. At 20 °, the retardation in flower initiation was proportional to the cold given.

3. EXPERIMENTS WITH *HYOSCYAMUS*

All plants received 10 hours basic illumination per day from "daylight" fluorescent tubes at an intensity of 22000 erg/cm²/sec, and at a temperature of 20°. The several treatments employed were as follows: half the plants received only the 10 hour mentioned above, while the other half was supplied with additional illumination of 525 erg/cm²/sec for 14 hours per daily cycle. Various cold treatments were introduced at the beginning of the experiments, *viz.*, 0, 15, or 30 cold nights at 2 °C. At the end of this cold treatment the plants were again divided into 4 series and subjected to a night temperature of either 2°, 13°, 20° or 27 °C. In order to test any possible devernalising effect of the sudden change from 2° to 27°, one series of 4 plants was transferred gradually, the change extending over a period of 4 nights. They were first transferred from 2° to 9° for 2 nights and then were given one night at 13° and one night at 20° before the final transfer to 27 °C.

When the experiment started, the plants were 59 days old. Since they had been raised during the dark time of the year and with rather cold nights, they grew slowly; the mean number of leaves was only 8.5 when the experiment started. (In the previous experiment with *Hyoscyamus* treatment had started when the plants had 16.5 leaves). Figure 10 shows the number of leaves formed before initiation of the first flower bud. This number increased with decreasing night temperature, thus confirming the previous experiment. When a cold treatment was given at the beginning of the experiment, however, the number of leaves formed before the first flower bud increased. The increase was relatively less in plants which had 30 cold nights than in those which received only 15. The series which was transferred from 2 ° to 27 ° gradually formed few more leaves than the plants that were changed over in one night. There was thus, no marked influence of the duration of the change-over from low to high temperature, on leaf number. Further, when the cold was given continuously for 15 days, the number of leaves was almost the same as when no cold was given.

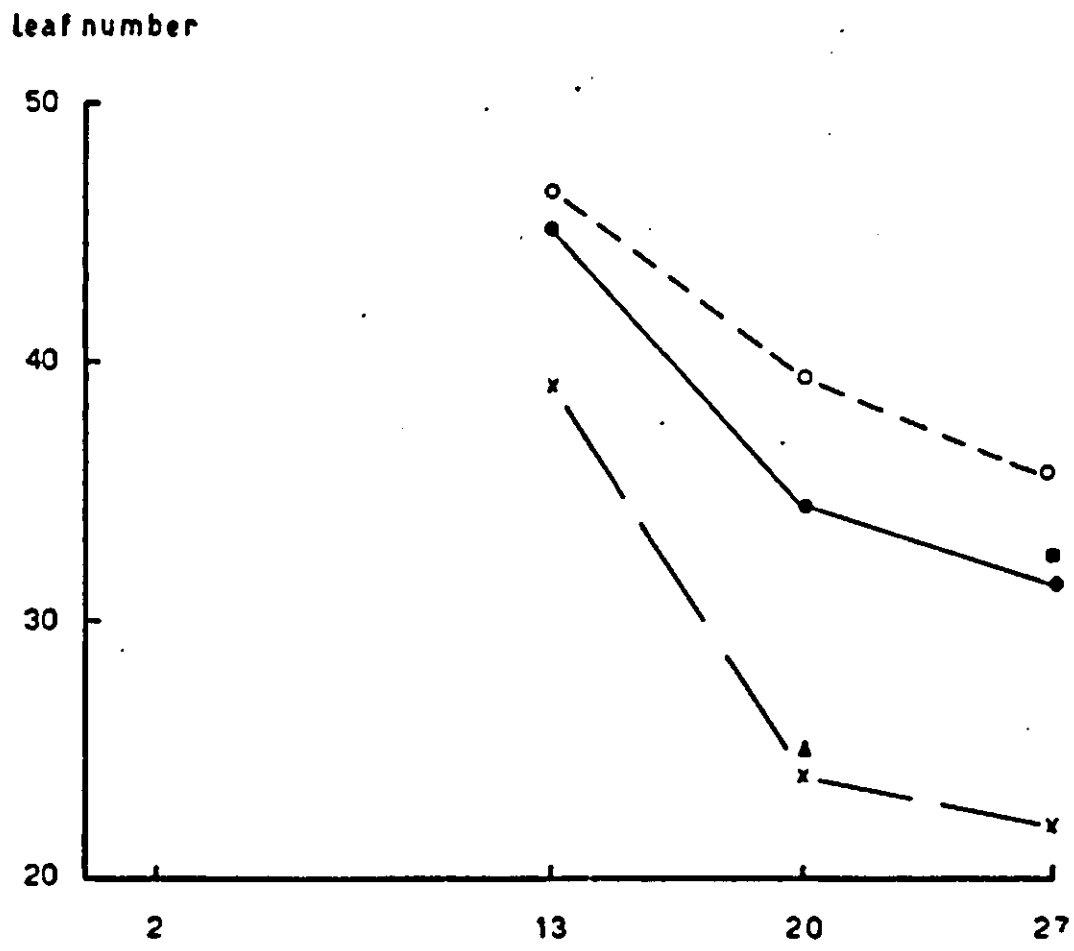


Fig. 10.
Leaf number (ordinate) up to formation of 1st flower in *Hyoscyamus*. Basic irradiation given for 10 hours at 20 °C. During the following 14 hours, different temperature treatments were given, consisting of 0 (x—x), 15 (•—•) or 30 (o—o) nights at 2° followed by nights at the temperatures indicated on the abscissa. ■ : Change-over from 2°–27° extended over 4 nights. ▲ : 15 continuous days at 2° at the beginning of the experiments. Supplementary irradiation was given for 0 or 14 hours at an intensity of 525 erg/cm²/sec.

The number of days before elongation is given in Table 17. When no cold was given, plants elongated at 20 ° and 27 ° after 30 and 26 days respectively. When cold was given for 15 or 30 nights, plants started elongation relatively later. However, at 13 ° the plants receiving 15 and 30 cold nights started to elongate respectively 10 and 14 days later than the plants receiving no cold.

When cold was given for 15 days and nights, elongation started a few days later than when no cold was given.

TABLE 17

Number of days before commencement of elongation (1) and flowering (2) in Hyoscyamus. *Basic irradiation given for 10 hours at 20 °C. During the following 14 hours, different temperature treatments were given, consisting of 0, 15 or 30 nights at 2° followed by nights at the temperatures indicated. In one lot the change-over from 2°–27° was extended over 4 nights, in another lot 15 continuous days at 2° at the beginning of the experiment were given. Supplementary irradiation was given for 0 or 14 hours at an intensity of 525 erg/cm²/sec.*

treatment \ night temperature	13°	20°	27°
1. 0 cold nights	82.0	25.0	22.0
15 cold nights	91.5	55.0	33.0
15 cold nights, change-over in 4 nights			35.0
30 cold nights	92.8	64.0	56.0
15 days continuous cold		44.0	
2. 0 cold nights	–	–	52.0
15 cold nights	–	–	56.0
15 cold nights, change-over in 4 nights			59.0
30 cold nights	–	--	67.0
15 days continuous cold		–	

Some of the plants at 20 ° flowered, but insufficient did so to enable the determination of a reliable average value for the time before flowering. At 27 ° all plants flowered. When the plants had had cold nights the average number of days before flowering increased; but the increase was not proportional to the number of cold nights. Thus after 15 cold nights the plants flowered 4 days later than the control plants, while after 30 cold nights they flowered 15 days later than the control plants. An influence of cold treatment was found after the start of elongation. The numbers of days between the beginning of elongation and of flowering at 27 ° for 0, 15 and 30 cold nights were 30, 23 and 11 respectively (Table 17).

The growth of the plants was followed by measuring the number of leaves every 4 or 5 days. The results are shown in fig. 11–13. The behaviour of plants at a night temperature of 27 ° was remarkable. We can assume that the increase in leaf number of the plants that received no cold was linear during the course of the experiment. Fig. 11 shows that the increase in leaf number during the first 24 days following 30 cold nights, was the same as when no cold nights had been given; since, however, during the cold treatment, the rate of increase in leaf number was depressed, the total number of leaves formed was smaller. There is, moreover, a change in rate of leaf number increase after these first 24 days following cold treatment. When no cold was given, namely, the first flower was found in the axil of the 31st leaf, formed after 40 days of treatment. When 30 cold nights were given, the first flower was found at leaf number 43.5. Assuming a linear increase in leaf number throughout the period of the experiment, one would expect this leaf to have been formed after 75 days; thus, the difference in time of flower bud formation would amount to somewhat more than 30 days. In fact, however, 54 days after the beginning of the experiment, there was a sudden increase in the rapidity of new leaf formation, so that the

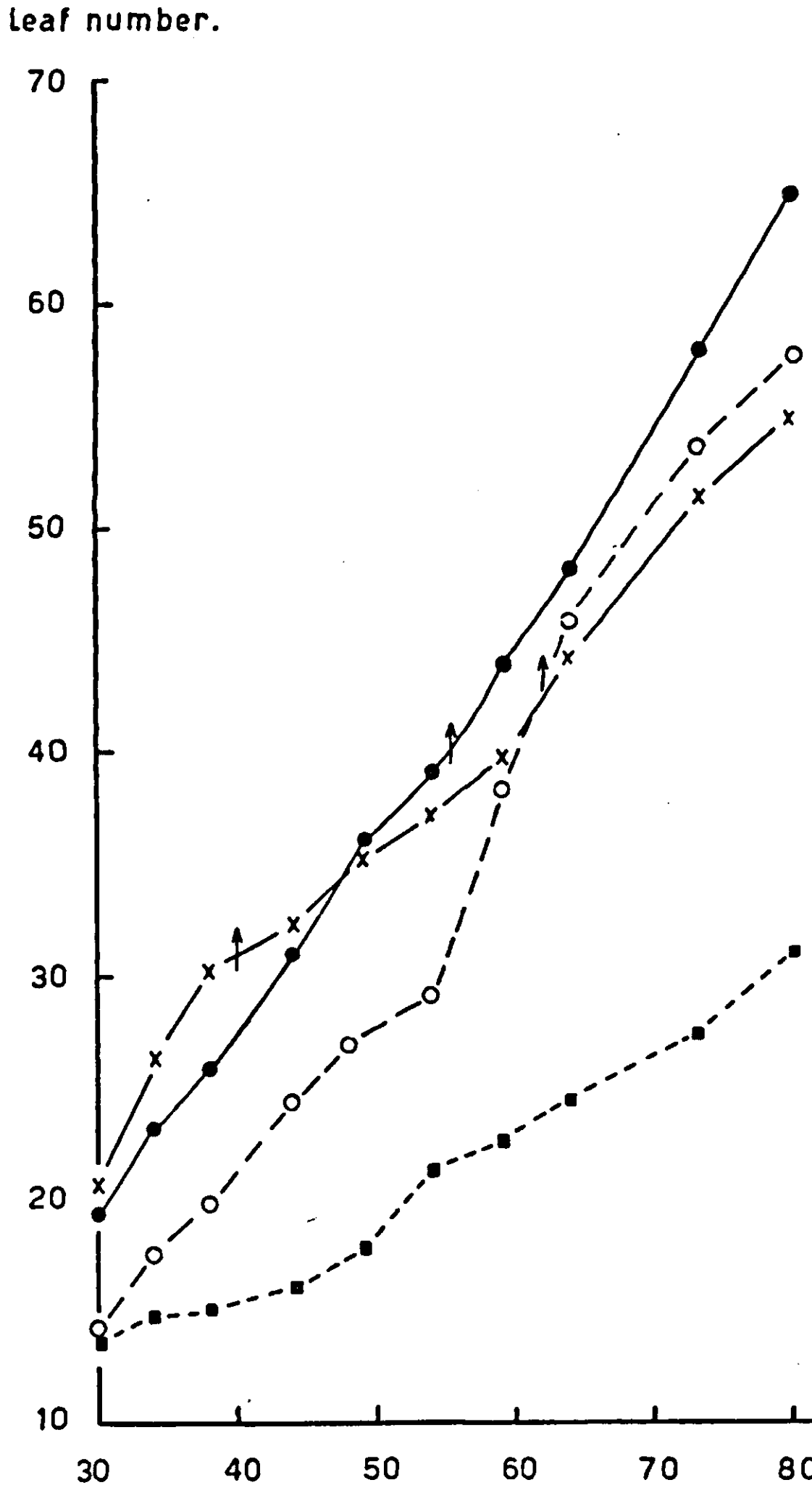


FIG. 11.
Leaf number (ordinate) after different duration of treatment. Same experiment as in fig. 10. x——x = 0; •——• = 15; o---o = 30; ■---■ = all nights at 2°. Appearance of 1st flower. Temperature (abscissa) during supplementary irradiation 27°.

required number of leaves was reached after 59 days. The initiation of flowers was going on when the plants started elongation after 56 days. Thus, the effect of cold was a more rapid development of leaves during and after flower initiation.

When 15 cold nights were given followed by a night temperature of 27 ° an average of 40.3 leaves was formed before the appearance of the first flower buds. On the assumption of a linear relation between leaf number and time, this should have been after 60 days, i.e. 20 days later than when no cold was given. Again, however, there was an increase in the rate of formation of new leaves which began after 38 days, so that the first flower bud appeared 53 days after the commencement of the experiment. Stem elongation started after 35 days. The influence of this cold treatment, therefore, started almost at the same development stage as that of the one in which 30 cold nights were given. The increase in rate of formation of new leaves, however, was less, so that the influence of cold on time of flowering was smaller.

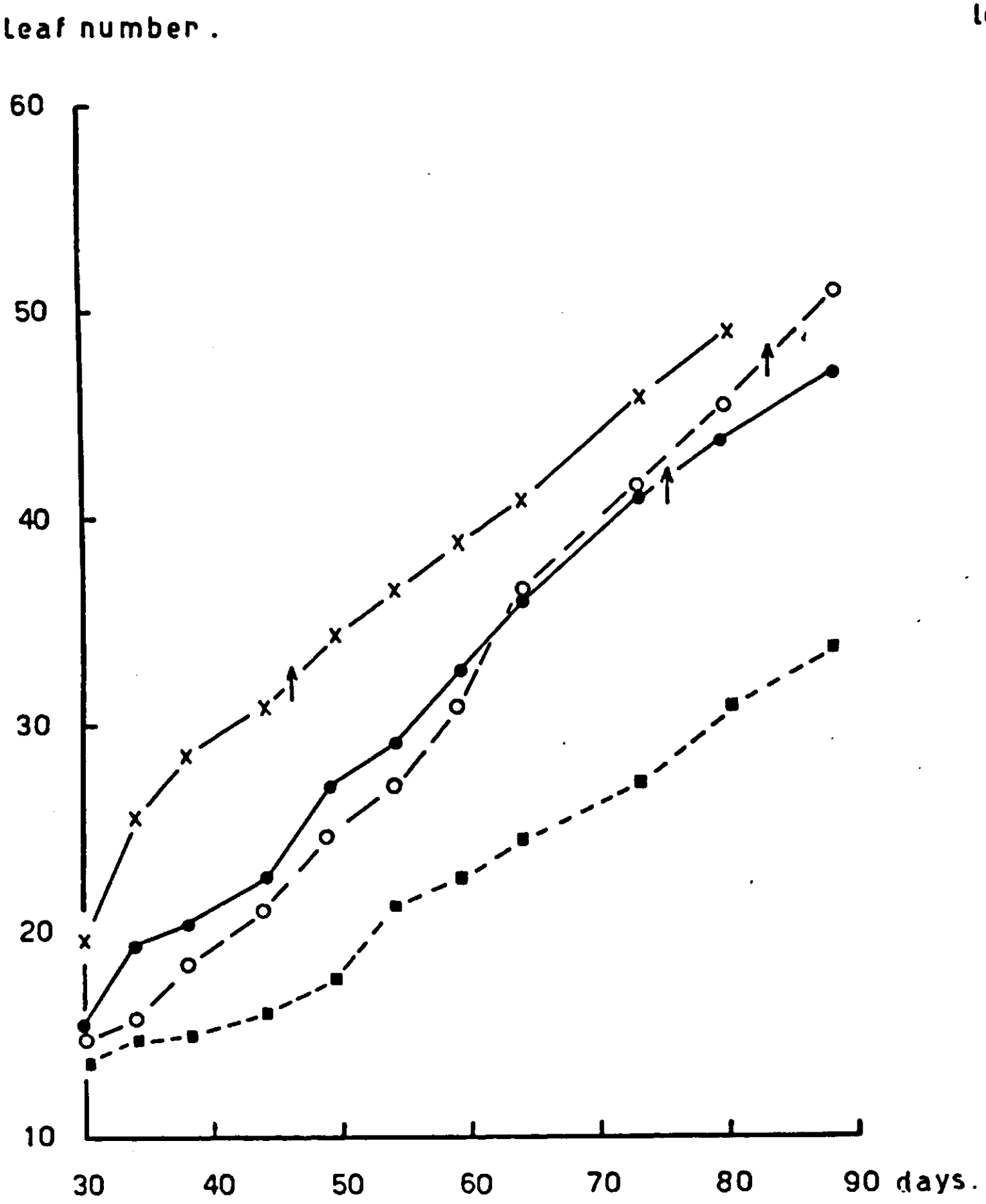


FIG. 12. Legend as for fig. 11, but night temperature 20°.

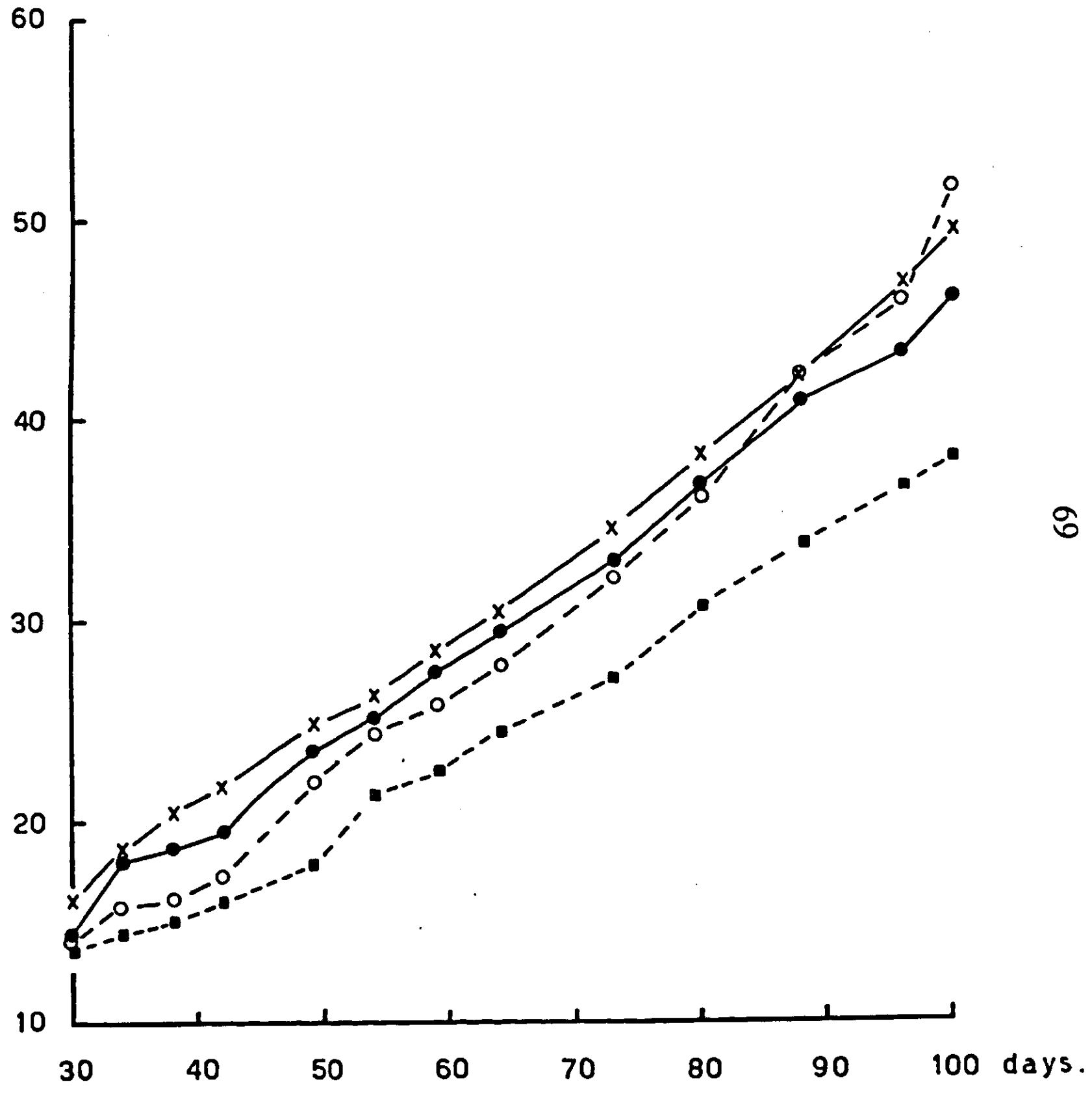


FIG. 13. Legend as for fig. 11, but night temperature 13°.

Unlike 27 ° a night temperature of 20 ° did not effect a sudden change in the rate of new leaf formation. The same trend, however, could be detected. After 88 days, the plants that had received 30 cold nights had formed the same number of leaves as the control plants so that, later on, an increase in the rate of leaf formation must have taken place. The influence of 15 cold nights was again smaller, and the leaf number of the control plants was not reached. Continuous cold for 15 days appeared to have a greater influence than the other cold treatments; the same number of leaves as in the control plants was already found after 64 days (figure 12).

At 13 °, only a few more leaves were formed than at 2°. When 30 cold nights were given, leaf formation surpassed that of the controls after 80 days of treatment. Elongation started after 93 days of treatment; thus the influence of the cold treatment started relatively earlier than at the higher temperatures (figure 13).

When the cold treatments were given to plants which were illuminated for 10 hours per day, flowering did not take place, although there was an increase

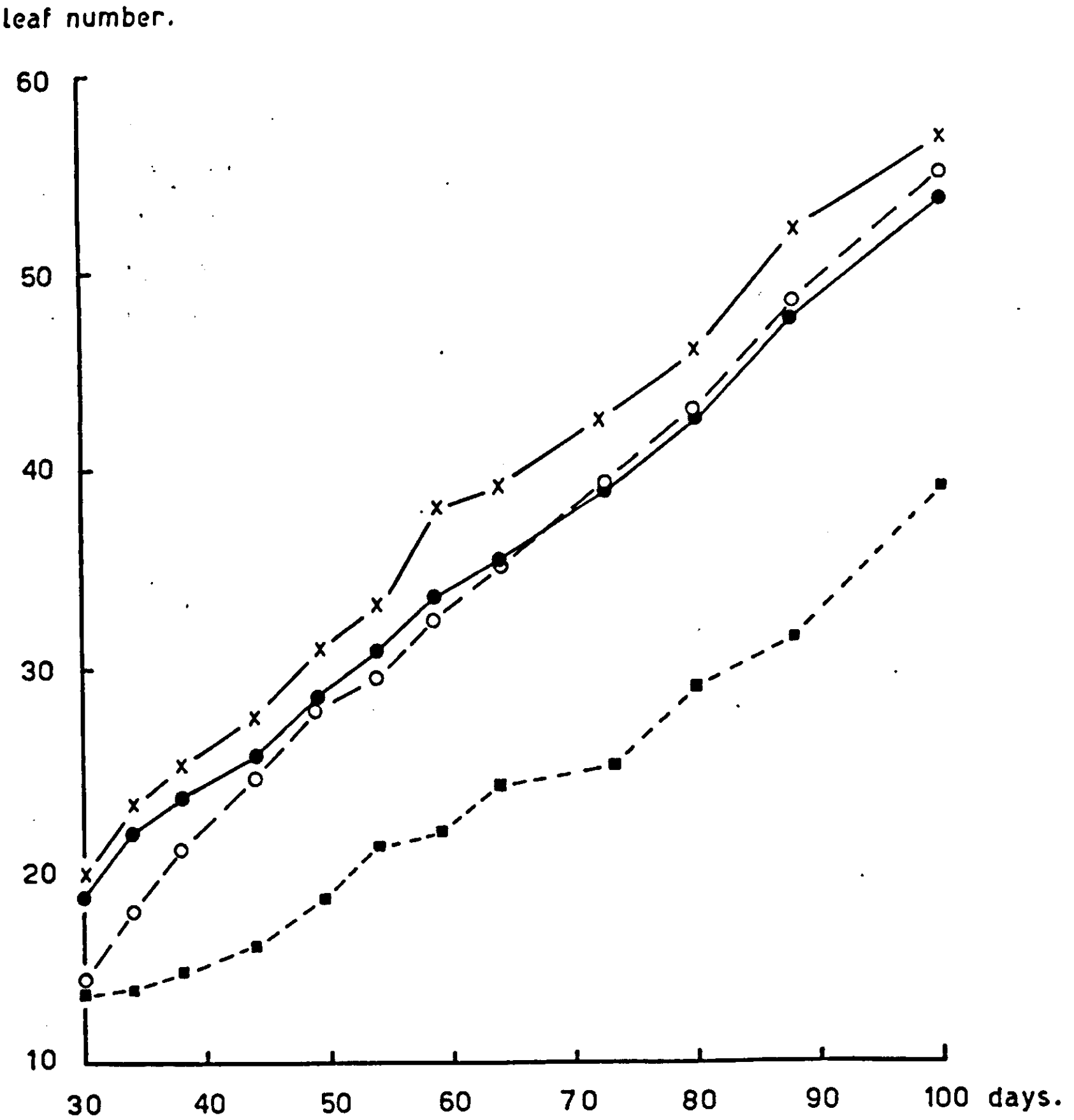


FIG. 14. Leaf number (ordinate) after different durations of treatment (abscissa). Same experiment as in fig. 10. x—x = 0; •—• = 15; o—o = 30; ■ - - - ■ = all nights at 2°. Temperature during darkness 27°.

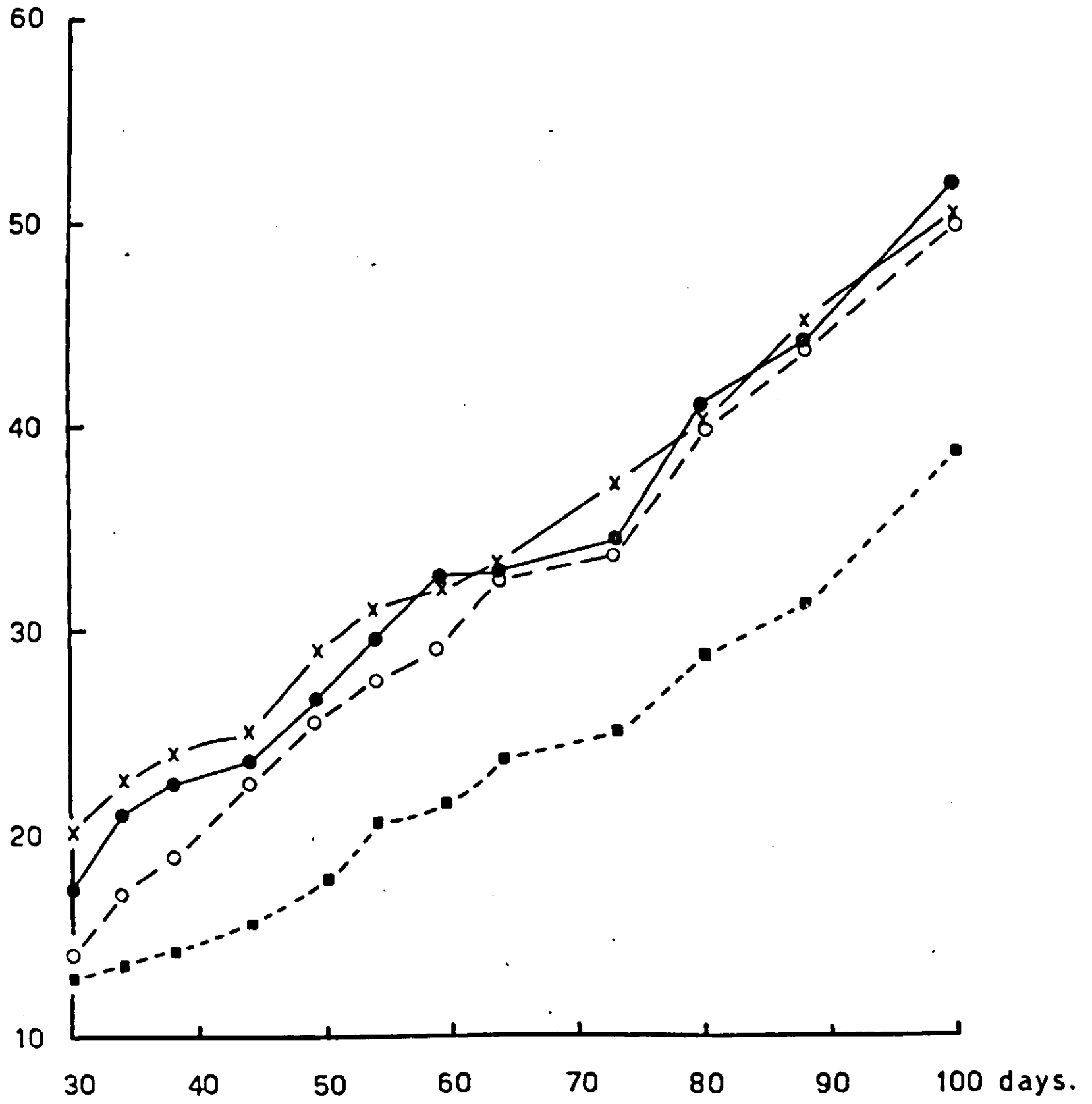


FIG. 15. Legend as for fig. 14, but temperature during darkness 20°.

in rate of leaf formation as can be seen from the figures 14–16. This increase, however, was less pronounced than when the plants received continuous illumination.

Stem elongation is illustrated in figures 17 and 18. When no cold was given the plants started elongation at 27 ° after 28 days; those which received 15 cold nights started, after 38 days, and those having 30 cold nights, after 56 days. However, at the end of the experiment, after 100 days, all the plants had reached almost the same length. It follows that the later stem growth was more rapid when cold nights had been given in the beginning of the experiments. When cold was given for 15 days continuously, elongation started 10 days later than in the control plants. During the first 24 days of elongation, plants reached the same length as those without cold. After that time they grew at the same rate. There was no elongation at 13 °.

Leaf area was measured, as in the previous experiment. At a daylength of 10 hours as well as at a daylength of 14 hours, it increased with increasing temperature, as can be seen from Table 18.

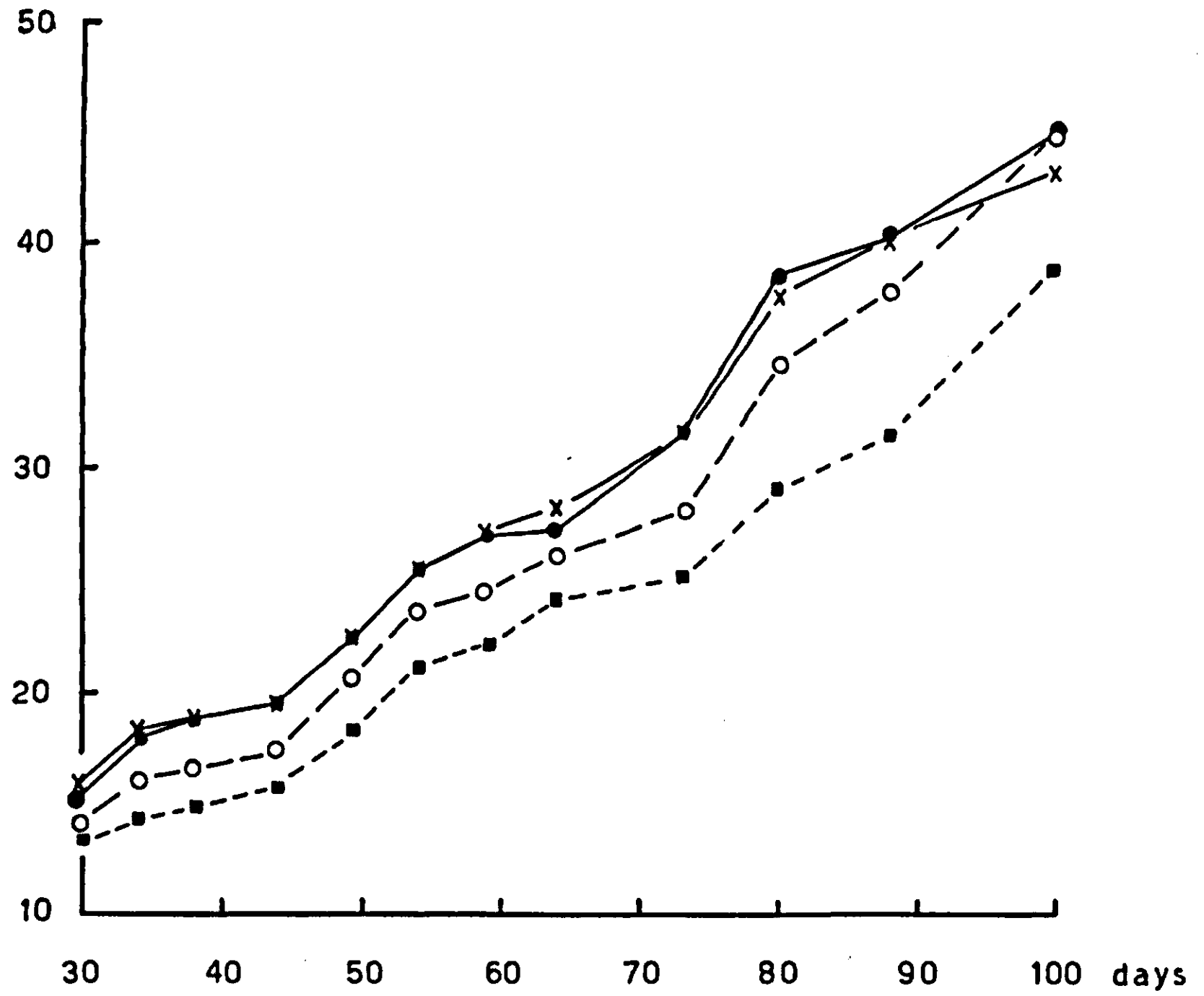


Fig. 16. Legend as for fig. 14, but temperature during darkness 13°.

TABLE 18

Leaf area (cm²) in Hyoscyamus *after 82 days of treatment. Same experiment as in Table 17; 0 cold nights*

night temperature \ daylenght	10	14
2°	135	117
13°	162	388
20°	302	469
27°	442	–

From this experiment we may conclude that the cold treatment influenced a variety of processes. In the first place more leaves were formed before the development of the first flower than in the control plants when cold was given. The increase in leaf number was relatively smaller at 30 cold nights than at 15 cold nights. So if the cold treatment is prolonged a promoting influence comes in. This phenomenon bears a resemblence to vernalisation; but is difficult to decide whether the same processes are involved, since virtually nothing is known about the mechanism. It is known that when plants are suddenly subjected to high temperatures they may be devernalised (GREGORY and PURVIS [1936]). This prompted the investigation into whether the cold treatment had a different effect when the change over to high temperature was brought about gradually.

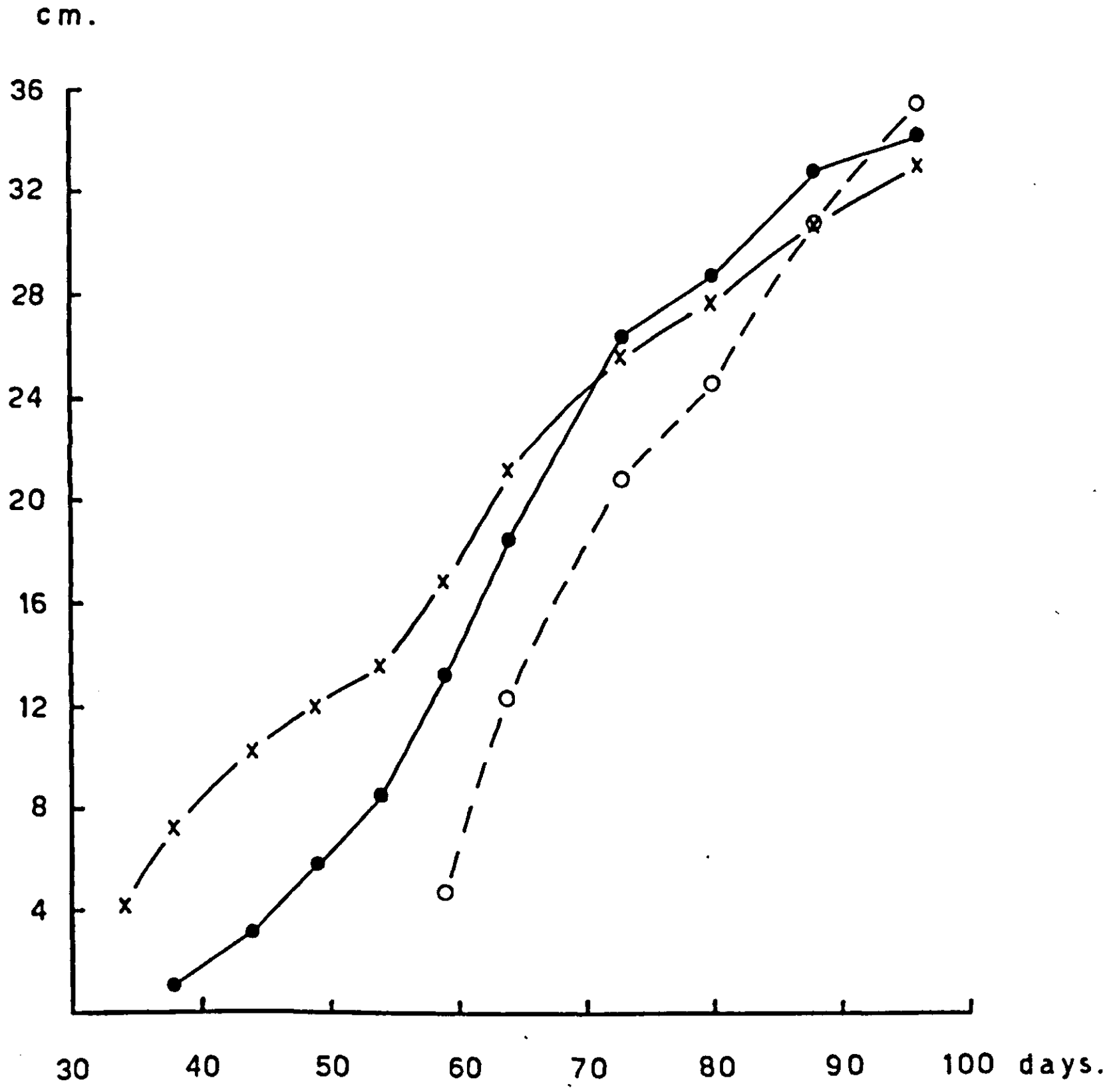


Fig. 17. Stem length (ordinate) after different durations of the treatment (abscissa), indicated in fig. 11.

Generally, there was a slight retarding influence on flowering if the change-over was extended over a number of nights, thus leading to the conclusion that under the conditions used, no processes comparable with devernalisation went on. It may be of course, that the only reason why no process such as devernalisation took place was that the constant day temperature of 20 ° acted as a buffer between the two extreme night temperatures. Secondly the plant growth rate continued to increase for a long time after the cold had been given. At 20 ° and 27 ° this increase started when flowers were initiated. It expressed itself in more rapid development of new leaves and faster stem elongation. When 30 cold nights were given and, followed by nights at 27 °, both processes increased to the same degree, as can be seen from the following example: when no cold was given, 1 cm of stem was formed in an average of 1.9 days; when 30 cold nights were given, 1 cm was formed in 1.1 days; this represents an increase in growth of 44 %. One leaf was made in an average of 1.7 days when no cold was given, and in 1.0 days, when 30 cold nights were given, this again represents an increase of 44 %.

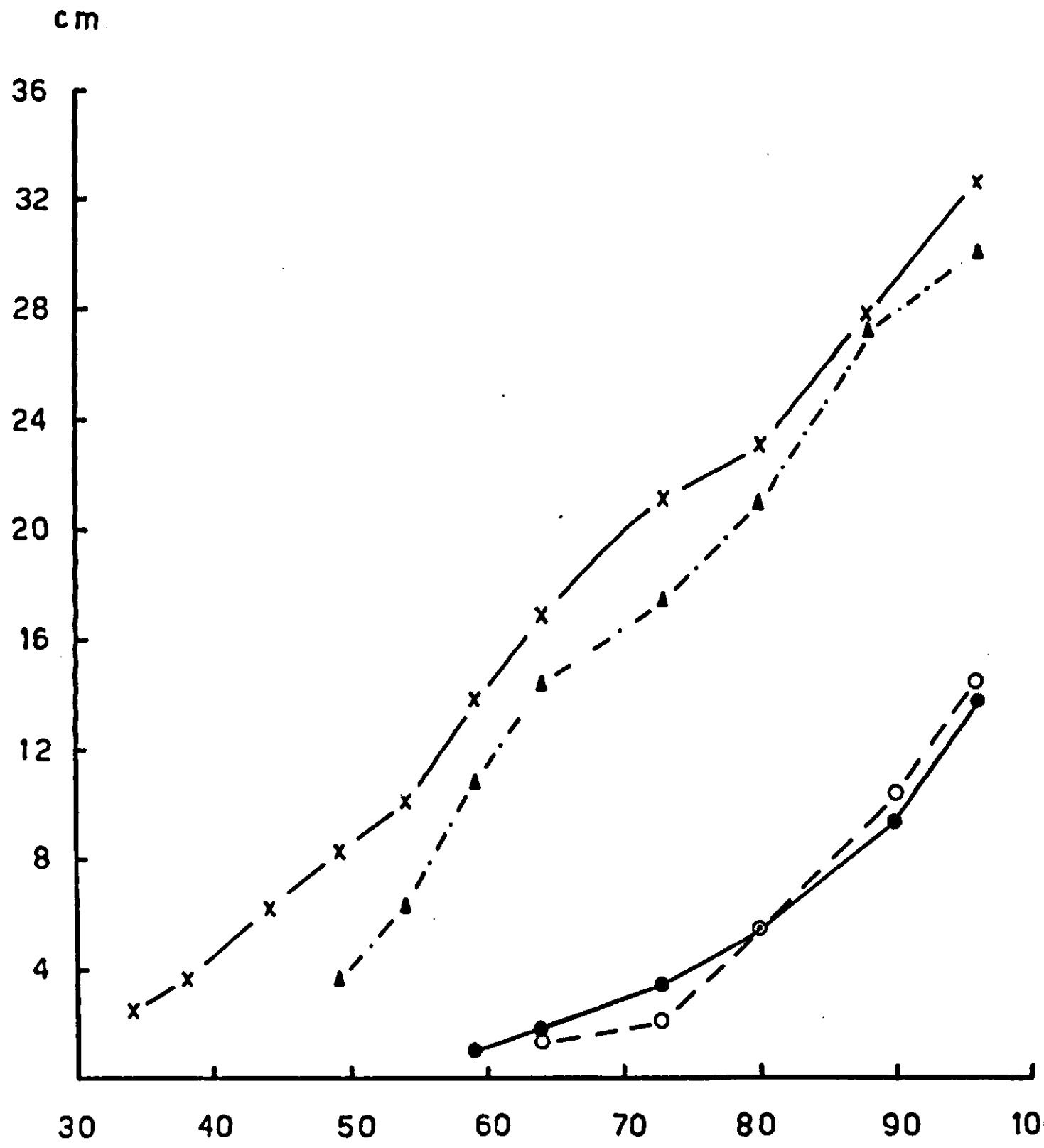


FIG. 18. Legend as for fig. 17, but night temperature 20°. ▲-·-·-▲ 15 days continuously at 2°.

4. EXPERIMENTS WITH *BRASSICA*

The plants were given a basic illumination period at an intensity of 22000 erg/cm²/sec, at a temperature of 20° C. for 10 hours per day. Supplementary illumination was given for 0, 4 and 14 hours in the experiment, and for 0 and 14 hours in the second one, at temperatures of 2°, 9°, 13°, 20°, or 27°. In the second experiment, 9° was omitted. During darkness, the plants received the same temperature as during the period of supplementary illumination. In the first experiment plants received 0, 3 or 6 nights at 2°; 0, 4, or 14 hours during these periods were given at an illumination of 525 erg/cm²/sec. In the second experiment 0 or 5 cold nights were given under these conditions. In the first experiment 4 plants per treatment were used, in the second 6 plants. Because of the greater number of plants per treatment, the number of treatments in the second experiment had to be less than in the first.

In the experiments described in chapter II, section 6 *Brassica* plants were treated in the same way and at the same time as in the experiments described here, except that they did not receive any cold nights. It was possible, therefore, to regard them as controls of plants which received cold nights, i.e. as the treatment in which 0 cold nights were given. The number of days before flowerbuds were visible in all experiments, is given in Table 19. It appeared that the influence of the cold treatment depended on the daylength used. In the first experiment, when supplementary irradiation was given for 4 hours, the accelerat-

ing influence of the initial cold treatment was greatest at a night temperature of 9 °; with 14 hours supplementary irradiation, however, the maximal effect was observed at 13 °. There was little difference between the influence of 3 cold nights and that of 6 cold nights. The results of the second experiment were essentially similar, but the promoting influence of the cold treatment was found at a higher temperature. Thus, with a daylength of 10 hours, the cold treatment was most effective at a night temperature of 13 °; while with a daylength of 24 hours, flowering was earliest at a night temperature of 20 °. In both experiments, therefore, the influence of the cold treatment was found at a higher temperature if the daylength was increased.

TABLE 19

Number of days before flowerbuds were visible in Brassica. Basic illumination given for 10 hours at 20 °C. During the following 14 hours, different temperature treatments were given, consisting of different numbers of nights at 2°, followed by nights at the temperatures indicated. Supplementary irradiation was given for 0, 4 or 14 hours during the temperature treatment at an intensity of 525 erg/cm²/sec. I = first experiment, II = second experiment

number of cold nights	night temperature / daylength	2°	9°	13°	20°	27°
I 0	10 h.	44.0	33.5	33.5	28.3	24.5
0	14 h.	41.0	31.3	26.8	29.8	23.0
3	14 h.		27.5	26.0	26.0	23.0
6	14 h.		29.0	27.5	29.8	23.0
0	24 h.	35.0	31,3	26.0	23.0	23.0
3	24 h.		26.0	23.0	23.0	23.0
6	24 h.		33.5	23.0	24.5	23.0
II 0	10 h.	39.8	–	38.7	43.5	35.6
5	10 h.		–	33.8	39.7	37.2
0	24 h.	31.0	–	23.3	23.3	17.7
5	24 h.		–	24.2	17.7	20.0

5. CONCLUSIONS

It has been demonstrated that, in spinach and *Hyoscyamus* cold nights at the beginning of an experiment have a retarding influence on flowering. In spinach the influence of the cold treatment on flowering was proportional to the number of cold nights given. When the cold treatments were given to plants that received a daylength of 14 hours the relation of flower initiation to temperature appeared to be essentially the same as when they were grown under a photoperiod of 24 hours. No influence of the cold treatment on stem length, total leaf number and leaf area was found. In *Hyoscyamus*, however, the retarding effect of the cold treatment on the initiation of flower buds is relatively smaller when given for 30 nights than when given for 15 nights. This means that, apart from the retarding influence of the cold treatment on flower initiation a promoting influence must be operating which becomes manifest if the cold treatment is continued for a sufficiently long time. A second influence of cold treatment on *Hyoscyamus* was found after flower initiation. From that point onwards, the plants started growing more rapidly than plants which had not received cold at the beginning of the experiment; the faster growth being manifest in a greater increase of stem length and a more rapid leaf development.

The influence of low night temperature in the beginning of the experiment

on flowering of *Brassica*, differed somewhat from that in both spinach and *Hyoscyamus*. It appeared that the influence of cold treatment depended on the daylength used. Thus with night temperatures of between 9 ° and 20 °, the promoting influence of cold was found at higher temperatures with increasing daylength.

To sum up, the only conclusion possible on this evidence is that the influence of cold treatment on flower initiation is subject to considerable specific variation. Thus, while it retards flowering in spinach and *Hyoscyamus*, it has an accelerating effect in *Brassica*. The effect is directly proportional to the amount of cold given in spinach, while in *Hyoscyamus* this relation is inverse. Again, in spinach and *Hyoscyamus* there is no apparent interaction between cold treatment and either daylength or night temperature; in *Brassica*, on the other hand, both daylength and night temperature exert a marked modifying influence on the action of cold. In view of this variation, it is impossible at this stage to conjecture what may be the precise effect of cold nights, or even what processes they may influence.

CHAPTER IV

THE INFLUENCE OF TEMPERATURE DURING A BRIEF PERIOD OF IRRADIATION IN THE MIDDLE OF THE DARK PERIOD ON FLOWERING OF SPINACH

1. INTRODUCTION

In the experiments described in previous chapters the temperature treatments were given for 14 hours per day. It is clear that, during this relatively long time, processes other than flowering must also be influenced and some of them (e.g. formation of leaves and growth of stems) may interfere with the primary processes determining flowering. It is desirable, therefore, to reduce the exposure to low temperature to a minimum. According to HARDER and BODE (1943), a long-day effect can be produced by interrupting the dark period by a short light period. The effect of this irradiation increases, the nearer it is given to the middle of the night. When exposures to light are given for one or two hours in the middle of the night, flowering depends on the total energy the plants receive. This period is the most suitable for examining the influence of temperature during the short irradiation. Thus, HARDER, WALLRABE and QUANTZ (1944) illuminated *Kalanchoe* for 1 minute in the middle of the night at different temperatures. They concluded that temperatures had no influence. Nevertheless it seemed of interest to carry out experiments of this type in the course of this study.

Before this could be done with spinach, data were required on the most suitable time of the night for illumination, and on the energies required for flowering.

2. THE INFLUENCE OF THE PART OF THE DARK PERIOD DURING WHICH A BRIEF PERIOD OF IRRADIATION IS GIVEN ON FLOWERING OF SPINACH

At different times of the dark period, plants were illuminated with "daylight" fluorescent tubes for a quarter of an hour; in the first experiment, at an intensity of 37800 erg/cm²/sec, and

in the second one, at an intensity of 25200 erg/cm²/sec. Basic irradiation was given for 10 hours per day at 22000 erg/cm²/sec; temperature during day and night was 20 °C. The supplementary light was given 3, 5, 7, 9 and 11 hours after darkness had started. Per treatment, six plants were used.

In these experiments, the number of elongated plants after so many days of treatment was used as a measure of flower initiation (see Table 20). In both experiments, stem elongation was found first when the illumination was given in the middle of the night. In spinach, a flower bud is surrounded by a large number of small leaves. Since all these leaves were counted in these experiments,

FIG. 19.

Leaf number in spinach (ordinate). Basic irradiation for 10 hours. Temperature 20 °C throughout. Plants were illuminated for 15 minutes at an intensity of 37800 erg/cm²/sec after different dark periods (abscissa). Measurement after 38 days of treatment.

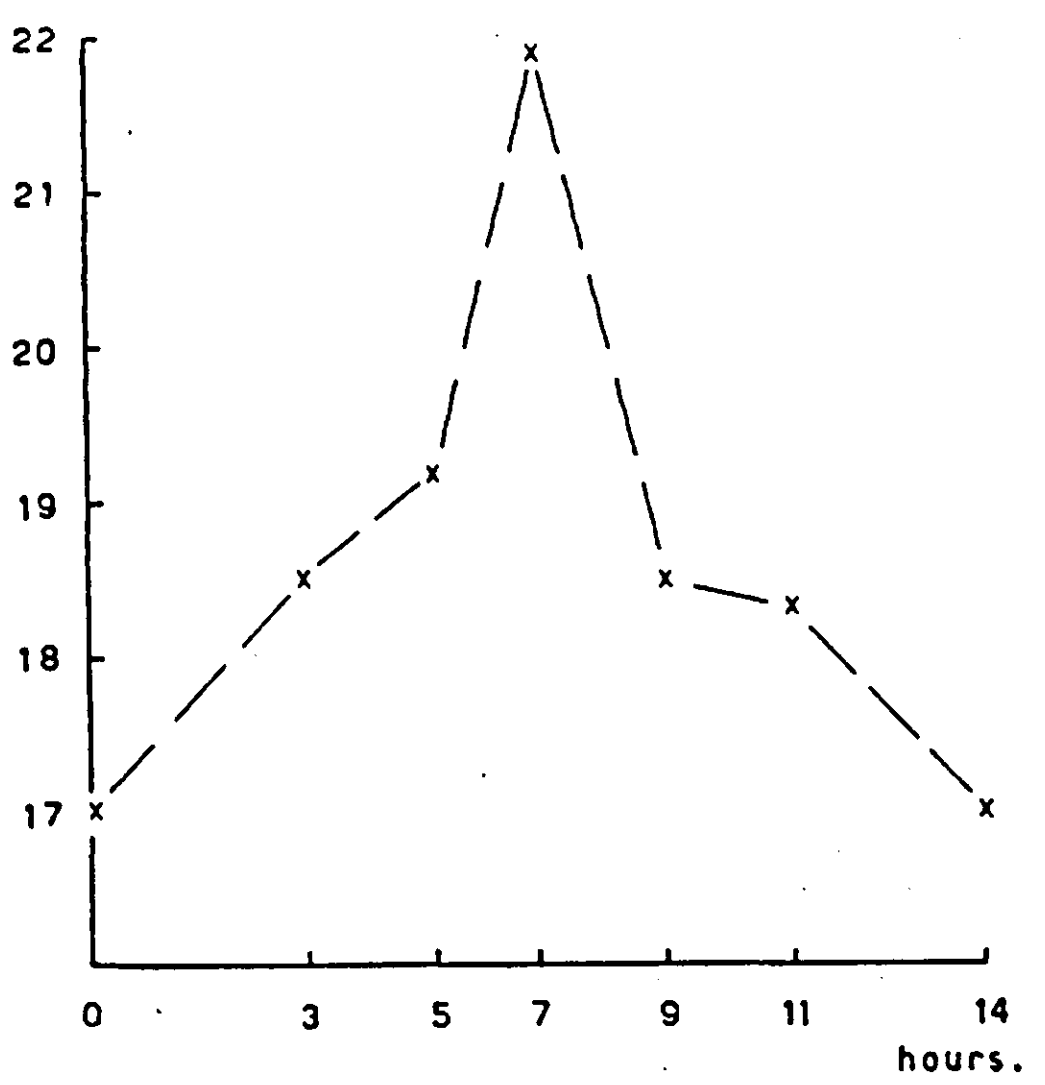
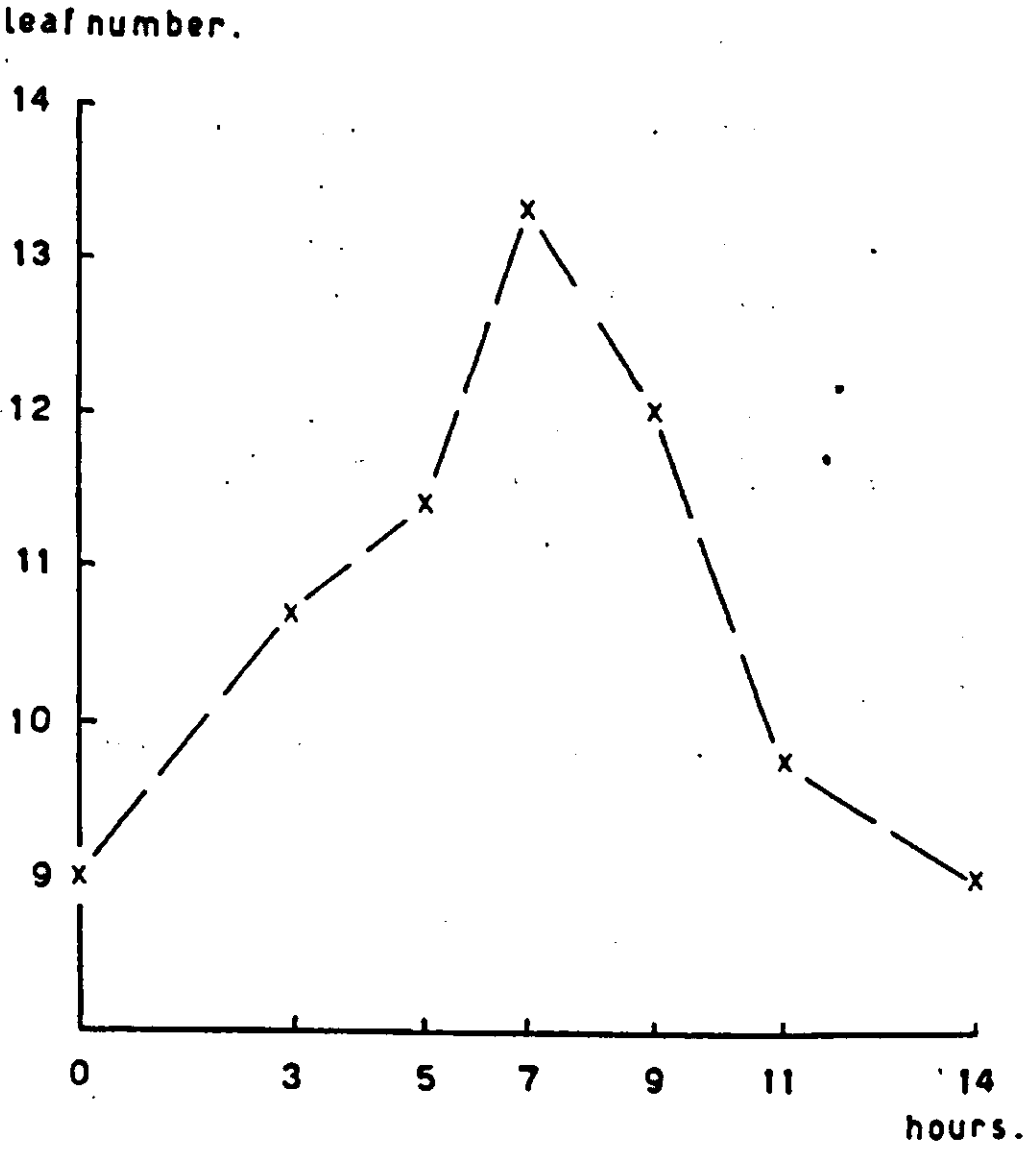


FIG. 20.

Leaf number in spinach (ordinate). Basic irradiation for 10 hours. Temperature 20 °C throughout. Plants were illuminated for 15 minutes at an intensity of 25200 erg/cm²/sec after different dark periods (abscissa). Measurement after 18 days of treatment.



a greater number of leaves than in the controls indicates the formation of flower buds. In both experiments as with stem elongation, the greatest number of leaves was found when the plants were illuminated in the middle of the dark period (figure 19 and 20). There was little difference in the effect of illumination during either the first or the second half of the dark period. Spreading the illumination over half an hour in the middle of the night, therefore, would be expected to have only a negligible influence; while extended over one hour, this influence would still be unimportant.

TABLE 20

Number of elongated plants and stem length (mm) in spinach. Basic irradiation for 10 hours. I: first experiment, plants were illuminated for 15 min. at an intensity of 37800 erg/cm²/sec after different dark periods. Measurement after 23 days of treatment. II: second experiment, plants were illuminated for 15 min. at an intensity of 25200 erg/cm²/sec. Measurement after 8 days of treatment

hours of darkness	elongated plants		stem length	
	I	II	I	II
3	2	1	1.6	0.1
5	5	3	8.6	2.0
7	6	3	14.9	4.0
9	2	1	2.3	1.2
11	1	0	0.2	0.0

3. THE INFLUENCE OF DURATION AND INTENSITY OF IRRADIATION IN THE MIDDLE OF THE DARK PERIOD ON FLOWERING SPINACH

a. First experiment

Basic illumination was given for 10 hours per day at an intensity of 22000 erg/cm²/sec. During the middle of the dark period plants were illuminated for 5, 15, 30, or 60 minutes with 22000 erg/cm²sec. "Daylight" fluorescent tubes were used throughout. Other plants received 30 minutes 525, 4200, 12600 or 37800 erg/cm²/sec from the same light source. Temperature during day and night was 20 °C.

Stem elongation was measured after 23 days. With exposures of 15 and 30 minutes per night at 22000 erg/cm²/sec, stems elongated almost at the same time; seven of a total of 8 plants elongated (Table 21 and 22). With 60 minutes, however, only 2 plants elongated. Thus, flower initiation was retarded by illuminations of long duration. Stem length was maximal at intermediate light intensities (Table 23).

In figure 21 leaf numbers have been ranged according to the total energy ($I \times t$) received in erg/cm² during the illumination in the middle of the night. It is obvious that leaf number depends largely on $I \times t$.

TABLE 21

Number of elongated plants and stem length (mm) in spinach after 23 days of treatment. Basic irradiation for 10 hours. Irradiation in the middle of the night at 22000 erg/cm²/sec during different times

min.	5	15	30	60
elongated plants .	7	7	7	2
stem length . . .	11.2	11.2	7.0	2.5

FIG. 21.

Leaf number in spinach (ordinate). Basic irradiation for 10 hours; irradiation in the middle of the dark period with different energies (abscissa). Measurement after 18 days of treatment. Temperature 20 °C throughout.

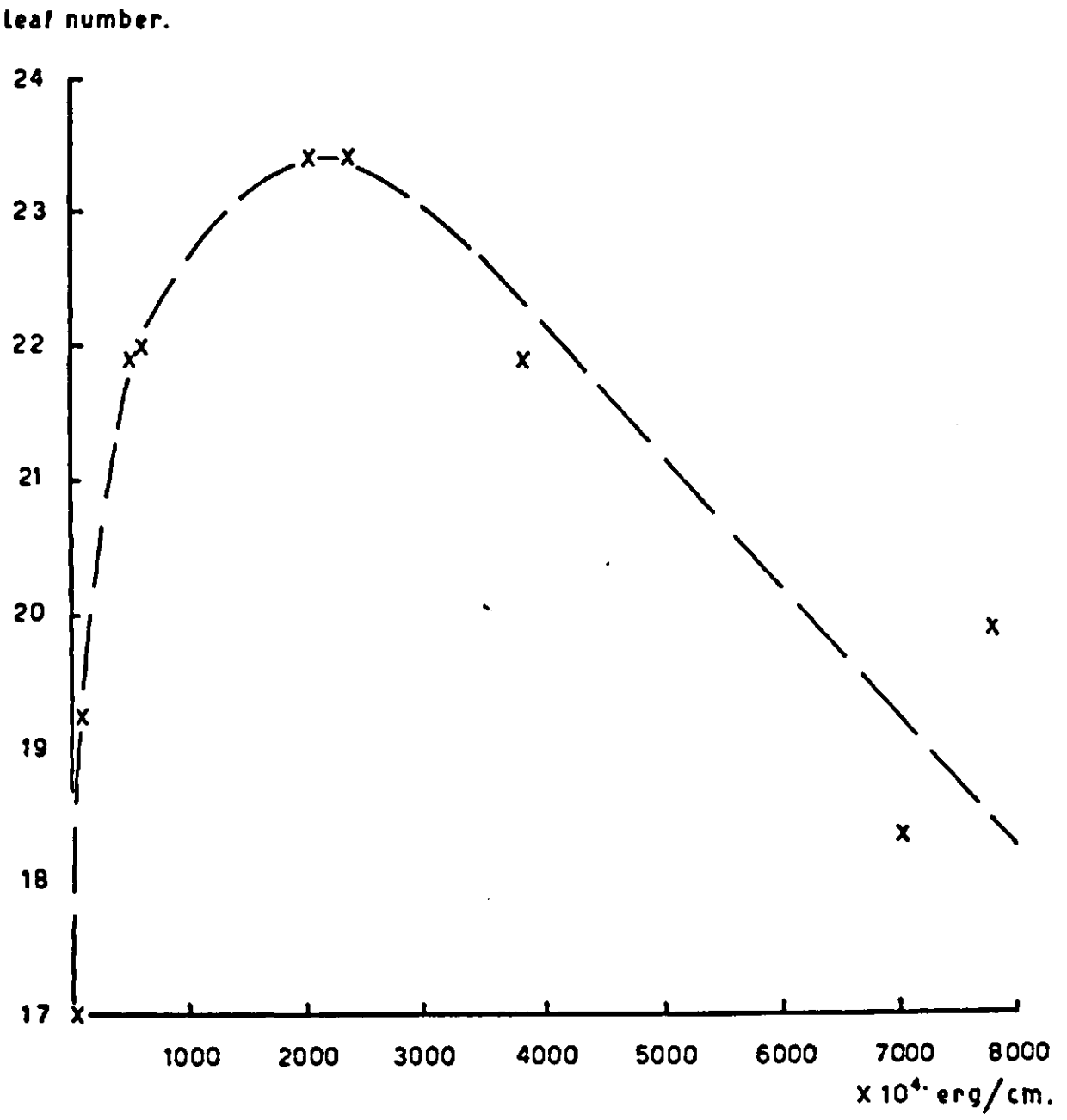


TABLE 22

Number of elongated plants and stem length (mm) in spinach after 23 days of treatment. Basic irradiation for 10 hours. Irradiation in the middle of the night at various intensities for 30 min.

light intensity erg/cm²/sec	525	4200	12600	22000	37800
elongated plants .	5	8	7	7	1
stem length . . .	7.8	12.5	9.0	7.0	0.3

TABLE 23

Number of elongated plants after 7 days of treatment, and stemlength (mm) after 9 days of treatment and leaf number after 18 days of treatment in spinach. Basic irradiation for 10 hours per day. Irradiation in the middle if the night at various intensities and during various times (sec)

light intensity erg/cm²/sec	time	I × t	elongated plants	stem length
525	1800	94.5×10^4	1	3.0
4200	1800	756×10^4	1	4.3
25200	300	756×10^4	1	11.8
12600	1800	2268×10^4	2	12.3
37800	900	3402×10^4	5	13.8
25200	1800	4536×10^4	4	14.0
18900	2700	5103×10^4	2	14.8
16800	3600	6048×10^4	1	10.0
37800	1800	6804×10^4	1	5.2

b. Second experiment

Basic illumination was given for 10 hours per day at an intensity of 22000 erg/cm²/sec. Plants were illuminated for 30 minutes in the middle of the dark period, at intensities of 420, 4200,

12600, 25200 and 37800 erg/cm²/sec, while in other experiments 25200 erg/cm²/sec were given for 60 minutes and 18900 erg/cm²/sec were given for 45 minutes. For all treatments six plants were used. During day and night, the temperature was kept constant at 20 °C. Control plants were illuminated for 11 hours consecutively.

With irradiation in the middle of the dark period, stem elongation was again found to be earliest at moderate values of I × t (Table 23). After 7 days of treatment some of the elongated plants already had small flower buds. At the lower and higher I × t values, smaller numbers of leaves were found, as can be seen in figure 22. Similar differences in stem length can be seen in Table 23.

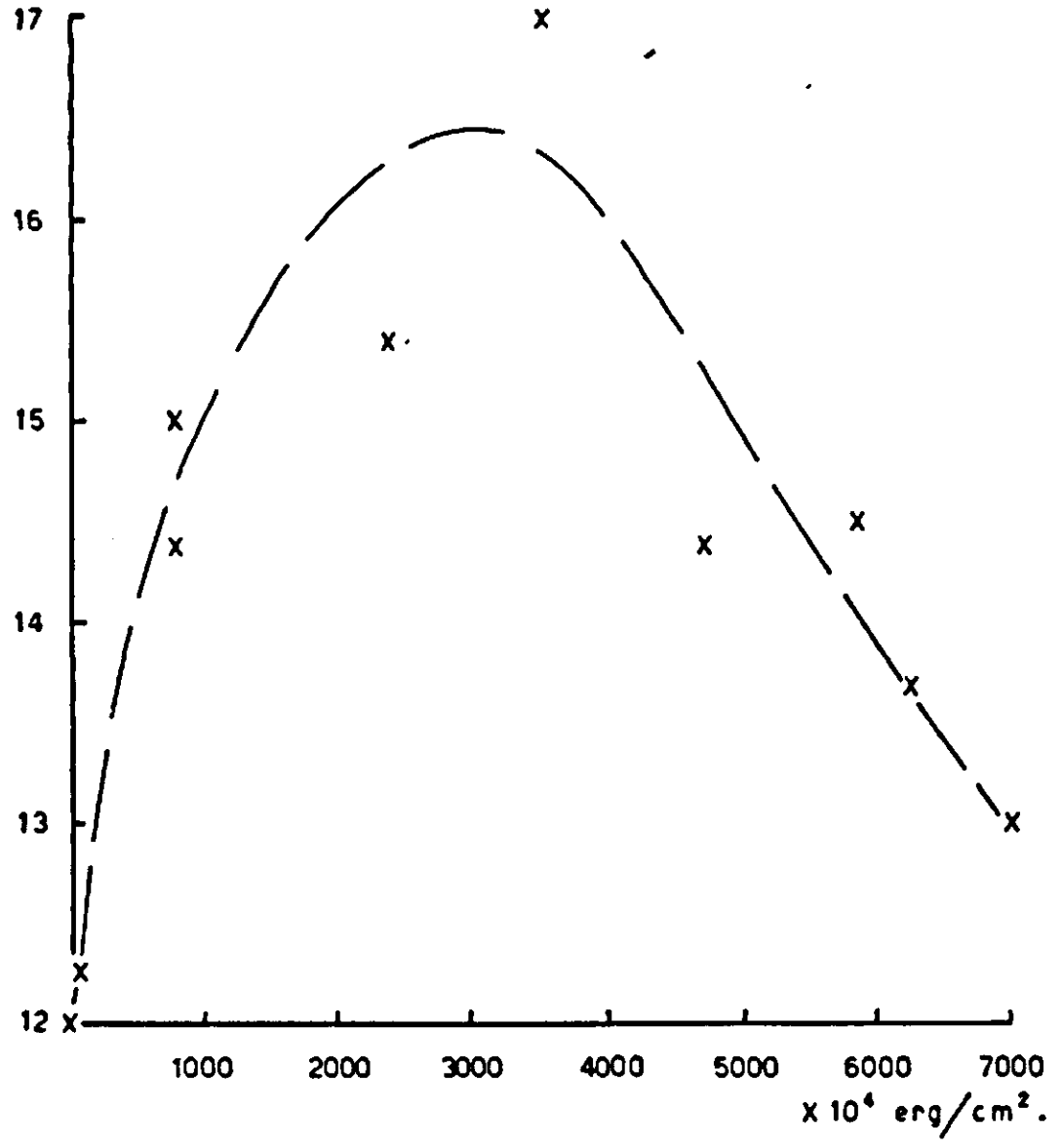


FIG. 22.
Leaf number in spinach (ordinate). Basic irradiation for 10 hours. Irradiation in the middle of the dark period with different energies (abscissa). Measurement after 23 days of treatment. Temperature 20 °C throughout.

4. THE INFLUENCE OF A BRIEF PERIOD OF IRRADIATION DURING THE MIDDLE OF THE DARK PERIOD AT DIFFERENT TEMPERATURES ON FLOWERING OF SPINACH

a. Preliminary experiment

Basic irradiation of 22000 erg/cm²/sec was given for 10 hours at a temperature of 20 °C. Seven hours after the beginning of the dark period the plants received a brief period of irradiation at different temperatures. Fifteen minutes before the light was put on, some of the plants were brought to temperatures of 2°, 10° and 27 °C, while others remained at 20 °C. The time of acclimatisation was short, since it was desirable to eliminate as much as possible any effect of temperature during darkness. The brief period of irradiation was given with the aid of a single "daylight" fluorescent tube, at an intensity of 12600 erg/cm²/sec, for 30 minutes. This irradiation was given at 2°, 13°, 20°, and 27°. Control plants received the different temperatures for three quarters of an hour at the same time at which the other plants were being illuminated. In the control treatment, 4 plants were used, in each of the other treatments, 7 plants were used.

Irradiation was photoperiodically most effective at 10 ° and 20 ° in that flowering was most abundant at these temperatures. Stem elongation was found at almost the same time in plants at 10 °, 20 ° and 27 °, but somewhat later at

2 °. Flower initiation therefore, was inhibited by very low temperatures during the brief period of irradiation. Flower buds appeared at almost the same time at 10 ° and 20 °; at 27 °, they were visible a few days later; thus, development of flower buds was also retarded by high temperatures. The retardation in development, found at 2 °, increased during the formation of flower buds (figure 23). Difference in leaf number and stem length were of the same nature, as can be seen from Table 24.

FIG. 23. Number of days (ordinate) up to stem elongation (•——•) and visible flower buds (x——x) in spinach. Basic irradiation for 10 hours. Plants were irradiated in the middle of the dark period for 30 minutes at an intensity of 12600 erg/cm²/sec at various temperatures (abscissa).

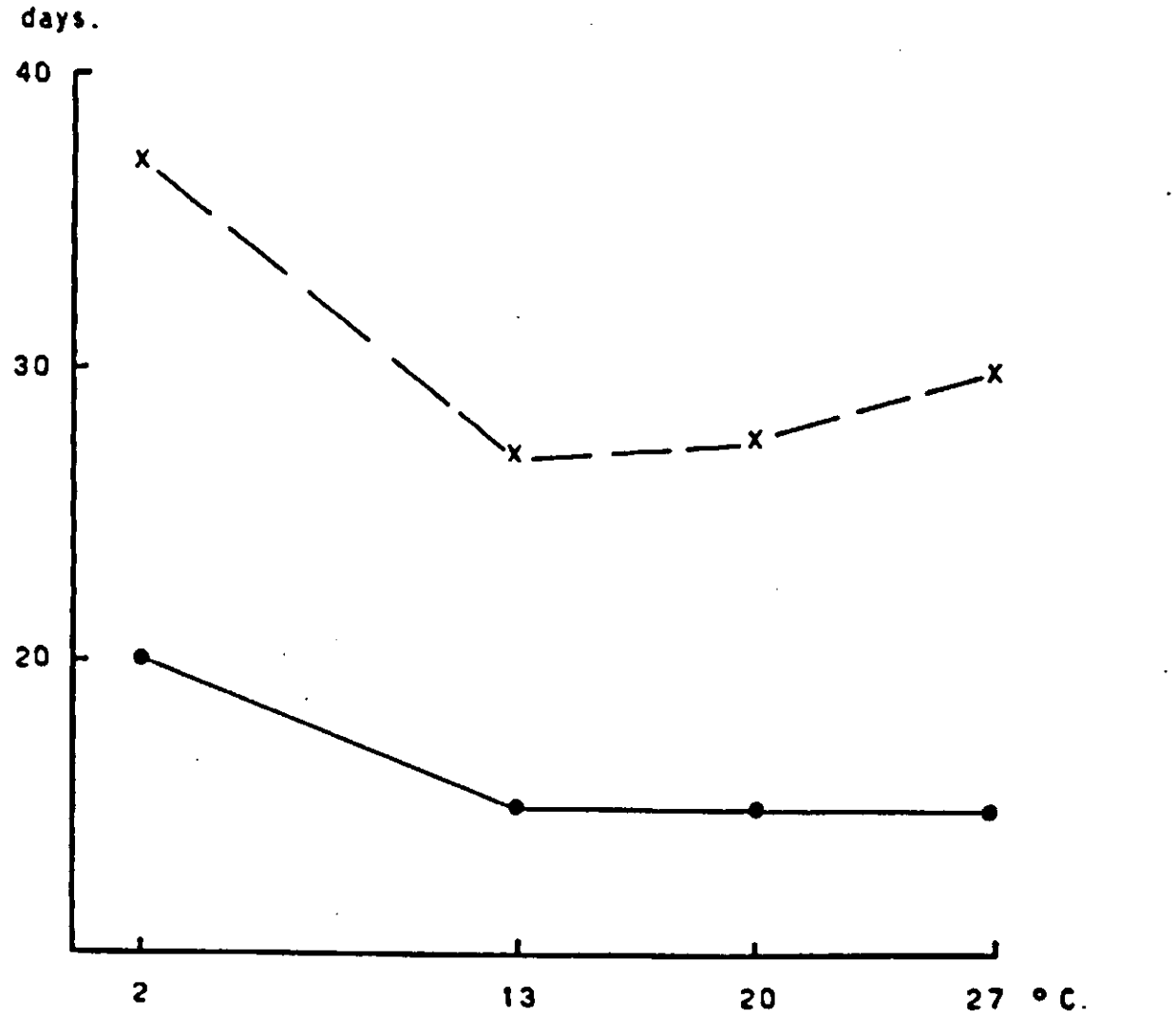


TABLE 24

Number of flowerbuds after 19 days of treatment, leaf number and stemlength (mm) in spinach after 23 days of treatment. Basic irradiation for 10 hours. Irradiation in the middle of the dark period at an intensity of 12600 erg/cm²/sec for 30 min. at various temperatures

temperature	2°	10°	20°	27°
flower buds	5	3	2	5
leaf number	18.4	20.6	22.0	19.8
stemlength	6.2	19.2	19.2	17.6

Control plants in the same temperature series (which had received three quarters of an hour darkness) showed no mutual differences in leaf number, or any other influences of a brief temperature treatment during darkness.

b. Further experiments

The previous experiment was performed, in all, three times. In the second experiment plants were illuminated in the middle of the dark period for 30 minutes with the aid of mercury lamps (HO 2000) at an intensity of 11400 erg/cm²/sec; in the third experiment incandescent lamps of 1000 watt were used at an intensity of 12600 erg/cm²/sec. Again the plants were illuminated for 30 minutes in the middle of the dark period, at 2 °, 10 °, 20 ° and 27 °C, having been brought to these temperatures 15 minutes before illumination started. Control plants were in darkness at the different temperatures from one hour until a quarter of an hour before the middle of the dark period, after which they were transferred to a temperature of 20 °C. At the middle of the dark period illumination was given for 30 minutes at an intensity of 25200 erg/cm²/sec by "daylight" fluorescent tubes. In all experiments, 4–5 plants were used per treatment.

TABLE 25

Number of elongated plants (1), stem length (2), leaf number (3) and size of flowerbuds (4) in spinach. Basic irradiation 22000 erg/cm²/sec. Plants were illuminated for 30 minutes in the middle of the dark period at temperatures as indicated in the Table. I: first experiment, supplementary light from mercury lamps HO 20000, II: second experiment, supplementary light from incandescent lamps of 1000 watt. III: Control plants received the various temperatures before they were illuminated at 20°. 1 was measured after 19 days of treatment in the first experiment and after 14 days in the second; 2–4 were measured after 30 and 34 days respectively, the control plants were measured after 34 days of treatment

temperature	2°			10°			20°			27°		
	I	II	III	I	II	III	I	II	III	I	II	III
(1) no. of elongated plants	1	1	–	3	–	–	3	3	–	3	3	–
(2) stem length	21.5	44.3	58.0	37.8	–	–	38.0	61.5	55.0	31.6	53.5	53.8
(3) leaf number	22.0	17.0	19.3	23.2	–	–	24.0	18.5	19.8	23.0	18.0	19.5
(4) size of flowerbuds	5	12	15	7	–	–	10	18	15	8	10	15

In Table 25 the influence of treatment on the different aspects of growth connected with flowering is illustrated. It appeared that in all cases at 2 °, stem elongation was somewhat later than at the other temperatures, the same tendency being found for stemlength, leaf number and size of the flower buds. Thus, the influence of temperature on illumination during the middle of the dark period was the same for the various light sources and intensities used. In the control treatment, flower buds were of the same size at all temperatures, while stem length and leaf number were also the same under the different treatments. The fact that low temperature influenced flowering response only when applied together with illumination may be ascribed to an interaction between these two variables.

5. CONCLUSIONS

In the experiments described here, the results of HARDER, WALLRABE and QUANTZ (1944) have not been confirmed. These authors found no interaction between temperature and illumination in the middle of the night, whereas, in the experiments described here, a marked interaction was demonstrated. From the results of the present experiments, using a wide range of light intensities, it appears that the greatest flowering response occurred at an energy of 25.10^6–30.10^6 erg/cm² from „daylight" fluorescent tubes. At this energy, moreover, temperature modified the response. As well as „daylight" fluorescent tubes, mercury lamps (HO 2000) and incandescent lamps (1000 watt) were used, so as to provide almost the same energy. In the three light sources mentioned, 20%, 3% and 52% respectively of the visible light, or 2500, 350 and 6300 erg/cm²sec, represents energy in the wavelength region, 6100–7000 Å, which is photoperiodically the most effective region. In the wide range of intensities, the influence of temperature on the effect of irradiations was the same. One of the most interesting features of the present experiments is the finding that when plants received a brief temperature treatment before being illuminated in the middle of the dark period, no influence of temperature on flower initiation was found. Thus, since temperature modifies only the light reaction, it seems reasonable to conclude that the photochemical reaction is closely connected with a biochem-

ical one. It is remarkable that the influence of temperature during a brief period of irradiation in the middle of the night is similar to the influence of temperature during the whole night. Whether this is incidental or not, cannot at present be decided. To investigate this point at greater length, it seemed desirable to use other long-day plants. Unfortunately experiments undertaken with *Hyoscyamus* failed, because the intensity of illumination used (25.10^6 erg/cm^2) proved to be inadequate for flowering in this species.

The experiments relating to the influence of intensity of irradiation in the middle of the night on flowering in spinach demonstrated that the optimum for flower initiation is at 25.10^6–30.10^6 erg/cm^2. Lower or higher energies retarded the unset of flowering. This suggests that at least two processes may be involved in the "photochemical" reaction, or at any rate, in the process going on during the light period.

CHAPTER V

PRELIMINARY STUDIES OF METABOLISM IN SPINACH

1. INTRODUCTION

Since the object of this study was to examine the relation between photoperiodism and metabolism it seemed desirable to attempt to relate the environmental factors which influence photoperiodic behaviour with known metabolic processes. As a first attempt in this direction some preliminary observations on the influence of daylength and temperature on starch hydrolysis, respiration rate and dry matter accumulation were made.

The influence of the length of the night on starch hydrolysis was first studied by GRAINGER. His original view (1938) was that, in short-day plants, starch hydrolysis begins relatively late in the night. In 1939, however, he found that in some short-day plants, starch hydrolysis goes on during the whole night. The influence of temperature on the disappearance of starch has been investigated by WASSINK (1953). He found a minimum in the disappearance of starch, at 10 ° and suggested that the starch content of leaves under different conditions is determined by the balance between hydrolysis, respiration and re-synthesis. It is clear that temperature and daylength may affect the amount of starch present in leaves.

For the same reason, some measurements of respiration rates and photosynthesis were made. The temperature dependence of both these processes has been throughly investigated in the past, but a detailed review of previous work is not proposed here. With regard to respiration, the work of VAN DER PAAUW (1932), WASSINK (1934) and MACALISTER (1937) deserves mention. All these found a decreasing Q 10 with increasing temperature between 5 °–25 °.

Direct measurements of photosynthesis of plants are accompanied by great difficulties. When natural conditions are used light intensity and temperature during the measurements are rarely constant, so that it is difficult to interprete results. It is not sufficient merely to maintain the temperature of the environment constant, since depending on light intensity the temperature of the leaf may increase during the illumination, even though the temperature 1 cm from the leaf remains steady. (WAGGONER and SHAW 1952). When the absolute photosynthetic capacity of plants is to be determined no mutual shading should

occur, and light should fall on all leaves from the same direction. These requirements are only easily fulfilled when single leaves are measured. Since, however, photosynthesis is influenced by the age of the material it is inadvisable to compare plants grown under different conditions on the basis of experiments carried out with single leaves. To overcome objections of this sort, indirect methods of measurement have often been used. Such as indirect measure of photosynthesis during a particular experiment is the increase in dry weight. From experiments of this type, GREGORY (1917) outlined the concept of Net Assimilation Rate (N.A.R.), which is the increase in dry weight per leaf area. WILLIAMS (1936) replaced leaf area first by leaf dry weight, and more recently by protein content (1939). He suggested the latter on the grounds that the rate thus expressed remains constant for a longer time than if referred to dry weight of leaf area. However, the N.A.R. on a basis of protein content was closely related to temperature, while it was almost independent of temperature on dry a weight basis. His experiments were performed under field conditions, so no adequate data are available about the light intensities reaching the leaves, and it is, therefore, difficult to assess the influence of temperature on N.A.R. In 1946 WILLIAMS concluded that protein content can be used as a reference for N.A.R. only when plants grow at low nitrogen concentrations. It is clear that data concerning N.A.R. must be considered cautiously and in the present study, no attempt was made to use this concept.

Closely allied to consideration concerning photosynthesis is the question of the rôle of sugar in plant development. Sugar is among the few substances which have been found to have a promoting effect on flowering. From the work of MELCHERS and LANG (1942), however, it appears that it only influences flowering when the daylength used is close to the critical daylength for flowering. From this work the question arises as to whether under suitable conditions sugar can be replaced by illumination at high intensities and, in the present investigation an attempt was made to obtain an answer to this question.

2. STARCH HYDROLYSIS UNDER VARIOUS CONDITIONS OF TEMPERATURE AND DAYLENGTH

a. Methods

A few days before the experiments started, the plants were brought indoors. They received 10 hours basic illumination at 22000 erg/cm²/sec, from "daylight" fluorescent tubes. Subsequently various treatments were given, as described in each experiment. For illumination during the dark period, "daylight" fluorescent tubes were again used.

The starch content was measured in leaf discs of 14 mm diameter, using the method described by WASSINK (1953). Measurements are made of light transmission of leaf discs, stained in an iodine solution after the extraction of chlorophyll. The starch content was measured at the beginning and the end of the treatment periods, and the difference between successive measurements of comparable leaf discs was used as a measure of starch hydrolysis. There is no linear relation between transmission value and starch concentration. It follows, therefore, that no quantitative values can be found and the data collected in the present experiments must be regarded as merely indicating qualitative relation-

ships. Standard errors of the treatment means were calculated according the formule S.E. $= \sqrt{\frac{\varepsilon x^2}{n(n-1)}}$.

b. Reliability of the method

In order to test whether this method gives reproducible results, the following experiments were carried out:

α. Spinach

From each of 3 plants 7 leaf discs were measured at the end of the basic illumination period. From equivalent leaves of 6 other plants, one disc was measured. From these same leaves discs were measured after 14 hours of darkness at 20 °. The results of these measurements are recorded in Table 26. It can be seen that the difference at the end of the day between discs from one leaf and those from different leaves, is almost the same. The individual difference in starch content measured are greater at the end of the dark period than at the beginning. At the end of the dark period less starch is present and factors other than starch content e.g. thickness of the leaves, become relatively more important.

TABLE 26

Light transmission in iodine stained leaf discs as a measure of starch content. In 7 discs of one leaf (1) and 6 discs of different leaves (2) of spinach at the end of the day; (3) represents 6 other discs of the same leaves as in (2) at the end of a 14 hour dark period

1	1	1	2	3
3.0	2.5	2.1	2.8	4.2
3.2	2.4	2.4	2.9	6.0
2.9	3.0	2.1	3.0	4.0
2.8	2.8	3.1	2.8	5.0
3.5	2.5	2.3	3.1	6.9
2.9	2.9	3.4	2.3	4.5
3.5	3.0	2.1		
3.1 ± 0.11	2.7 ± 0.09	2.5 ± 0.19	2.8 ± 0.11	5.1 ± 0.46

β. Hyoscyamus

The starch content of different leaves was compared at the beginning and at the end of the night. It appeared (Table 27) that the individual variation at the end of the day was greater than in the case of spinach, while at the end of the night, it was of the same magnitude.

It must be admitted that these preliminary experiments did not hold out much promise of succes. Difference between individuals were great and obviously too variable for much reliability to be placed on the results. Nevertheless, in the absence of a method that gives qualitative results in so short a time, it was decided to continue the experiments. Since spinach gave more easily reproducible data than *Hyoscyamus*, this species only was used.

TABLE 27

Light transmission in iodine stained leaf discs as a measure of starch content. In 6 discs of different leaves of Hyoscyamus. *Samples were of the same physiological age and taken at the end of the light period (1) and after 14 hours of darkness (2)*

1	2
3.8	15.0
7.0	11.0
6.4	7.2
3.8	10.4
9.5	11.9
8.2	13.1
6.5 ± 0.9	11.4 ± 1.1

c. The influence of temperature on starch hydrolysis

The influence of temperature was measured in two different ways. In the first, leaf discs were removed from the plants prior to different temperature treatments, and in the second, leaf discs were taken from leaves attached to the plants which had undergone temperature treatment for some days. In the first experiments, well-grown healthy leaves were taken from plants raised in the greenhouse at a daylength of 10 hours. Measurements began at the end of the daily light period. From each leaf 7 discs were cut. Some discs were killed immediately; the others were divided into 6 groups, placed on gauze in a petri dish containing filter paper, and then exposed to one of a series of temperatures, *viz.*, 2 °, 9 °, 13 °, 20 °, 27 ° and 33 °. In the first experiment, 4 discs were exposed to each temperature, in the second experiment, 6 discs were used. After 15 hours of darkness they were killed and starch content estimated. The difference between the initial and final values was taken as a measure of hydrolysis. Results are given in Table 28. Minima in the hydrolysis were found at 9 ° and at 27 ° with a maximum somewhere between 13 ° and 20 °.

TABLE 28

Difference in light transmission in iodine stained leaf discs as a measure of starch hydrolysis in spinach at various temperatures during a 14 hour dark period; I = first experiment. II = second experiment

temperature / experiment	2°	9°	13°	20°	27°	33°
I	5.3	2.9	3.4	2.0	1.5	2.4
II	4.4	1.9	3.9	1.7	1.3	2.2

For the second series 5 plants were brought indoors and grown under a 10 hour day. During the night they were kept at 2 °, 9 °, 13 °, 20 ° or 27 °. After 4 days of this treatment, measurements of starch content were begun. One fullgrown leaf of each plants was used. At the end of the day a disc was taken from one half of each of 10 leaves and killed. The plants then were exposed to the various temperatures. After a further 15 hours a disc was taken from the other halves of each of the 10 leaves and the starch content of all the discs was estimated. The results are shown in Table 29. With the exception of the reading at 20 °, the values show the same trend as when detached leaf discs were used.

TABLE 29

Difference in light transmission in iodine stained leaf discs as a measure of starch hydrolysis in spinach treated for 4, 40 and 81 days respectively. Basic irradiation 10 hours per day; the temperature during the night was varied as indicated. Leaf discs were taken at the beginning and the end of the dark period

temperature / days of treatment	2°	9°	13°	20°	27°
4	4.5	2.2	4.4	6.7	1.7
40	3.1	2.2	4.2	6.1	6.0
81	3.1	0.5	2.6	2.9	0.7

A second determination was done with plants from the experiments described in chapter II, section 2. The control plants used were grown under 10 hour day and exposed to various night temperatures. For each temperature treatment, 5 plants were used. From each plant, one leaf disc was taken from a full grown plant at the end of the day and killed. After 14 hours of darkness a disc was taken from the other half of the leaf. The starch content of all the discs was estimated at the same time. This experiment was performed twice, after 40 and 81 days of treatment respectively. From Table 29 it can be seen that the same trend was found as in previous experiments. After 40 days of treatment the maximum in hydrolysis between 13 ° and 20 ° was less pronounced than after 81 days of treatment.

It seems, therefore, the experiments have shown that starch disappearance shows the same trend when the leaves remaine on the plants as when they were detached before the temperature treatment. Under all conditions a minimum in hydrolysis was found at 9 ° and a maximum somewhere between 13 ° and 20 °. Illumination at low light intensity during temperature treatment also had no effect on starch hydrolysis.

d. The influence of illumination in the middle of the dark period on starch hydrolysis

For these experiments plants were brought indoors and illuminated for 10 hours per day with 22000 erg/cm²/sec at a constant temperature of 20 °C. Six plants were illuminated for a further 14 hours per day at an intensity of 525 erg/cm²/sec, six other plants were illuminated for half an hour in the middle of dark period at an intensity of 12600 erg/cm²/sec, while a final series of 6 plants remained in darkness for the remaining 14 hours. Starch content was measured at the end of the basic illumination period, in the middle and at the end of the dark period; when illumination was given in the middle of the dark period, starch content was measured just before and just after this illumination. Estimations of starch content were made on the first, forth and nineth day of treatment. From each plant, a full grown leaf was used for each determination.

It can be seen from Table 30 that there is hardly any difference in starch hydrolysis between plants receiving light periods of 10 and 24 hours respectively. Since there is little difference between plants receiving 14 hours darkness and those receiving 14 hours weak illumination, it may be concluded that little or no starch is synthesized at an illumination of 525 erg/cm²/sec. When the plants

were illuminated in the middle of the night at an intensity of 12600 erg/cm²/sec some synthesis of starch was found.

TABLE 30

Light transmission in iodine stained leaf discs as a measure of starch content in leaf discs of spinach taken after different numbers of hours in darkness or weak illumination. The "brief illumination" values refer to measurements made at the stated number of hours after the end of the day, the plants receiving half an hour of illumination in the middle of the night

treatment / no of days of treatment	darkness			weak illumination			brief illumination			
	0 h	7 h	14 h	0 h	7 h	14 h	0 h	6½ h	7½ h	14 h
1	3.2	3.8	5.3	2.5	3.7	4.3	2.4	4.6	4.1	6.1
4	2.3	3.5	6.6	2.7	3.6	5.0	2.7	3.6	3.1	6.2
9	2.1	2.6	5.1	2.1	2.9	5.1	1.8	2.7	2.3	4.0

3. RESPIRATION OF DETACHED LEAVES OF SPINACH AND *HYOSCYAMUS* IN RELATION TO TEMPERATURE

a. Methods

Respiration of detached leaves was measured by means of the diaferometer technique developed by NOYONS (1922) and used in botanical studies by AUFDEMGARTEN (1939) and VAN DER VEEN (1949). This technique involves the determination of changes in a gas mixture by measuring the corresponding changes in their thermal conductivity. Since the conductivity of CO_2 is less than that of air and that of O_2 is approximately the same as that of air, a change in the CO_2 content of air will change its heat conductivity; these changes can be measured with the diaferometer. The apparatus used in the present work has been described in detail by SPIERINGS, HARRIS and WASSINK (1952). Briefly, a thin platinum wire is heated by a uniform electric current from a 6 volt battery, regulated by a rheostat. The wire is surrounded by the gas under investigation, to which it loses heat. Any change in the thermal conductivity of the gas causes a change in the rate of heat loss from the wire and, consequently, in its temperature. Changes in temperature of the wire, effect commensurate changes in its resistance which can be measured by means of a sensitive galvanometer.

Leaves were placed in a glass chamber kept at constant temperature in a water bath. A gas flow of constant velocity passed over the leaf and through the diaferometer, where its heat conductivity was measured against that of gas which had by-passed the leaf and, thus had not changed in CO_2 content. The air was saturated with water before entering the diaferometer.

b. Results

In all experiments full-grown leaves were used. The respiration was measured in 8 leaves from *Hyoscyamus*. The respiration was calculated both on a area basis (figure 24) and on a basis of dry weight (figure 25). In all cases respiration increased markedly with temperature, although the CO_2 output per dry weight was smaller for *Hyoscyamus* then for spinach; per leaf area it was about the same for both plants between 4 °–25 °. The Q 10 between 5 °–15 ° was. 2.55 and between 15 °–25 ° 2.44, thus it decreased very slightly with increasing temperature.

FIG. 24.
Galvanometer deflection as a measure of respiration (ordinate) in relation to temperature (abscissa) in detached leaves of spinach (x——x) and *Hyoscyamus* (•——•) calculated on the basis of leaf area.

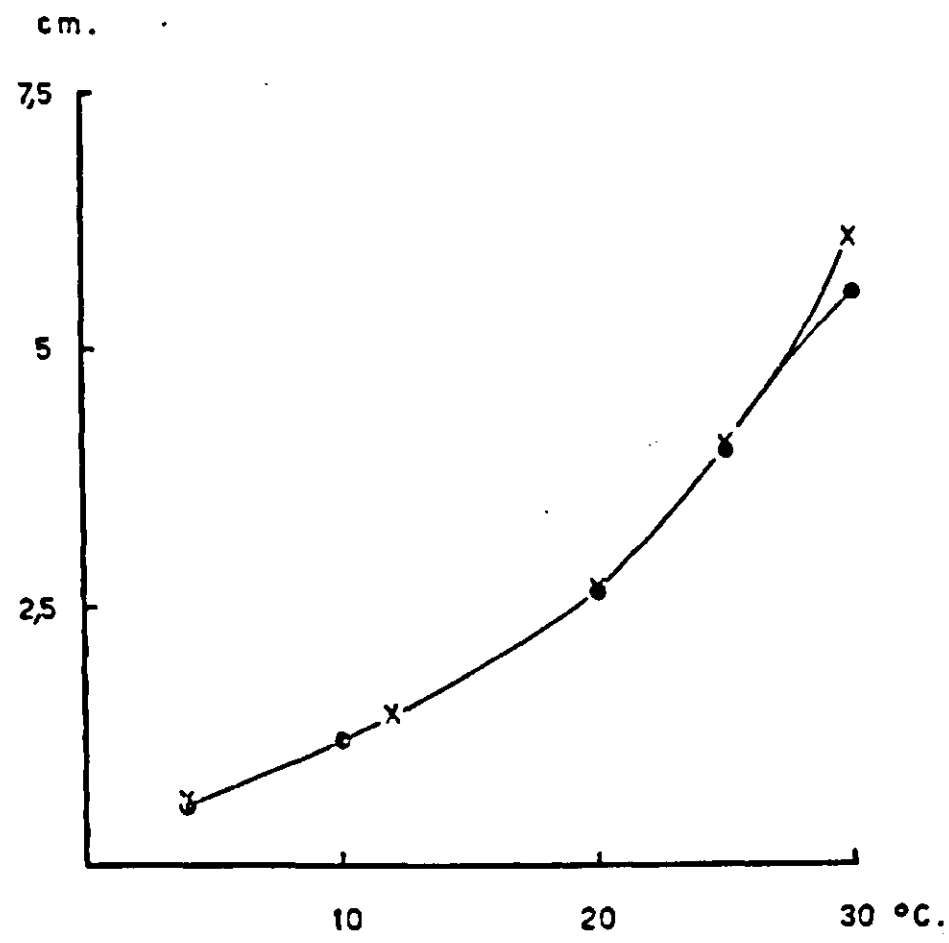
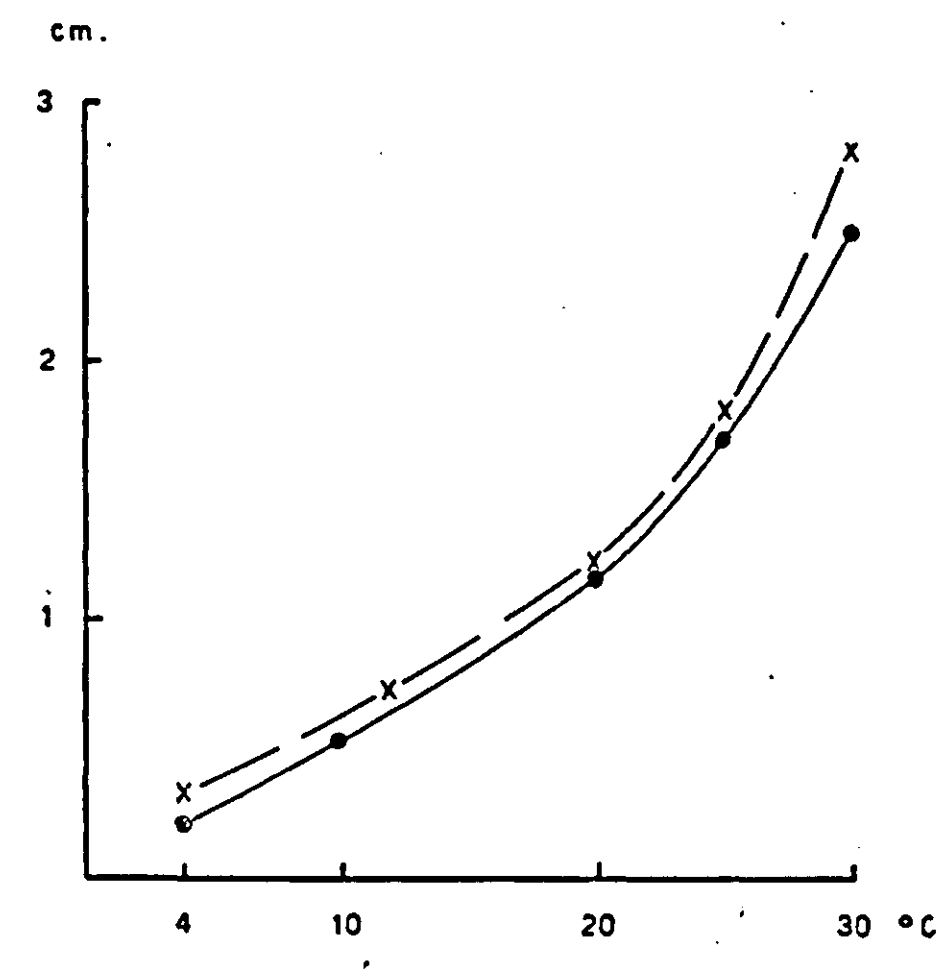


FIG. 25.
Same measurements as in figure 24, but on basis of dry weight.



4. DRY MATTER PRODUCTION OF SPINACH UNDER DIFFERENT CONDITIONS

a. The influence of various temperatures during a brief period of illumination in the middle of the dark period

α. Methods

To obtain the data presented here, plants were oven dried to constant weight and weighed to the nearest 0,01 g. Measurements of dry weight were made on the plants used in the experiments described in chapter IV section 4. These plants were illuminated in the middle of the night at an intensity of 12600 erg/cm²/sec for half an hour and at different temperatures, *viz.*, 2 °, 10 °, 20 ° or 27 °. Control plants were kept in darkness during the same times and at the same temperatures. The measurements were made after 38 days of treatment. Standard errors were calculated as in the previous experiment.

β Results

In Table 31 leaf number, dry weight, stem length and flower bud size are presented by treatments. It can be seen that there is very little effect of treatment on dry weight and, because of the magnitude of the standard errors, what differences there are, are not significant. Nevertheless, it appears that, in general, there is a correlation between dry weight and leaf number. No clear relation

TABLE 31

Number of leaves, dry weight (g), stemlength (cm) and size of flowerbuds in spinach, after 48 days of different treatments. Illumination of 126000 erg/cm²/sec given in the middle of the night at the different temperatures indicated (1). Control plants received no illumination (2)

	2°				10°				20°				27°			
	leaf number	dry weight	stem length	bud size	leaf number	dry weight	stem length	bud size	leaf number	dry weight	stem length	bud size	leaf number	dry weight	stem lenght	bud size
1	22	1.29	9	2	32	2.09	18	3	30	2.15	6	3	23	1.01	9.5	2
	25	2.43	5	2	27	2.08	8	3	32	2.09	8	3	24	1.26	5.5	3
	27	2.12	3	3	19	1.02	4.5	1	32	2.17	7.5	3	18	1.68	10.5	2
	20	1.38	1	1	20	1.14	7	1	30	1.87	11	3	20	1.45	6.5	1
	27	1.97	20	3	32	1.25	7	3	32	1.80	11	3	23	1.37	11.5	2
	17	0.74	2.5	1	27	1.64	12	3	25	1.72	5	2	20	1.62	2	1
	23	1.62	6.5	2	23	1.71	8	2	32	1.96	17	3	32	2.36	15	3
									30	1.28	5	3	25	1.32	7	2
	23.0	1.65 ± 0.22	6.7	2.0	25.5	1.56 ± 0.16	10.4	2.1	30.4	1.88 ± 0.10	8.8	2.9	23.1	1.50 ± 0.14	8.4	1.9
2	14	1.18	–	–	14	1.27	–	–	14	1.13	–	–	14	1.20	–	–
	16	1.65	–	–	15	1.35	–	–	15	1.35	–	–	14	1.24	–	–
	17	1.88	–	–	17	1.88	–	–	17	1.88	–	–	17	1.80	–	–
	15.6	1.57 ± 0.20	–	–	15.3	1.50 ± 0.19	–	–	15.3	1.39 ± 0.20	–	–	15.0	1.38 ± 0.15	–	–

was found between dry weight and either stemlength or flower bud size. The dry weight of the control plants did not differ significantly from that of the plants which received illumination in the middle the dark period. One may conclude, therefore, that the level of photosynthesis was the same under all the different treatments, but that the energy was used in different ways.

b. The influence of different daylengths at constant temperature

α. Methods

Plants were sown in large wooden boxes, and brought indoors when 4 weeks old. Basic illuminated by "daylight" fluorescent tubes was given for 10 hours per day at an intensity of 22000 erg/cm²/sec. The temperature was held constant at 20 °. Supplementary illumination was given for 8 hours at an intensity of 850 erg/cm²/sec from 15 watt incandescent lamps. This additional irradiation overlapped the basic irradation period by varying amounts, so that at all daylength used, the plants received the same amount of energy. Thus, for a daylength of 10 hours, the incandescent lamps were on for the last 8 hours of the illumination period; for a daylength of 14 hours, they burned for the last 4 hours of the basic illumination and for the first 4 hours of the dark period; while at a daylength of 18 hours, they were on for the first 8 hours of the dark period.

β. Results

At various times, 10 representative plants from each daylength were selected and measured. Flowering responses are shown in Table 32. It can be seen that the plants at a day length of 18 hours had flower initials and flowers somewhat earlier than those at 14 hours, and that no flower initials were found at a day-length of 10 hours.

TABLE 32

Number of days before flower initials, flowers and seeds were found after treatment with different daylengths in spinach

daylength	flower initials	flowers	seeds
10 hours	–	–	–
14 hours	15	55	–
18 hours	7	30	52

Plants at a daylength of 10 hours had small stems, which had developed during their initial growth period in the greenhouse in December, when the light intensity was low. This etiolation was not connected with flower initiation. At the other daylengths, stems elongated more rapidly and produced flower buds.

Changes in leaf area are presented in Table 33. The leaf area of plants at day-lengths of 10 hours and 14 hours was about the same during the first 52 days of treatment. After 65 days of treatment, however, the area was greatest at 14 hours. At a daylength of 18 hours, leaf area was greater than at the other daylengths from the 19th day of treatment onwards. Leaf number was also greater than at the other daylengths.

TABLE 33

Leaf number, stem length (mm), dry weight (mg), leaf area (cm^2) and ratio of dry weight to leaf area in spinach after different periods of treatment. Basic irradiation for 10 hours at 22000 erg/cm^2/sec. Supplementary irradiation for 8 hours, at an intensity of 850 erg/cm^2/sec. applied in such a way that the total daylengths were 10, 14 and 18 hours

treatment / number of days	leaf number			stem length			dry weight			leaf area			dry weight / leaf area		
	10 h	14 h	18 h	10 h	14 h	18 h	10 h	14 h	18 h	10 h	14 h	18 h	10 h	14 h	18 h
7	4.0	5.0	4.5	8.1	11.3	13.1	–	–	–	10.3	15.1	14.2	–	–	–
19	6.9	6.6	8.3	14.7	36.1	56.4	91	93	126	31.9	35.0	46.3	29	27	27
27	19.3	8.4	9.2	14.9	49.0	118.0	119	116	149	30.8	30.5	44.5	31	38	31
37	9.8	11.2	15.1	17.8	109.0	224.0	198	195	298	47.6	52.4	79.4	41	37	36
52	10.2	12.7	22.1	18.0	148.0	305.0	299	272	386	67.1	66.6	107.1	44	45	36
65	11.8	15.0	–	22.0	265.0	–	351	628	–	72.4	89.1	–	48	70	–

Shoot dry weight showed the same trends as leaf area which also can be seen from Table 33. Up to the 52nd day there was no difference between plants at a daylength of 10 hours and those at 14 hours, but after 65 days of treatment the dry weight of plants at a 14 hour day was greater than that in the 10 hour series. This increase in dry weight, however, was greater than could be expected from the increase in leaf area, as is demonstrated in figures about the ratio of dry weight to leaf area. With one exception this ratio increased with time for all daylengths to the same degree. The increase in dry weight per unit leaf area can only be explained by assuming an increase in photosynthesis per unit leaf area.

The rate of photosynthesis in very young and in old leaves is low (FREELAND [1952]). When the number of leaves increases with a high rate of photosynthesis the total photosynthesis of a plant will also increase. The exception referred to above is the value for a daylength of 14 hours, after 65 days of treatment. It is of some interest that, in unpublished experiments, VAN OORSCHOT also found this result. If it is real, it can only be explained on the assumption that there is an increase in photosynthesis per unit leaf area, associated with flowering. In which case, it is difficult to see why a similar effect was not observed in plants growing at a daylength of 18 hours.

Soon after the experiment started a great difference was noted in the appearance of the plants. Those at 18 and 14 hours had much longer stems than those at 10 hours. After 30 and 52 days at the daylengths of 18 and 14 hours respectively, flowers were found. Nevertheless, the relation between dry weight and leaf area was about the same for all treatments, which means that the leaves formed at a daylength of 10 hours had larger dry weights than those formed at daylengths of 14 and 18 hours. Thus, as in the previous experiment, the light energy supplied was the same in all daylengths, but it was used in different ways.

5. THE INFLUENCE OF APPLIED SUCROSE AND HIGH LIGHT INTENSITY TREATMENT ON SPINACH

a. Methods

All plants received a daylength of 10 hours. Those treated with sucrose were illuminated at an intensity of 22000 erg/cm²/sec from "daylight" fluorescent tubes at a temperature of 20 °C. The other plants were given intensities of 57000 erg/cm²/sec and 114000 erg/cm²/sec from high pressure mercury vapour lamps (HO 2000). In view of the high temperature of the mercury lamps a glassplate was mounted above the plants and the surrounding air cooled by three 40 watt fans. In this way the temperature was maintained at a level no more than 1 °–2 ° higher than under the fluorescent tubes.

The supply of sucrose to plants proved to be difficult. Thus, when carried out according to the methods described by WENT and CARTER (1948) (i.e. spraying or shoot immersion), it appeared that the uptake of sucrose was small. The greater part of the sugar remained on the outside of the leaves. At the same time the petioles were too brittle to be bent. It was found, that sugar was absorbed best when administered through the leaf petioles. Leaves were therefore cut, and small glass cylinders fixed to them by short lengths of rubber tubing. Spinach petioles are not cylindrical, so it was necessary to close the joints with vaseline. Sucrose solutions of 0, 10 and 15% were applied through the glass cylinder, together with 0.025% sulphanilamide, to prevent growth of micro-organisms. Control plants had no cylinders attached and the leaves were not cut. Three cylinders per treated plant were used. Every three to four days a section of each petiole had to be removed, because the ends of the petioles died, while every 10 days a new petiole had to be taken. At concentrations of 15% sucrose new petioles had to be taken after 8 days. Each treatment started when about 14 leaves were present, this number being sufficient to complete the experiment. At the end of the experiment plants were three months old, and the growing points were no longer always strictly vegetative. Thus, if sucrose has, in fact, a promoting influence on flowering, it should have been demonstrated under the conditions of these experiments.

b. Results

The results of the experiment are given in Table 34. After 45 days of treatment, the growing points were examined under 40-fold magnification. In plants with 0% sucrose 1 out of 4 growing points were in the reproductive state; in the control plants, 2 out of 5; in the plants with 10% sucrose, 4 out of 7; and in the 15% sucrose plants, 4 out of 6. Thus the sucrose treatment had a noticeable, though slight promoting effect on flower initiation. High light intensities, however, had no influence on the initiation of flowers. Thus, when plants were illuminated at an intensity of 57000 erg/cm²/sec, 2 out of 6 plants had reproductive growing points and at an intensity of 114000 erg/cm²/sec 2 out of 5 plants.

TABLE 34

Leaf number, stem length (mm), dry weight (mg), and leaf area (cm²) in spinach. I = plants treated with sucrose; basic illumination 22000 erg/cm²/sec for 10 hours; control plants were not treated. II = illumination for 10 hours from high pressure mercury vapour lamps at different intensities

treatment	leaf number	stem length	dry weight			leaf area	
			roots	shoots	total	total	mean
I 22000 erg/cm²/sec							
0% sucrose	19	22	0.46	1.83	1.80	210	11.4
10% sucrose	22	26	0.57	1.87	2.47	339	16.2
15% sucrose	15	30	0.55	2.10	2.65	229	15.7
control	24	16	0.53	2.12	2.65	360	15.6
II 57000 erg/cm²/sec	18	20	1.02	4.05	5.07	628	34.9
114000 erg/cm²/sec	19	16	1.21	4.78	5.92	729	34.8

The dry weight of the roots and shoots was determined separately. The dry weight of the plants receiving 0% sucrose was smaller than that of the control plants, since several leaves had been removed. With 15% sucrose the dry weight reached that of the control plants. By comparing the volume reduction in a cylinder attached to a plant with that of an essential similar one, not attached to a plant, an approximate estimation of the sucrose taken up by the plant could be made. In this way it was shown that during the experiment, 14 cc solution was taken up by the plants; when 15% sucrose was given this represents 2.1 g. Since leaf areas of plants with 15% sucrose were almost the same as in the 0% plants, the difference in dry weight may be ascribed to this 2.1 g of sucrose. The dry weight was by 0.83 g. If these calculations are correct, therefore, it seems that utilisation of applied sucrose was by no means great.

With the plants illuminated at different intensities dry weight increased with intensity. The increase was relatively greater from 22000 erg/cm²/sec to 57000 erg/cm²/sec than from 57000 erg/cm²/sec to 114000 erg/cm²/sec. The greater dry weight of the plants resulting from increased photosynthesis did not result in the initiation of flowers. This may have been due to differences in the quality of the light.

The influence of treatment on leaf number and on the total and mean leaf areas is also demonstrated in Table 35. Since the leaves were somewhat irregular areas were determined by tracing on to squared paper, cutting out the tracing and weighing it. When plants were treated with 15% sucrose, the number of leaves present at the end of the experiment was smaller than at the other sucrose

concentrations. This is because 15% had a more damaging effect on the petioles than the other concentration. When 0% sucrose was given, the mean leaf area was relatively small, but at 10% it was equal to that of the control plants. The total area per plant was somewhat smaller than in the control plants, because fewer leaves were present.

The greater dry weight of plants at high light intensities was largely due to the greater leaf area per plants under these conditions. It appeared that the mean leaf area was twice that in plants grown at 22000 erg/cm²/sec.

When plants were given sucrose, their stems were somewhat longer than in plants without sucrose. The intensity of the illumination, however, had no such influence.

From these experiments, the conclusion may be drawn that when energy is supplied in the form of sucrose, it is used for flower initiation and stem elongation; when supplied in the form of illumination at high light intensity with mercury lamps, it is used to increase leaf area. It must be remembered, however, that the latter effect may be due not to high light intensity *per se*, but to the quality of the radiation.

6. CONCLUSIONS

Starch hydrolysis during the dark period in spinach appears to be unaffected by weak illumination. During illumination in the middle of the night at 12600 erg/cm²/sec there was some synthesis of starch, and this illumination results in flowering, though no causal relationship is necessarily implied by this. CLAES and MELCHERS (1949) found no influence of illumination in the middle of the dark period on starch synthesis or hydrolysis in *Xanthium*, although the flowering response of the plants was affected by the illumination. Starch hydrolysis during the dark period at different temperatures always shows a minimum at 9 ° and an optimum between 13 ° and 20 °. Flowering response, however, had no minimum at 9 °. It seems reasonable to conclude, therefore, that flowering response is at any rate not directly determined by the starch-sugar balance. Indeed, it is doubtful whether this balance even plays a subordinate rôle in flowering, but the evidence presented here is insufficient, for any categorical remarks to be made on this point.

Respiration in both spinach and *Hyoscyamus* was similar. It increased with temperature, but there was a small decrease in Q 10 with increasing temperature. Between 2 ° and 25 ° CO_2 out put per unit leaf area was the same for both species, though at a dry weight basis, that of *Hyoscyamus* was smaller than that of spinach. Since flowering response in relation to temperature was different for both species, we may conclude that, as in the case of the starch-sugar level, respiration plays no predominant rôle in the processes governing flowering.

Since the ratio between dry weight and leaf area was the same at different daylengths, it follows that the increase in dry weight of spinach, measured per unit leaf area, was not influenced by daylength. This ratio increased with age, however, thus indicating that photosynthesis per unit leaf area increased during the experiment. Flower initiation had no influence on the ratio. Although the ratio of dry weight to leaf area was not influenced by the daylength, the appearance of the plants at different daylengths varied greatly; those at daylength of 14 and 18 hours both made stems and flowers, while in those at a daylength of 10 hours leaves only were found. It can be concluded from this, that a

mechanism exists which controls the distribution and use of photosynthates. This conclusion is reinforced by a comparison of the influence of sucrose application with that of illumination at high light intensities. When the plants were fed sucrose, the energy supplied was used to increase the length of the stems, and, to a lesser degree, to initiate flowers. High light intensity, on the other hand, chiefly effected an increase in mean leaf area and, as a conseqence of this, plants dry weight, to a much greater extent than when the plants were treated with sucrose.

CHAPTER VI

DISCUSSION AND SUMMARY

1. GENERAL DISCUSSION AND CONCLUSIONS

In this discussion the principal aim will be to review, against the background of previous work, those results of the present investigation which may throw some light on the relation between photoperiodic effects and metabolism in plants. There are several reasons for supposing that flower initiation is the result of several interrelated reactions (HAMMER [1940], GREGORY (1948], LANG [1952]). In the present investigation an approach to a closer examination of these reaction was made by studying their temperature dependence. For a number of reasons attention was paid primarily to the influence of temperature on processes going on during the dark period; it has been repeatedly demonstrated that temperature during the day has little effect on flower initiation (HAMNER and BONNER [1938], PARKER and BORTWICK [1939]). The influence of photosynthesis, for example, is probably not of fundamental importance *per se*. Nevertheless, although the absolute level of photosynthesis may have only a modifying influence on the photoperiodic reaction, there are indication that processes connected with photosynthesis may play a more decisive rôle. HARDER and VON WITSCH (1941) induced flowering in *Kalanchoe* by exposing a single leaf to a short day. When this leaf was deprived of CO_2, no flowers were initiated. In this case, the agent normally formed in the leaf under short day conditions, was either not produced in, or not transported from, the other parts of the plants which received a long day in the presence of CO_2. Similarly HAMNER (1940) destroyed the flowering impulse by giving the short-day plant soya, cycles of 3 minutes light and 3 hours darkness. Plants treated in this way flowered, if a long dark period was preceded by a short day. PARKER and BORTHWICK (1941) illuminated soya for different periods of the day at low CO_2 concentrations or at low light intensities. By this treatment the number of flower primordia decreased. When low light intensities or a CO_2 limitation were applied for 7 to 8 hours per day, no flowers were initiated. HARDER and GÜMNER (1952) were able to induce flowering in Kalanchoe by an illumination of only 1 second per day. MELCHERS and LANG (1941) found that when sugar was applied to *Hyoscyamus*, flower initiation was slightly promoted. In the present study this indication was confirmed in the case of spinach. The results of all these experiments suggest that if photosynthesis indeed is involved in flower initiation, its rôle is not in any way quantitative. Other experiments in this study demonstrated that the way in which energy is supplied may have a more marked influence on flower initiation than the amount of energy received, and may also

affect the form of the plant. Thus there was a difference in flowering response between plants receiving energy in the form of sucrose, and those receiving relatively more energy in the form of high light intensity. Those receiving sucrose initiated flowers somewhat earlier than those receiving high light intensity. Further, there was no difference in flowering response between plants at high and at low intensities, but at a high light intensity mean leaf areas were twice as great as at a low intensity. The energy supplied, thus, was used not to promote flowering, but to increase leaf area. In these experiments, for low light intensities, fluorescent tubes were used, while high intensity illumination was supplied by high pressure mercury vapour lamps. In view of the differences in wave length of the radiation from these two light sources, it may be that the reactions of the plants were influenced more by the spectral composition of the lamps used, than by the amount of energy supplied.

The independence of photosynthesis and daylength was also demonstrated in another way, by measuring plants dry weight in relation to leaf area and flower development. It then appeared that the ratio between dry weight and leaf area was the same for plants at widely different developmental stages, resulting from different daylength treatment. Since photosynthesis determines the amount of dry matter produced, it must be assumed that the photosynthesis per unit leaf area in these plants was the same under all daylength conditions. It can be concluded that, in this case, the way in which the energy supplied was used depended on the daylength.

Turning to the experiments in which the influence of temperature during the night was studied, it has been adequately demonstrated in the present study that processes going on during the dark period have a profound influence on flower initiation. When spinach was illuminated for either 10 or 11 hours consecutively, together with 1 hour in the middle of the dark period, no flowers were initiated. Thus, during the intervening dark hours processes must go on which cause the illumination to become flower inducing. During the dark period a marked influence of temperature was found, but only when extended over a long period. When, however, temperature treatments were given for only $^3/_4$ of an hours, no influence could be detected. It follows that the dark processes probably run at a low rate. The finding that supplementary illumination before or after the middle of the dark period diminishes its effect, suggests that the concentration of the products of these reaction changes during the night, and that it is greatest at the mid-point of the dark period. VAN DE SANDE BAKHUIZEN (1951) has evolved a theory of the photoperiodic mechanism in which he suggests that after the middle night, flower inducing substances are transformed into leafforming substances. This is a difficult hypothesis to test, since under most conditions flowering and leaf number are related. It would be of interest to know whether the influence of temperature is the same on processes occurring before and after the middle of the dark period. Spinach, however, is not a very suitable species for this purpose, because differences in flowering response between 13° and 27° are very small. In the present work, for example plants flowered at about the same time over a range of temperatures during supplementary illumination and during darkness between 13° and 27°.

Further evidence of the complexity of the reaction preceding flower initation, can be deduced from the experiments in which spinach, *Hyoscyamus* and *Brassica* were exposed to various night temperatures. It was found that the wide range of specific variation in reaction to night temperature precluded the formu-

lation of any simple hypothesis to account for the effect observed. Thus, flower initiation was almost the same in spinach between 13° and 27° C. at all daylengths used. At lower temperatures flowering response decreased with temperature. In *Hyoscyamus*, however, in all cases flowering response decreased greatly with decreasing temperature. At a daylength of 14 hours only plants at 20° and 27° had started to initiate flowers at the end of the experiment. At a daylength of 24 hours all plants at 27° flowered, while at 20° few plants flowered, and at 13° plants had started to initiate flowers. In *Brassica* also flowering response increased with temperature, but all plants flowered at the various temperatures and at all daylengths used.

It is known that in spinach and *Hyoscyamus* (KNOTT [1939] and LANG [1941]) a cold treatment in the beginning of the experiment has a promoting influences on flowering. It was investigated whether the plants also differed in their response to low temperature treatment applied during the first nights of the experiments. Indeed the plants behaved differently. In spinach almost no influence of the cold treatment on any process was found. In *Hyoscyamus* the influence of the cold treatment appeared to be complicated. In every case a retarding influence on flowering was found, but when the cold treatment was prolonged the inhibition decreased relatively. After flowers were initiated a sudden increase in the rate of developing of new leaves was observed, and stems elongated faster than those in the plants that had received no cold treatment in the beginning of the experiment. In *Brassica* the influence of the cold treatment depended on the daylengths used. Thus, a promoting influence of the cold treatment was found if subsequently the plants received night temperatures between 9°–20°; the promoting influence was found at higher temperatures with increasing daylength.

It cannot be claimed that out attempt to identify the processes going on during the dark period, and which determine flowering, have so far met with much success, though a certain amount of evidence as to processes which are *not* primarily involved, has been accumulated. Thus, it has been demonstrated that at any rate in spinach, the temperature relations of starch hydrolysis are quite dissimilar from those of flowering. By inference, therefore, starch hydrolysis is not one of the processes which determine flowering. Similarly, respiration does not appear to play a very important rôle; while spinach and *Hyoscyamus* showed virtually the same relation between temperature treatment and respiration, the same temperature treatment resulted in quite different flowering responses. A relation between respiration rate and photoperiodic reaction has been suggested by ELLIOTT and LEOPOLD (1952). They found that in both long-day and short-day plants, the respiration rate as measured at midday was changed by illumination in the middle of the dark period; in day-neutral plants there was no effect of such illumination. Differences in respiration rate parallel to those in flowering, however, were only found during the first days of treatment and not later. It must be concluded, therefore, that although metabolic reactions may be involved in the processes which lead to flower induction during supplementary illumination and during darkness, there is no direct relation between flower initiation and either starch hydrolysis or respiration.

One of the most striking features of the present work is the demonstration of interaction between illumination during the dark period and temperature in spinach. Thus, while temperature treatment just before a short period of illumination in the middle of the dark period has no effect on flowering response, the same temperature treatments during this illumination period exercise a

profound influence. It follows that at least two processes must be involved during this illumination period, one photochemical and the other biochemical. The photochemical reaction depended on the total amount of energy the plants received; thus, on the product of intensity and time and not on the absolute amount of one or the other. Furthermore it appeared that the greatest effect of the illumination was found if it was given at an energy of 25.10^6–30.10^6 erg/cm^2. When lower of higher amounts of energy were applied, flowering response was less. The influence of temperature on the biochemical reaction appeared to be the same as on those going on during the preceding dark period.

From the consideration of the various experimental results obtained in the present investigation, together with those of previous workers in this field, there can be little doubt about the complexity of the processes of flowering. Not only do a number of interrelated processes go on at the same time, but there may well be specific differences in the balance between these various processes; the limiting reaction may vary not only between different conditions but also between species under the same conditions; such difficulties make it improbable that the relation between photoperiodic effects and metabolism will ever form the subject of a simple theory. The present investigation has demonstrated realistically that the many problems involved will not be solved without much further work.

Finally, the present investigation has demonstrated the importance of the advice of GREGORY (1937): "The method of maintaining all factors at a constant level with the exception of the one studied, is capable of yielding precise information but is necessarily limited to the actual condition of the experiment. As the aim of all investigators is to make generalisations of wide application, the consequences of changes in one factor should be studied at many levels of the other factors; in a word, the interaction of factors is of equal importance to the study of single factors in isolation."

SUMMARY

In this report, experiments concerning the relation between photoperiodic effects and metabolism are described. An attempt was made to investigate the interaction of daylength and night temperature, and some preliminary observations were made to relate observed effects of temperature on plant growth and development to various metabolic functions. The following features emerged:

1. In spinach plants, flowering response increased with night temperature up to 13° C. Between 13° and 27°, influence of night temperature on flowering was small. In *Brassica* and *Hyoscyamus*, flowering response increased with night temperature, over the range 2°–27°.

2. The optimum temperature for leaf development lay between 13° and 20° in spinach and *Brassica;* in *Hyoscyamus* leaf area increased with temperature up to 27°.

3. An initial treatment with nights at 2° inhibited flowering in spinach, proportionally to the number of cold nights given. In *Brassica* flowering was promoted by cold nights, if the subsequent night temperature was between 9° and 20°; the effect of night temperature then increased with daylength. In *Hyoscyamus* flowering was retarded by cold nights; the retarding influence decreased with increasing duration of the treatment. After flower initiation a sudden

increase in the rate of formation of new leaves was found, together with an increase in the rate of stem elongation.

4. In spinach illumination during the night had the greatest influence on flowering if it was given in the middle of the dark period at an energy of 25.10^6–30.10^6 erg/cm^2. If higher or lower energies were given, the flowering response decreased.

5. Temperature modified the response to illumination in the middle of the dark period. Thus, at low temperatures, flowering was retarded. It was concluded that at least two processes must determine flowering; one photochemical and the other biochemical.

6. There were no differences in respiration rates of detached leaves between spinach and *Hyoscyamus* although there were differences in flowering response.

7. In spinach, starch hydrolysis had minima at 9° and 27°, with an optimum between 13° and 20°. This points to the absence of any direct relation between starch hydrolysis and flowering.

8. The ratio between dry weight and leaf area in spinach was not influenced by the length of day.

9. Sucrose applications had a small promoting influence on the initiation of flowers in spinach plants at a daylength of 10 hours. The same influence could not be reproduced by increasing photosynthesis by means of increased light intensities.

The possible interaction between flowering and metabolic processes was discussed, and the conclusion reached that, so far, no evidence has been found suggesting direct relations with any of the processes studied.

ACKNOWLEDGEMENTS

The investigation was supported by a grant from the Netherlands Organization for Pure Scientific Research (Z.W.O.). I am indebted to Dr A. LANG, Pasadena, Dr A. F. SCHOOREL and Ir J. SNEEP, Wageningen who furnished me with seed. I wish to express my great thanks to Mr S. D. RICHARDSON M.A., B.Sc. for correcting the english manuscript and making many valuable suggestions. The study was carried out at the suggestion and under the direction of Prof. Dr E. C. WASSINK to whom I am grateful for continuous interest and stimulating advice.

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