SOME EFFECTS OF LOW, NON-FREEZING TEMPERATURES ON PLANTS

by

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ABSTRACT

Low, non-freezing temperatures can cause both harmful and beneficial effects on plants, and this research was carried out to survey some effects on starch and pigment accumulation. Four species were selected on the basis of photosynthetic biochemistry and major systematic grouping. Zea and Gomphrena possess the C_4-dicarboxylic acid pathway typical of certain families of tropical origin, while Triticum and Phaseolus contain the Calvin cycle alone which is typical of plants originating in temperate regions. Zea and Triticum are Monocotyledoneae while Gomphrena and Phaseolus are members of the Dicotyledoneae.

Plants of each species were subjected to 10 days of cold treatment starting when they were 10, 21 or 35 days old (15, 26 and 40 days old for Gomphrena), and spectrophotometric measurements of starch, chlorophylls a and b, and carotenoids were carried out during the treatments.

The effects of cold temperature depended on species, age, and duration of treatment. All of the species exhibited a significantly higher level of starch in the cold temperature for at least two of the three ages tested. The most dramatic effect of low temperature occurred in Gomphrena when the starch concentration increased to over 2000 per cent of the concentration attained at the warm temperature. Variations in the effect of cold treatment between the different ages tested were more pronounced in the monocots used than in the dicot species studied and variations due to the duration of cold treatment were observed in Gomphrena and Triticum.

Cold treatment also caused significant reduction in total chlorophylls, chlorophyll a and chlorophyll b in all the species except Triticum. In Zea, the response to cold decreased as the plants aged, and the duration of cold

treatment had a significant effect in Zea and Gomphrena. When the youngest plants only are considered, the response of starch and chlorophyll levels to cold treatment was well correlated with the typical photosynthetic pathway of the species tested.

Low temperature had no significant effect on total carotenoid concentration.

The effect of low temperature on light transmission by young Zea leaves during the first 48 hours of greening was also examined. Chlorophyll a concentration and leaf light transmission were highly correlated and the more convenient transmission measurements can therefore be used to predict leaf chlorophyll concentration. At the warm temperature used, there was a linear increase in chlorophyll concentration after a 2 hour lag period. Preceding cold treatment caused a longer lag period before chlorophyll began to accumulate at the warm temperature. Also, no chlorophyll accumulated, or there was net chlorophyll breakdown at low temperature. Kinetin treatment did not prevent the decrease in chlorophyll concentration at the low temperature.

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ACKNOWLEDGEMENTS

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1. GENERAL INTRODUCTION

In a comprehensive review of the effects of temperature on plant growth, Went (1953) mentioned that perhaps half of the published papers in plant physiology could be quoted since so many contain references to temperature, if only in their description of the experimental conditions. Nevertheless, there is relatively little information available on the response of plants to low, non-freezing temperatures. Furthermore, in spite of the fact that the effects of temperature are largely mediated by their effects on chemical reactions, in only some cases has the biochemistry of the plant been studied in relation to low, non-freezing temperatures. Nevertheless in many cases physiological responses to low non-freezing temperature have been attributed to postulated biochemical changes.

The temperature at which most plants grow ranges from 0° C to 40° C. At very low temperatures, reactions can be inhibited by an inadequate supply of energy because the lower kinetic energy of the reacting molecules results in a decreased rate of reaction. At very low and excessively high temperatures, protein denaturation can occur. As a result of this, a generalized temperature - response curve for the growth rate of plants would be expected to follow the shape of an enzyme temperature - response curve, rising rapidly at low temperatures, reaching an optimum at intermediate temperatures, and then falling at higher temperatures. Many examples of such responses of plant growth to temperature are known (Evans et al., 1964; Went, 1957). It is often found that at low temperatures an enzyme reaction possesses an apparent activation energy higher than expected (Langridge, 1963). This is usually attributed to an increase of intermolecular hydrogen bonding. By this hypothesis, such excessive bonding alters the structure of the enzyme so that the centers of activity lose their specific configurations, or they are

no longer exposed to the substrate (Langridge, 1963).

Temperature changes not only directly alter biochemical activity, but can also lead to physical changes in tissues which can change the equilibrium or rate of an enzymatic reaction. Substrate availability may be affected by permeability changes which occur at low temperature (Kuiper, 1964). Also, carbon dioxide solubility in water is higher than that of oxygen at low temperature, but at higher temperature the reverse occurs. This last effect may be one reason why the ratio of the rates of photosynthesis to respiration is so high at low temperatures. As temperature is increased, respiratory rate is increased relatively more than photosynthesis, thus resulting in lower ratios. This might explain, at least partly, why many plants grow better in temperate regions than in the tropics (Went, 1953).

At low temperatures, the increased amount of carbon dioxide in the cell sap increases its acidity. This will favour carbon dioxide fixation into organic acids which will further increase acidity (Pucher et al., 1948).

As a consequence of such changes in enzymatic activities, as well as alterations of the physical properties of the cell sap, the lowering of temperature may cause changes in the amount and nature of substrates available to the plant. The increase in sugar and acids may account for some of the observed effects of lowered temperature, especially the short term effects on growth.

Our knowledge of the effects of temperature on growth has been greatly expanded by the work of Went and his collaborators. His first major contribution to this area of knowledge was the discovery that temperatures at night were of specific importance in the growth of whole plants (Went, 1944). According to species, variety, and age of the plant, Went identified an

optimal night temperature which was different from and usually lower than the daily optimum (Went, 1956 and 1957). In Zea, the optimal night temperature depended on the diurnal temperature regime in which a particular plant was maintained. For example, a day temperature of 23° C was associated with a nocturnal optimum of 17° C, but when the day temperature was 30° C, the nocturnal optimum was shifted to 20° C. It is not certain whether the beneficial effect of lower night temperatures is due to the absolute values of temperature experienced by the plant or whether the simple experience of some flux of temperature is the beneficial item.

A suitable night temperature may also have beneficial effects of plant development. The earliness and intensity of flowering and fruiting can be affected by night temperature (Went, 1953). For example, in Lolium perenne, L. perenne L.X multiflorum, Dactylis glomerata, Agrostis tenuis, Holeus lanatus, Trifolium repens, T. subterraneium and Lotus uliginosus, lower day temperature gave a much greater relative reduction in growth of individual tillers than did a corresponding reduction in night temperature. In contrast, a lowering of the day temperature had a relatively small influence on the rate of tillering of most species, but in some cases lower night temperatures resulted in substantially higher rates of tillering (Mitchell, 1955 and 1956). In "chili pepper" plants, the optimum night temperatures for stem elongation decreased from 30° C to 8.5° C as the plant progressed to maturity (Dorland, and Went, 1947).

Low non-freezing temperatures during the day may also have beneficial effects on growth. For example, in Poa pratensis, growth at 15° C was greater than at 25° and 35° C (Darrow, 1939, Hiesey, 1953). Bromus carinatus, B. rigidus, B. rubens, Mellica imperfecta, Stipa lipita, Poa scrabella, all grew

well at day/night temperatures of 20°/3° or 6° C or 10° C, but their growth was restricted at 30°/3° (Ashby and Hellmers, 1959).

In many tropical plants death is caused by temperatures well above the freezing point (Went, 1953). This occurs in Coleus, Saintpaulia, Palisota and many other genera, and it is associated with loss of sugar and other changes in the tissues rather than with the formation of ice. By growing Coleus, Saintpaulia and Palisota for a long period at 12° C, they could be made more hardy and able to tolerate the effects of low temperatures (Spranger, 1941). In passing, it is interesting to note that some of the deleterious effects of low temperature on growth can be overcome by chemical treatments (Ketellaper, 1963). The nature of the effective metabolites depends on the species and on the temperature. For example, nicotinic acid stimulated the growth of tomato plants at 20/14° C. Cosmos was stimulated by a mixture of vitamin B at 17/10° C and eggplant by a mixture of ribosides at 20/14° C. These active substances did not promote growth at the optimal temperature (Ketellaper, 1963).

Many investigations have been made on the temperature responses of grasses. All festucoids so far examined grow relatively well at low temperatures, have an optimum temperature for growth below 27° C, and grow poorly at 35° C. The panicoid-chloridoid-eragrostoid grasses, on the other hand, have a higher optimum temperature for growth, growing vigorously at 35° C and extremely slowly at temperatures below 15° C (Cooper and Tainton, 1968; Evans et al., 1964). These differences were consistent when species for the two groups were grown under conditions of fluctuating night and day temperatures. Reductions in growth with lower night temperatures have been observed in many non-festucoid grasses but in festucoid species low night

temperatures often increase growth (Evans et al., 1964). These differences appear to be connected with the evolutionary origin and the region of climatic adaptation of the two groups. Panicoid and chloridoid-eragrostoid grasses are largely tropical in distribution, while festucoids are primarily temperate (Hartley, 1950; Stebbins, 1956; Brown, 1958).

Recent comparative biochemical studies have distinguished basic differences between tropical and temperate grasses in the type of carbon pathway in photosynthesis, the occurrence of photorespiration and the maximum rate of photosynthesis. The panicoid (excluding the temperate subgenus Dichanthelium, (Downton, et al., 1969)) and the chloridoid-eragrostoid grasses exhibit no photorespiration and have a high maximum rate of photosynthesis. They appear to fix most of their carbon through the C_4-dicarboxylic acid pathway (Hatch and Slack, 1966, 1967). They also possess a highly developed parenchymatous sheath around the vascular system in the leaves (Downton and Tregunna, 1968; Johnson and Hatch, 1968). These bundle sheath cells have relatively large specialized plastids which have few grana and tend to accumulate starch (Brown, 1958). Their mesophyll cells, on the other hand, contain chloroplasts which are similar in size and structure to those of plants employing the Calvin cycle only, but these chloroplasts do not accumulate starch.

The festucoid species, on the other hand, exhibit photorespiration, lack the C_4 pathway, and have a lower maximum rate of photosynthesis. They also lack a specialized parenchymatous sheath around the vascular system.

Similar differences in photosynthetic characteristics occur in several families in the Dicotyledoneae. Some members of the Amaranthaceae, Portulacaceae, Chenopodiaceae and certain other Centrospermae exhibit high photosynthetic rates, fix most of their carbon through the C_4 dicarboxylic acid pathway, and lack photorespiration. They also possess a well developed

parenchyma bundle sheath as well as a definite mesophyll layer arranged radially around the sheath. The plastids in the bundle sheath cells are specialized in accumulation of starch, and little or no starch is accumulated in the mesophyll cells (Downton and Tregunna, 1968; Tregunna and Downton, 1967).

Changes in starch and pigment content have been observed after exposure of certain plants to low temperatures, but these changes have not been extensively studied. To some degree, the effects so far observed appear to depend upon the species used and its developmental stage.

Many investigations have been carried out on the effects of low temperature on the accumulation of starch and other carbohydrates. Digitaria decumbens, a tropical grass, shows high starch content at 10° C and growth is severely reduced by this temperature (Hilliard and West, 1969). It was suggested that growth reduction was due to inhibition of night translocation. Similar effects were reported for Cynodon dactylon (Schmidt and Blaser, 1969; McKell et al., 1969), Poa pretensis (McKell et al., 1969) and Dactylis glomerata Eagles, 1967 and 1967 b; Colby and Drake, 1966; Blaser et al., 1966). The accumulation of fructosans at low temperature has been reported for several species including Agrostis tenuis (Schmidt and Blaser, 1969) and Lolium perenne (Sullivan and Sprague, 1949). In Poa pratensis and P. compressa, total carbohydrate accumulation increases under cold conditions (Brown, 1939).

As previously mentioned, the inhibition of the translocation of carbohydrates by low temperatures has been used to explain the accumulation of carbohydrates under these temperatures (Hilliard and West, 1969). There are several reports which attribute a Q10 of one or less to translocation,

and which indicate that the maximum translocation rates occur at low temperature (Went, 1946; Went and Engelberg, 1946; Went and Hull, 1949; Hull, 1951; Goodal, 1946). However, most of the accumulated experimental evidence on this point indicates that translocation of carbohydrates has a Q_{10} greater than one, and that it is greatest at temperatures about 25° C (Hewitt and Curtis, 1948; Swanson and Bohning, 1951; Curtis, 1929; Curtis and Herty, 1936; Veron and Aranoff, 1952).

Carbohydrates in plant tissues are involved in a dynamic system of energy balance. When demand for carbohydrates for growth exceeds photosynthesis, there is an energy loss; conversely carbohydrates accumulate in tissues when demand is low compared with photosynthetic carbohydrate production. Thus it may be postulated that any factor which restricts growth relatively more than photosynthesis, would cause a carbohydrate accumulation in the plants. As the relative rate of photosynthesis is greatest at about 25° C in temperate species and about 35° C in tropical species, Evans (1964) has suggested that accumulation of assimilates is unlikely to occur in tropical species maintained at low temperatures. When stems are chilled, translocation can be inhibited but only during a period of illumination (Hull, 1952). Experiments on the effects of petiole chilling on translocation demonstrated little or no long-term effects of cold treatment (Swanson and Geiger, 1967; Geiger, 1969).

Many tropical plants show severe symptoms of chlorosis and even death when germinated and grown at temperatures between 10° C and 15° C. In temperate plants, on the other hand, chlorophyll remains constant even at the freezing point (Cooper and Tainton, 1968).

In Zea, low night temperatures have been found to have a negligible effect on chlorophyll concentration (Went, 1957; Alberda, 1969). However, when the day temperature was reduced to 10° C, concentrations of chlorophyll dropped considerably in Zea, Pennisetum and Sorghum. In other studies with Zea, the concentration of chlorophyll was negligible at about 15° C (Friend, 1960 and 1966). An inbred mutant of Zea has been isolated which does not form chlorophyll at temperatures as high as 17° C (Millerd and McWilliam, 1968). The reduction of chlorophyll concentration in leaves of Zea was observed in those leaves which elongated during cold treatment (Alberda, 1969). The primary site of the effects of low temperatures appeared to be located near the apex of the shoot (Millerd and McWilliam, 1968). Studies of the effects of temperature on plastid morphology in Zea indicated abnormal grana development at low temperatures. A similar effect was observed in the light at 3° C and at 15° C in darkness (Klein, 1960; McWilliam and Naylor 1967; Millerd et al., 1969).

The chlorosis of thermophilic plants subjected to low temperature may occur because of a depression in the synthesis of chlorophyll or its immediate precursors, or it may be caused by their destruction once they are formed. In Zea, low temperature has been shown to cause a reduction in the rate of photochlorophyllide synthesis and hence in the rate of chlorophyllide accumulation (McWilliam and Naylor, 1967). It is also known that the enzyme that catalyzes the esterification of chlorophyllide to chlorophyll is sensitive to temperature as well as light (Wolff and Price, 1957). However, other studies have shown that in light at 3° C, Zea transforms protochlorophyll into chlorophyll (Klein, 1960). The in situ absorption maximum for chlorophyll at 3° C was shown to differ from the values obtained from plants

grown at 26° C, indicating a different behavior of the chlorophyll produced at low temperature. According to Millerd, the lack of chlorophyll in a mutant of Zea at 16° C was a consequence of a lesion affecting a relatively early stage of either chloroplast development or function (Millerd and McWilliam, 1968). On this basis, the change in the absorption maximum of chlorophyll under cold could be explained by the lack of ordered association of the chlorophyll with other components of the photosynthetic apparatus in the lamellae.

Another possibility is that photooxidation of chlorophyll (or its immediate precursors such as protochlorophyllide) may occur. Evidence for rapid photodestruction has been found in barley (Augustinussen and Madsen, 1965; Virgin, 1955), wheat (Virgin, 1956, 1958) and in corn (McWilliam and Naylor, 1967; Klein, 1960). In corn for example, rapid photooxidation of chlorophyll and carotenoids occurred at 16° C, but the combination of light and low temperature did not damage the early steps leading to chlorophyll production. Bleaching appeared to result not only from the photooxidation of chlorophyll, but of chlorophyllide breakdown at a rate greater than their formation (McWilliam and Naylor, 1967). Once chlorophyll is formed and presumably complexed within the chloroplast lamellae, it appears to be protected from photodestruction. A reduction in carotenoid content may also be a contributing factor in chlorosis because of its role in the protection of chlorophyll from photooxidation (Anderson and Robertson, 1960; Griffiths et al., 1955).

2. The Effects of Temperature and Age on Starch and Pigment Accumulation in *Triticum*, *Zea*, *Phaseolus* and *Gomphrena*

INTRODUCTION

The aim of this research was to survey some of the effects of low, non-freezing temperature on starch and pigment concentrations in plant leaves. It was hoped that the results of this survey would give some indication whether plant response to low temperature is correlated with plant distribution, photosynthetic metabolism and leaf anatomy, or plant age.

To carry out this survey, four species were selected. Two of the species belong to the Monocotyledoneae: *Triticum vulgare* which carrys out photosynthesis by the conventional Calvin cycle, and *Zea mays* which possesses the C_4-dicarboxylic acid pathway (Downton and Tregunna, 1968). Similarly, two species were selected from the Dicotyledoneae: *Phaseolus vulgaris* and *Gomphrena globosa* whose pathways of photosynthetic metabolism resemble those of *Triticum* and *Zea* respectively. Throughout the remainder of this thesis, the Calvin cycle species will be referred to as "temperate", while the C_4 species will be called "tropical". This terminology is used for convenience since the centers of distribution for Calvin cycle and C_4 species are located in the temperate and tropical regions respectively (Cooper and Taiton, 1968). It is nevertheless recognized that the correlation between photosynthetic pathway and the distribution of any particular species is not absolute.

MATERIALS AND METHODS

Seeds of Zea mays L. var. Pioneer, Gomphrena globosa L. var. Amaranth, Triticum vulgare L. var. Spring Thatcher and Phaseolus vulgaris var. Tender Crop Green Pod were obtained from Buckerfield's Seed Co., Vancouver, and were planted in pots in a mixture of two-thirds standard greenhouse soil and one-third sand.

Three kinds of growth chambers were used for this research. One Sherer-Gillett Cell 255-G Chamber and one Controlled Environments Ltd. EF7 Chamber were used for growing most of the plants until the experiment was started. The Sherer-Gillett Chamber contained eight 80 watt cool white fluorescent lamps and four 25 watt incandescent lamps. It had an interior floor space of 0.25 m^2 and an interior height of 0.65 m and had no humidity control. The Controlled Environments chamber contained eight 80 watt cool white fluorescent lamps and four 25 watt incandescent lamps. It had an interior floor surface of 0.65 m^2 and an interior height of 0.97 m. Some plants were also grown in the Percival chambers described below.

For the experimental treatments, the plants were transferred to two Percival Refrigeration and Manufacturing Co. PGC-78 plant growth chambers. The light system in these chambers was composed of sixteen 150 watt cool white fluorescent lamps and ten 25 watt incandescent lamps. A translucent light barrier was situated just below the light system. There was no humidity control, and, according to the manufacturer's specifications, the humidity varied from 50 to 70% R.H. depending on the ambient condition outside the chamber.

The light intensity during plant growth and during the experimental treatments was approximately 11000 lux at 30 cm above the top of the pots.

Measurements showing the vertical gradient in light intensity in the Percival growth chambers are given in Appendix I. The photoperiod was 16 hours throughout this experiment.

During growth in the Sherer-Gillett and Controlled Environment chambers, the day/night temperature conditions were 25-28°/18-22° C as indicated by thermometers suspended in the chambers. During the experimental treatments in the Percival chamber, 25-28°/18-22° C was used for the day/night regime for the warm temperature (control) conditions, and 7-11°/3-8° C were the cold temperature day/night conditions. The plants were watered daily, and NPK nutrient solution (20,20,20 per cent) was applied once a week.

For this experiment the plants were transferred to the warm or cold temperature conditions when they were 10, 21 or 35 days old (15, 26 or 40 days old for Gomphrena), and they were kept under treatment for ten days thereafter. Leaf samples were collected and measurements of pigment and starch content were carried out just before the plants were exposed to the treatment conditions, and at 5 and 10 days after the treatment was initiated.

The design of this experiment was a two factor (temperature and age) factorial, with split plot for the three times at which chlorophyll and starch measurements were made. The layout was a randomized complete block design. Each experimental unit had a replicate number of six. Results from preliminary trials with 3 week old Zea and Triticum plants, which had been exposed to cold or warm temperature treatments for one week, were used to determine the number of replicates in this experiment.

For starch determination, a modification of Pucher's method was used, in which starch is solubilized with acid and stained with iodine. (Pucher et al., 1948; Carter and Neubert 1954; Hassid and Neufeld, 1964). Since

starch content in plants varies during the day, preliminary tests were carried out to determine the best time for sample collection. Three tests a day were performed for each species when they were 10 and 21 days old, and grown under the warm condition. For these preliminary tests, starch was estimated out in the following way. Leaf samples were plunged into boiling water for 30 seconds and then placed in cold methanol to decolorize. After decolorization, the leaves were floated on an iodine solution prepared as follows: 11 gm of iodine and 22 gm of potassium iodide were dissolved in water and the solution was diluted to 500 ml. 2 ml of this stock solution and 20 gm of potassium iodide were then dissolved in water and made up to 500 ml. This dilute iodine solution constituted the test solution used (Sandsted et al., 1939). Triticum showed no starch in any of the tests. Zea showed a slight coloration in the oldest plants at any time in the day. Gomphrena and Phaseolus gave positive results at any age and at any time of the day. From this, it was decided to collect the samples for the main experiment at noon (six hours after the onset of daily illumination).

For the starch measurements, a different number of plants was used for each sample according to the species and developmental stages, as shown below:

TABLE 2-1 NUMBER OF PLANTS USED

Species	10 Days Old	21 Days Old	35 Days Old
Triticum	10	4	1
Zea	4	1	1
Phaseolus	3	1	1
Gomphrena*	20	10	1

*For this species the plants were 15, 26 and 40 days old.

After collection, the leaf samples were dried overnight in an oven at 85-90° C. The following morning they were ground in a mortar and pestle and weighed. 200 mg dry weight of plant material were used for each starch determination. Each weighed sample was then placed in a 50 ml centrifuge tube and mixed with 4 ml of distilled water. Following this, the tubes were placed in a boiling water bath for 15 minutes to allow the gelatinization of the starch. They were then cooled in an ice bath for approximately ten minutes. Three ml of 72% perchloric acid were added and mixed with the plant material to solubilize the starch. The mixture was allowed to extract for 20 minutes and was then centrifuged. The supernatant was poured into a 50 ml volumetric flask, and 3 ml of acid were added and mixed with the residue to complete the extraction. After 20 minutes, the samples were centrifuged again and the supernatants were combined and diluted to 50 ml with distilled water. Supernatants stored at 4° C showed no change in absorbance for two days after extraction. Extracts were further diluted when the soluble starch concentration was very high. 0.05 ml of iodine solution prepared from 15 ml of the stock solution described previously and 8 gm of potassium iodide dissolved in 200 ml distilled water, were added to 5 ml of the extract, and the absorbance of this mixture was immediately measured in a Beckman DU spectrophotometer at 620 nm. The amount of starch present was determined by referring to a standard curve, which was prepared using purified potato starch (ACS) treated according to the above methods (Figure 2-1).

The procedure for the extraction and measurement of pigments was based on the method of Bruinsma (1963). Leaf samples were collected and their surface area and fresh weight was determined. The size of the sample used depended on plant age and species as follows:

TABLE 2-2 FRESH WEIGHT (gm) OF LEAF SAMPLES FOR PIGMENT DETERMINATION

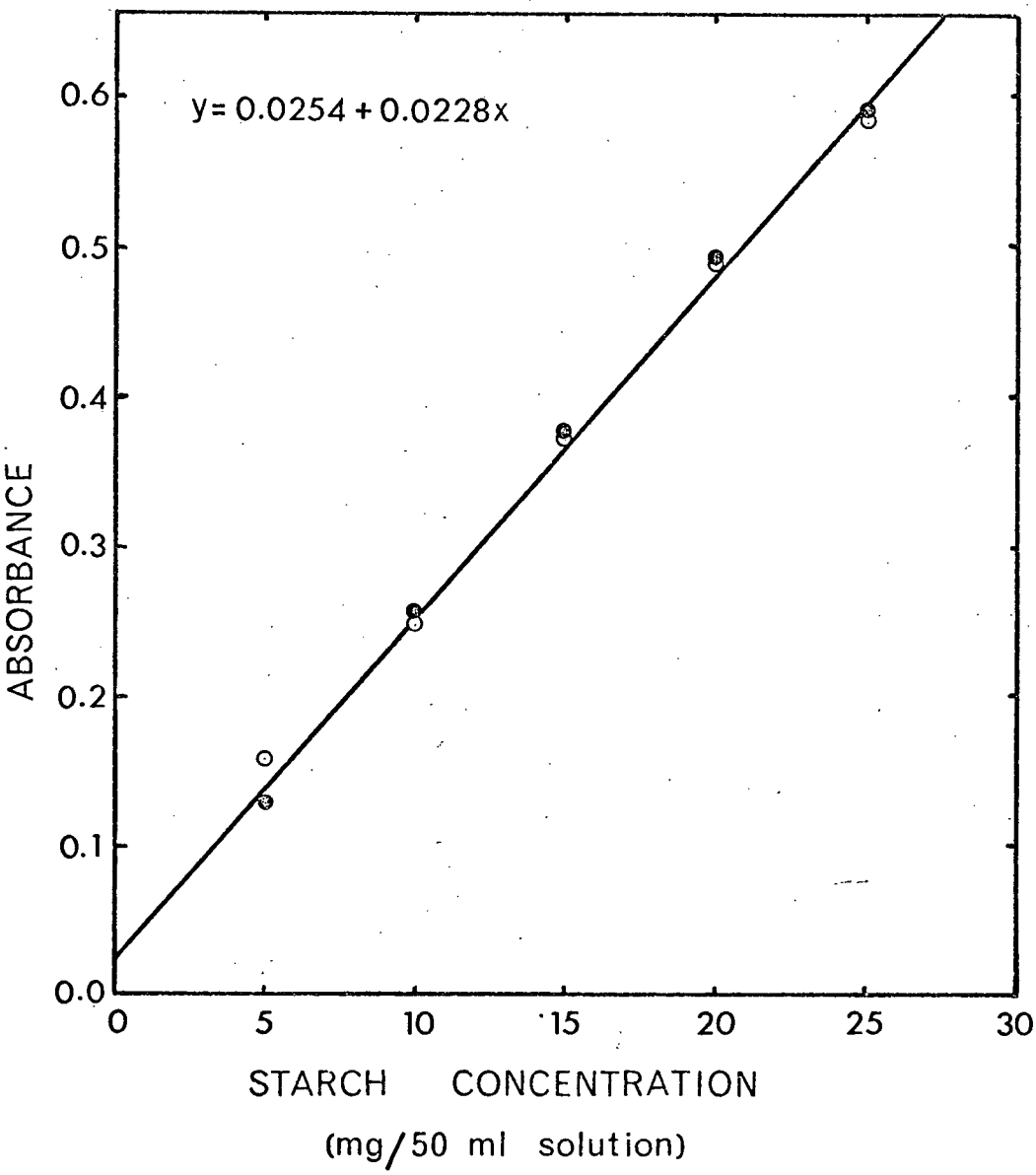
Species	Plant Age - Days 10	21	35
Triticum	0.3 (32.5)*	0.2 (32.5)	0.1 (50.0)
Zea	0.5 (32.5)	0.3 (53.5)	0.5 (95.0)
Phaseolus	0.5 (17.5)	0.3 (31.0)	0.5 (32.5)
Gomphrena**	0.2 (2.5)	0.3 (3.76)	0.2 (10.00)

*Numbers in parenthesis are the heights of the plants in cm.

**In this species, the plants were 15, 26 and 40 days old.

Figure 2-1

Effect of potato starch concentration on the absorbance of aqueous iodine-potassium iodide-starch mixture at 620 nm. Solid and open circles represent replicate determinations at each concentration tested.


y= 0.0254 + 0.0228x
ABSORBANCE
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
5
10
15
20
25
30
STARCH CONCENTRATION
(mg/50 ml solution)

For the ten day old plants, entire leaves were used, but for the older plants, the leaf samples consisted of segments cut from the central portion of the leaf (intermediate between the leaf base and tip).*

Each leaf sample was cut into small pieces and mixed with 50 ml of cold (4° C) 80% aqueous acetone (reagent grade ACS), the mixture was then blended at high speed in an Osterizer blendor for 0.5 to 2 minutes and the resulting slurry was quantitatively filtered into a volumetric flask. The residue on the filter was washed several times with additional cold 80% aqueous acetone until all the chlorophyll was extracted. The extract was diluted to 100 ml (200 ml for Zea samples) by adding more cold 80% aqueous acetone. The extracts were then stored in darkness at 4° C.** until spectrophotometric analysis was carried out.

To determine the concentration of chlorophyll a, chlorophyll b and total chlorophyll, 5 ml of the aqueous acetone extract was used and its absorbance was measured at 645, 652 and 663 nm using a Beckman DU spectrophotometer. The pigment concentrations were then calculated using the following equations (Bruinsma, 1963):

1) Chl. a (mg/l) = $12.7\ OD_{663} - 2.7\ OD_{645}$

2) Chl. b (mg/l) = $22.9\ OD_{645} - 4.7\ OD_{663}$

3) Total Chl. (mg/l) = $20.20\ OD_{645} + 8.02\ OD_{663}$

4) Total Chl. (mg/l) = $1000\ \frac{OD_{642}}{36.0}$

Where OD (optical density or absorbance) = $\log \frac{I_o}{I}$; I and I_o are the intensity of the transmitted and incident light respectively. Formula (3) resulted from the addition of 1 and 2. The absorption curves for chlorophyll a and chlorophyll b (specific absorption coefficient vs. wavelength) intersect at

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* See App. II

** See App. III

652 nm at a common specific absorption coefficient α of 36.0. The amount of total chlorophyll can therefore also be determined by measuring the absorbance at 652 nm and computing the concentration of total chlorophyll as given in Formula 4. The average of the results obtained using Formulae 3 and 4 were used to compute the total chlorophyll concentration.

The solution used for chlorophyll determination was also utilized for the total carotenoid estimation. Solution absorbance was measured in the spectrophotometer at 440.5 nm and the following equation was used (D. von Wettstein, 1957):

$$\text{Car. (mg/l)} = 4.695\ OD_{440.5} - 2.68\ C_{(a + b)}$$

Leaf pigment concentrations were expressed both on a fresh weight and leaf area basis. In this way, the more constant unit in the cold and warm conditions could be used to compare the data.

A multifactorial analysis of variance was carried out to determine the significant effects of age, temperature and duration of treatment. Whenever differences were detected at the 5% level, Duncan's new multiple range test was carried out to determine which conditions differed from each other.

For each set of replicates, 95% confidence limits were also calculated, and these are indicated by the vertical lines about sample means in many of the figures in the following sections.

RESULTS

(i) Effects of temperature and age on starch content.

Figures 2-2 and 2-3 show that in warm conditions, the accumulation of starch was consistently low except in Phaseolus throughout all the ages tested, and in Zea in the 20-21 day old plants. In Phaseolus, the mean starch concentrations ranged from 563 to 982 mg per g dry weight and were high in both the primary and trifoliate leaves. In Zea, a peak of over 300 mg starch per gm dry weight was observed at about 20 days, and younger and older plants contained significantly less starch. In all other cases, the mean starch concentrations were below 200 mg/g in the warm temperature conditions. In Triticum starch was practically absent; the highest concentration observed was about 30 mg/g in the youngest plants. In Gomphrena, the highest concentration found was 153 mg/g in the 26 day old plants, and the younger plants contained significantly less starch.

Low temperature tended to increase starch accumulation and the magnitude of the increase depended on plant species, age, and time of exposure to cold. Gomphrena showed a very great increase in starch concentration in response to cold, and the quantity of starch accumulated increased significantly when the time of exposure to cold was extended from 5 to 10 days. In Phaseolus, small significant differences in starch concentrations occurred in the youngest plants after 5 days of treatment, and in the intermediate age. In this case, there was no significant effect of duration of cold treatment. Triticum exhibited significantly higher starch accumulation in the cold except in the youngest plants used after 5 days of treatment. In the intermediate age, the concentration of starch accumulated increased with time of exposure to cold, but even under cold conditions, only quite low levels of starch content were attained. In Zea, cold had significant

Figure 2-2

Starch concentrations in 10, 21 and 35 day-old Triticum (a) and Zea (b) plants subsequently exposed to warm and cold conditions for 0, 5 or 10 days.

● Warm temperature

○ Cold temperature

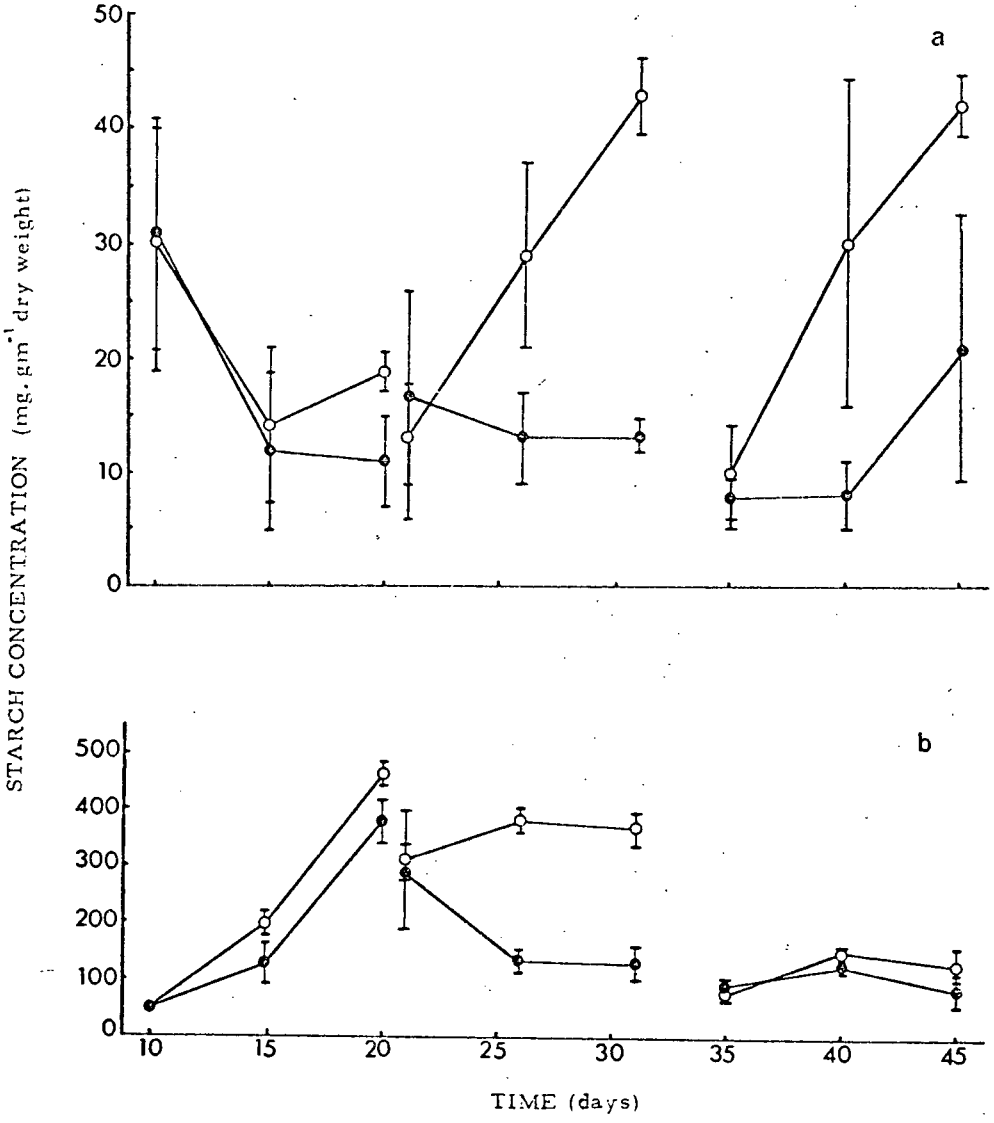
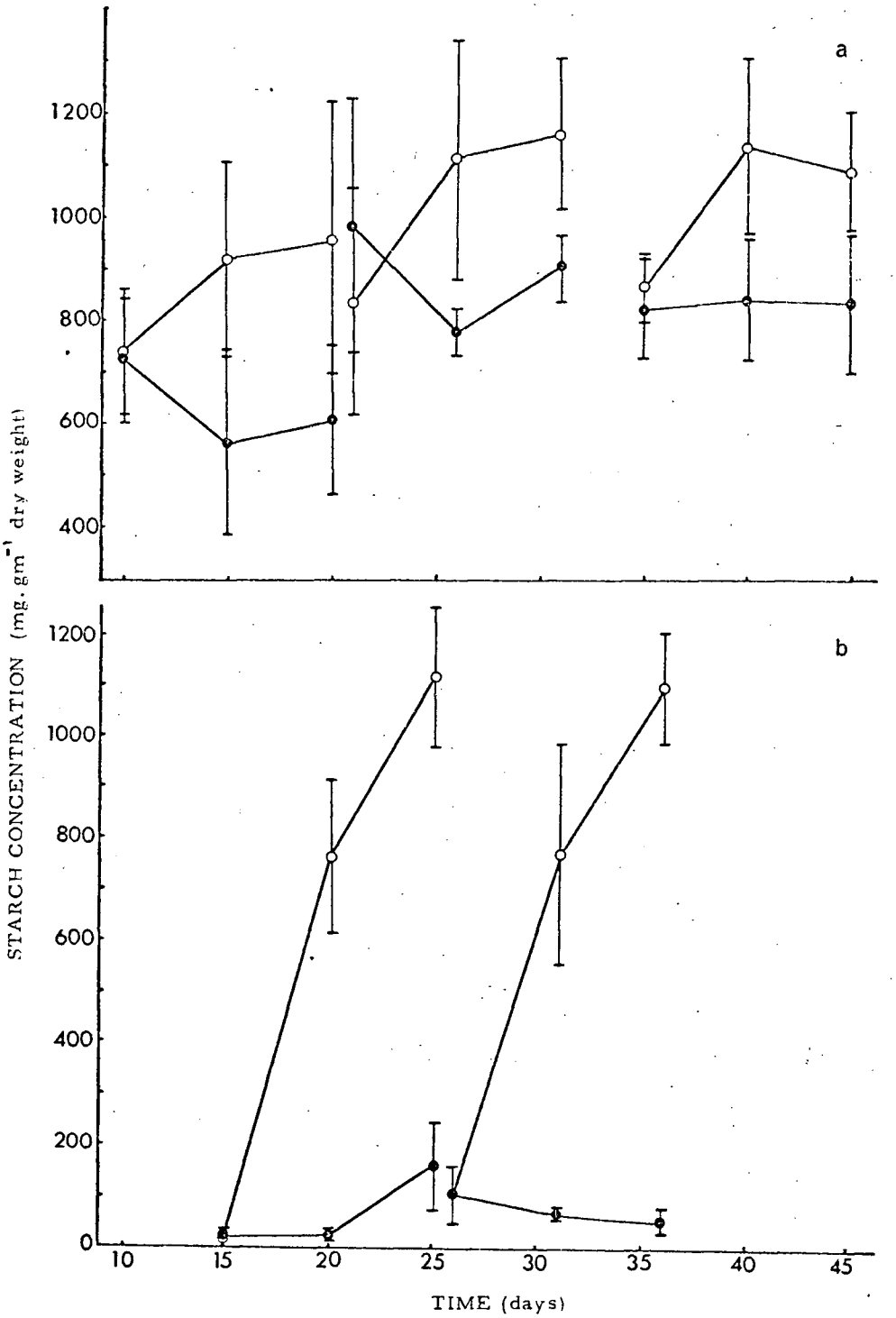

a
b
STARCH CONCENTRATION (mg.gm^{-1} dry weight)
TIME (days)
0
10
20
30
40
50
100
200
300
400
500
10
15
20
25
30
35
40
45

Figure 2-3

Starch concentrations in 10, 21 and 35 day-old Phaseolus (a) plants, and 15 and 26 day-old Gomphrena (b) plants subsequently exposed to warm or cold conditions for 0, 5 or 10 days.

● Warm temperature

○ Cold temperature


a
b
1200
1000
800
600
400
200
0
10
15
20
25
30
35
40
45
STARCH CONCENTRATION (mg.gm^{-1} dry weight)
TIME (days)

effects in the two youngest ages, but not in the oldest plants. In the youngest age, the differences between the warm and cold treatment were not great, but in the intermediate age, the starch concentration in the cold plants remained constant while in the warm plants it dropped appreciably, thus resulting in large differences.

(ii) The Effects of Temperature and Age on Pigment Accumulation.

a. Triticum vulgare

The behavior of Triticum depended to some extent on the units used to express chlorophyll concentration. As shown in Figures 2-4 and 2-5, the total chlorophyll and chlorophyll a and chlorophyll b concentrations per gram fresh weight increased greatly in the 26 to 31 day old plants. Following this increase, high levels of chlorophylls were maintained in the older plants. Although the chlorophyll concentrations per gram fresh weight were slightly lower for the cold-treated plants, the effect of temperature was not significant at the 5% level.

When the chlorophyll concentrations were expressed on a leaf area basis, however, no increase in the level of chlorophylls was evident until the plants were 40 to 45 days old. Once again, low temperature did not significantly affect the total chlorophyll or chlorophyll a and b concentrations.

The total carotenoid level was not significantly affected by temperature. Carotenoid content increased with age when expressed on a per gram fresh weight basis, but did not change appreciably when leaf area was the basis of expression.

Figure 2-4

Total chlorophyll (a) and total carotenoid (b) concentrations in 10, 21 and 35 day-old Triticum plants subsequently exposed to warm or cold conditions for 0, 5 or 10 days.

●▲ Warm temperature

○△ Cold temperature

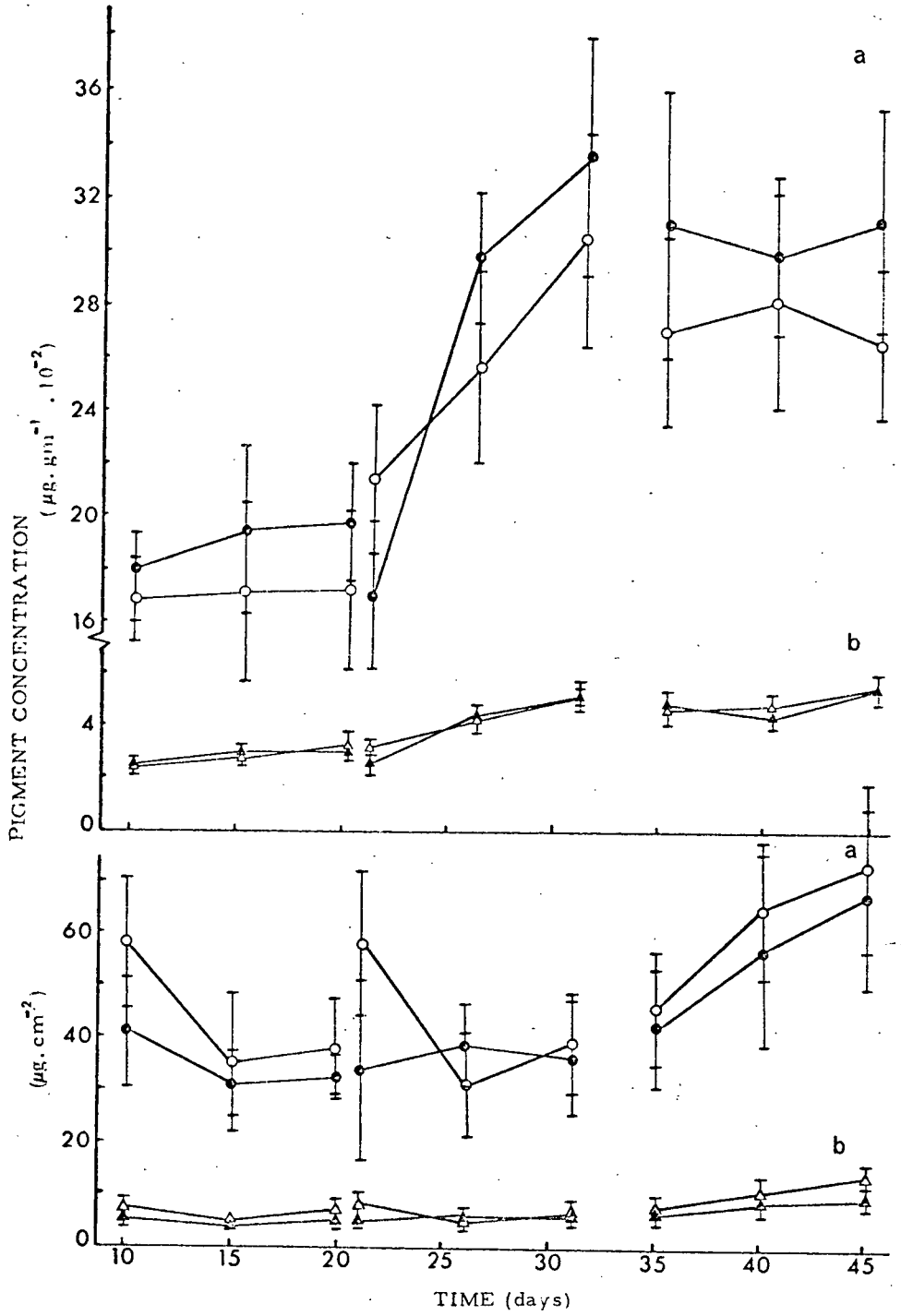
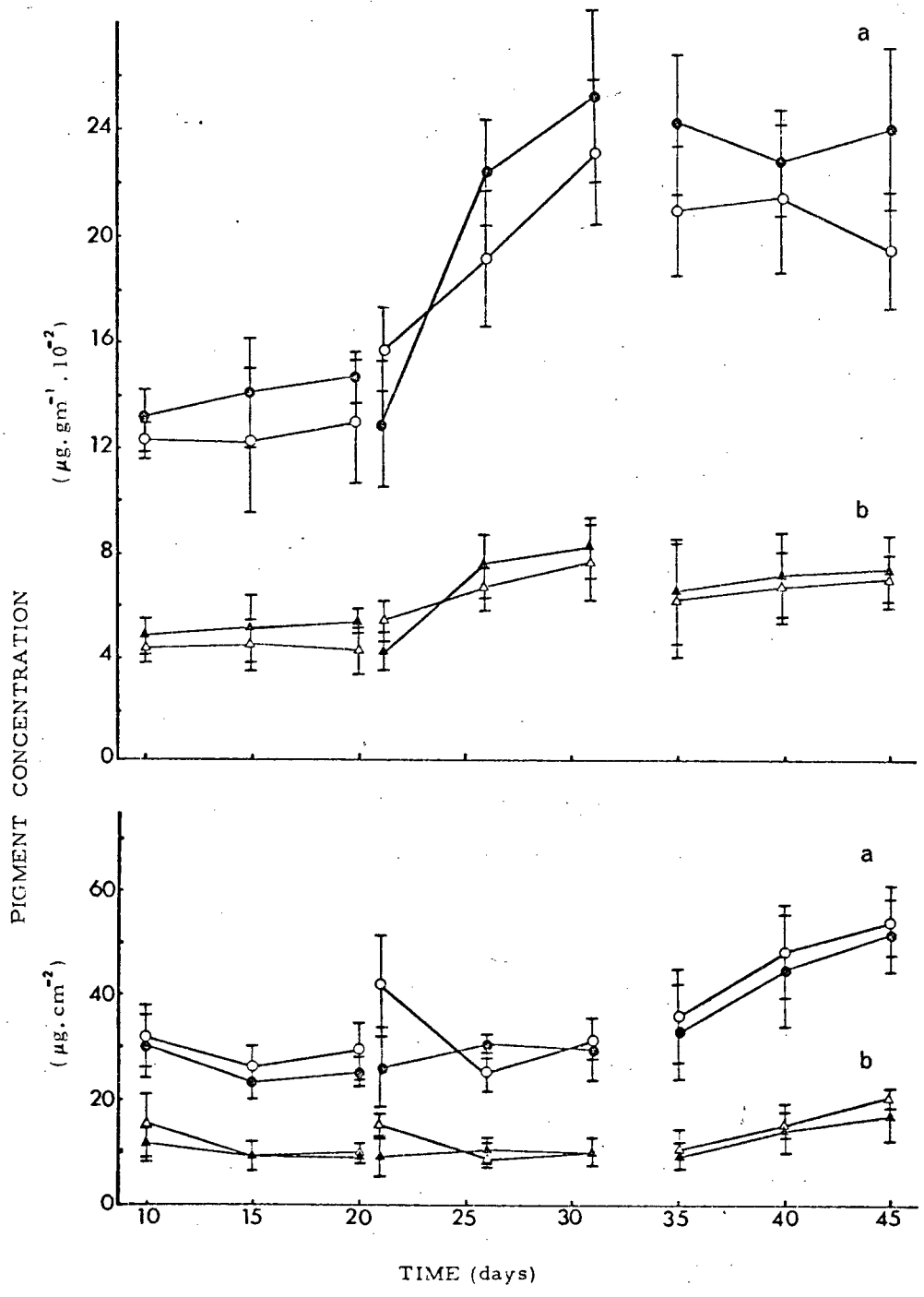

PIGMENT CONCENTRATION
(µg.gm⁻¹ .10⁻²)
36
32
28
24
20
16
4
0
a
b
(µg.cm⁻²)
60
40
20
0
a
b
10
15
20
25
30
35
40
45
TIME (days)

Figure 2-5

Chlorophyll a (a) and chlorophyll b (b) concentrations in 10, 21 and 35 day-old Triticum plants subsequently exposed to warm or cold conditions for 0, 5 or 10 days.

●▲ Warm temperature

○△ Cold temperature


a
b
a
b
24
20
16
12
8
4
0
60
40
20
0
10
15
20
25
30
35
40
45
PIGMENT CONCENTRATION
(μg.gm⁻¹.10⁻²)
(μg.cm⁻²)
TIME (days)

b. Zea mays

Figures 2-6 and 2-7 show the chlorophyll and carotenoid concentrations in leaves of Zea after warm or cold temperature treatments at different ages. At the warm temperature, the total chlorophyll concentration generally tended to decrease as the plants aged, with a particularly large drop occurring in plants which were older than 31 days.

On exposure to cold temperature, the total chlorophyll concentration was reduced in all the ages studied. Quite similar responses were found in the two youngest ages studied. For these plants, the difference in chlorophyll concentration between the warm and cold temperature treatments increased with the duration of the treatment and were significant at the 5% level. For the oldest set of plants, however, cold treatment had little or no significant effect on total chlorophyll accumulation. The small difference which was evident after 10 days of treatment of the oldest plants was not significant if the chlorophyll concentrations were expressed on a leaf area basis.

Figure 2-7 separates the effects of temperature and age on chlorophylls a and b. The changes in chlorophyll a closely followed the changes in total chlorophylls. This was expected since chlorophyll a constituted 60-90% of the total. Chlorophyll b, on the other hand, was less sensitive to temperature than chlorophyll a, although it also exhibited a decline with age. For chlorophyll b, low temperature caused a significant reduction only in the two youngest sets of plants, and then only after they had been under treatment for 10 days.

Figure 2-6 shows that carotenoid concentration remained relatively constant and was not significantly affected by temperature or age.

Figure 2-6

Total chlorophyll (a) and total carotenoid (b) concentrations in 10, 21 and 35 day-old Zea plants subsequently exposed to warm or cold conditions for 0, 5 or 10 days.

●▲ Warm temperature

○△ Cold temperature

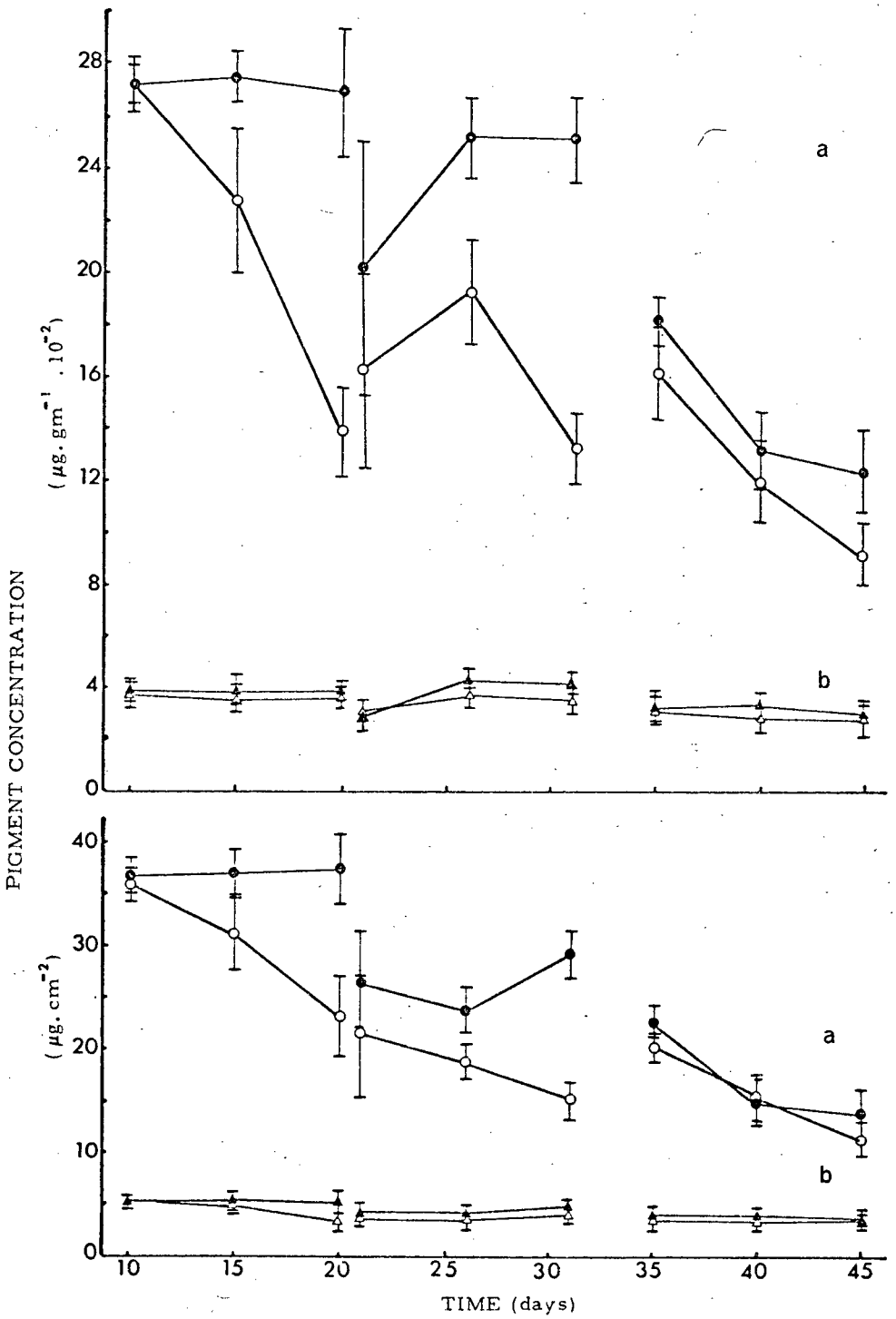
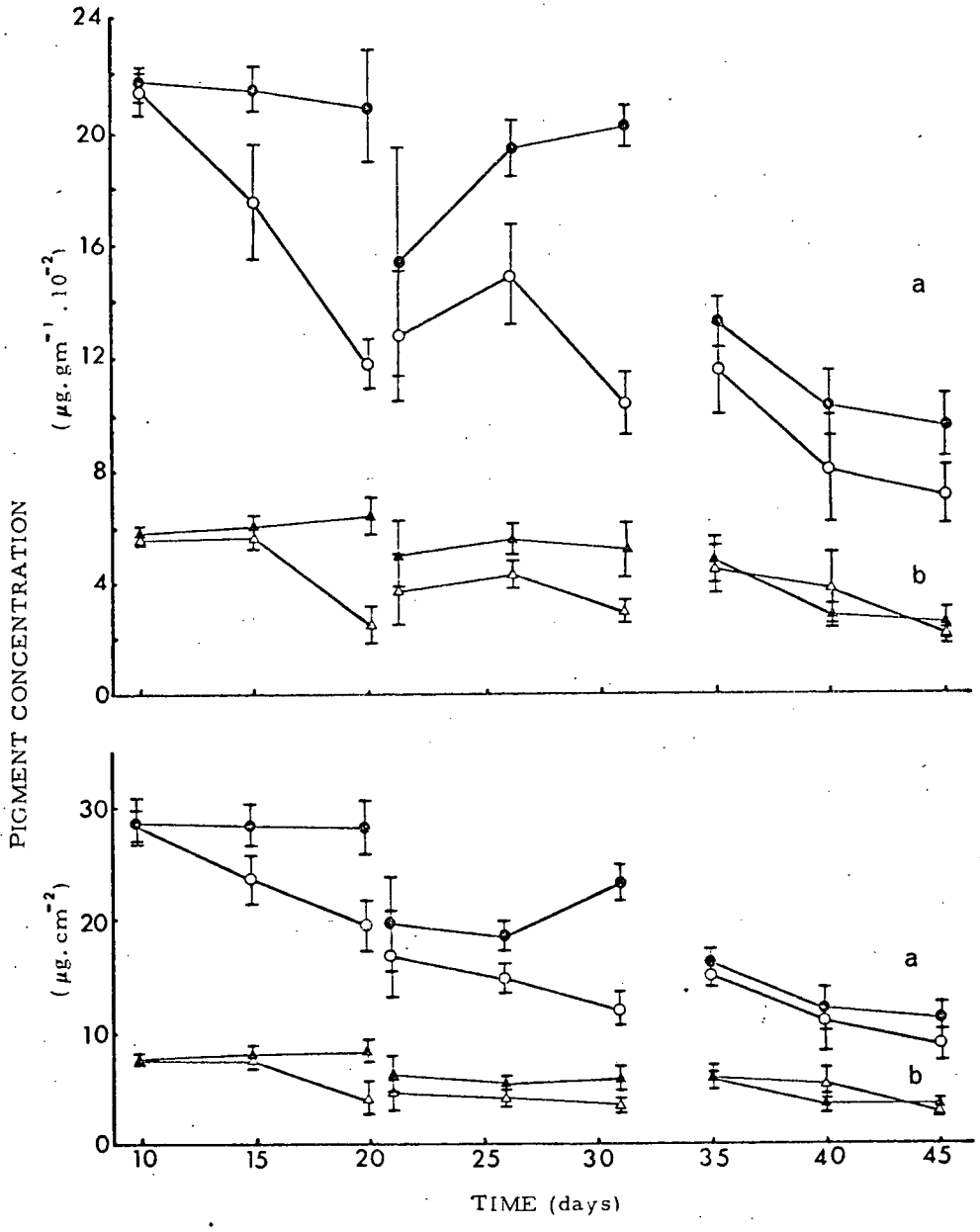

PIGMENT CONCENTRATION
(μg.gm⁻¹ .10⁻²)
28
24
20
16
12
8
4
0
a
b
(μg.cm⁻²)
40
30
20
10
0
a
b
10
15
20
25
30
35
40
45
TIME (days)

Figure 2-7

Chlorophyll a (a) and chlorophyll b (b) concentrations in 10, 21 and 35 day-old Zea plants subsequently exposed to warm or cold conditions for 0, 5 or 10 days.

●▲ Warm temperature

○△ Cold temperature


PIGMENT CONCENTRATION
(μg.gm⁻¹.10⁻²)
(μg.cm⁻²)
a
b
a
b
TIME (days)
0
4
8
12
16
20
24
0
10
20
30
10
15
20
25
30
35
40
45

c. Phaseolus vulgaris

Figure 2-8 indicates that the total chlorophyll concentration in the warm Phaseolus plants did not change greatly with age. The total chlorophyll level was lower in the cold-treated plants in all cases, but this reduction was significant only for the two oldest sets of plants after both 5 and 10 days of exposure to cold. The size of the reduction in chlorophyll concentration was similar for cold treatments of 5 or 10 days duration. Figure 2-9 shows that the reduction in total chlorophyll in Phaseolus may be attributed to reductions in both chlorophyll a and chlorophyll b. The total carotenoid concentration was not significantly affected by age or cold treatment.

d. Gomphrena globosa

Figure 2-10 shows that when the results were expressed on a per gram fresh weight basis, there was a general increase in total chlorophyll concentration during development of Gomphrena in warm conditions. Cold treatment reduced the total level of chlorophyll at all ages, and the reduction was greatest in those plants which were exposed to cold for 10 days. The difference in total chlorophyll concentration between the warm and cold treated plants were significant except for the two oldest sets of plants when they had been under cold treatment for only 5 days. This response of total chlorophyll to cold temperature was related to reductions in the levels of both chlorophyll a and chlorophyll b as indicated by the results in Figure 2-11.

When the total chlorophyll, chlorophyll a and chlorophyll b concentrations were expressed per unit leaf area, a very different pattern was obtained. Total chlorophyll content remained relatively constant except in the oldest plants

Figure 2-8

Total chlorophyll (a) and total carotenoid (b) concentrations in 10, 21 and 35 day-old Phaseolus plants subsequently exposed to warm or cold conditions for 0, 5 or 10 days.

●▲ Warm temperature

○△ Cold temperature

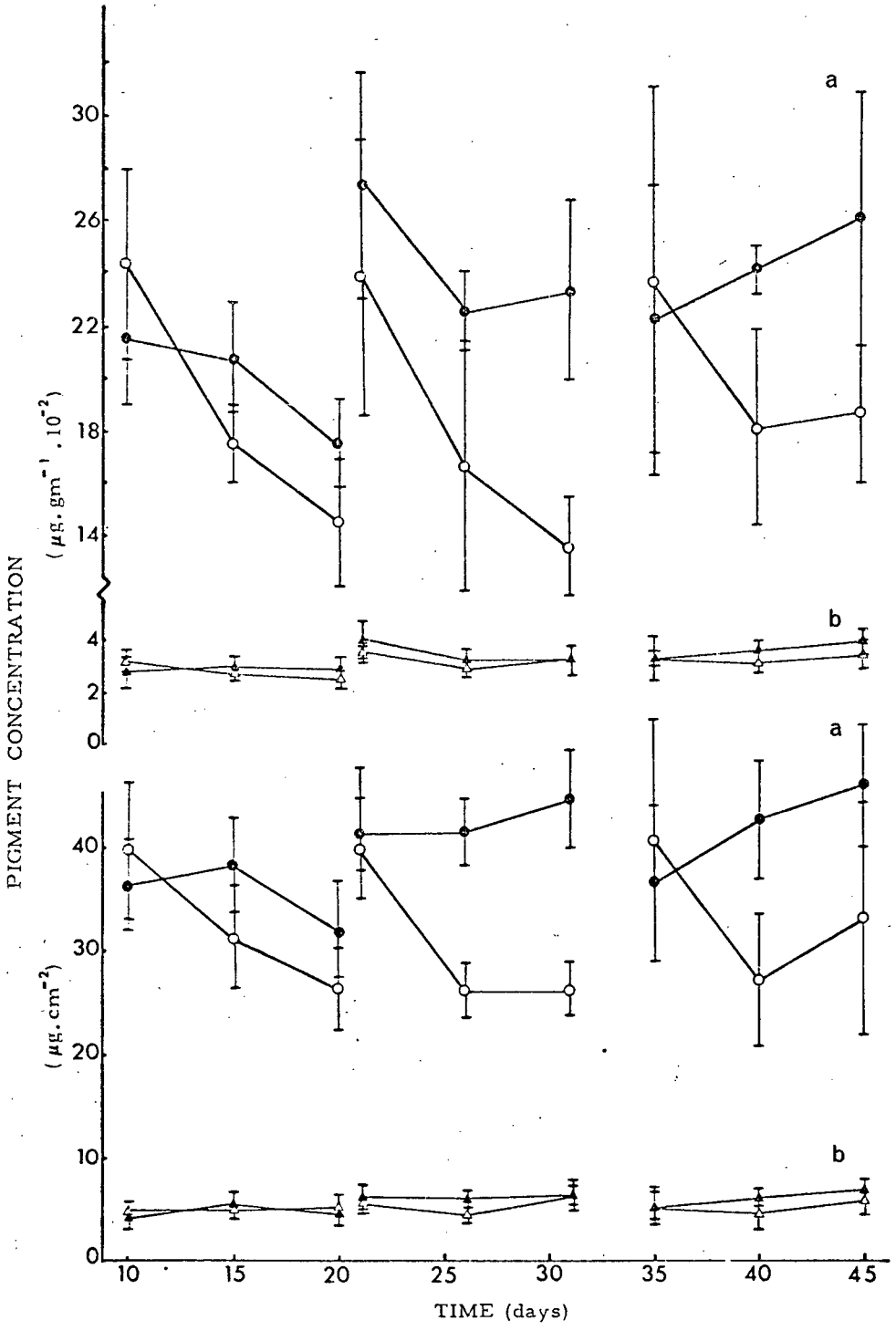

PIGMENT CONCENTRATION
(μg.gm⁻¹.10⁻²)
(μg.cm⁻²)
a
b
a
b
30
26
22
18
14
4
2
0
40
30
20
10
0
10
15
20
25
30
35
40
45
TIME (days)

Figure 2-9

Chlorophyll a (a) and chlorophyll b (b) concentrations in 10, 21 and 35 day-old Phaseolus plants subsequently exposed to warm or cold conditions for 0, 5 or 10 days.

●▲ Warm temperature

○△ Cold temperature

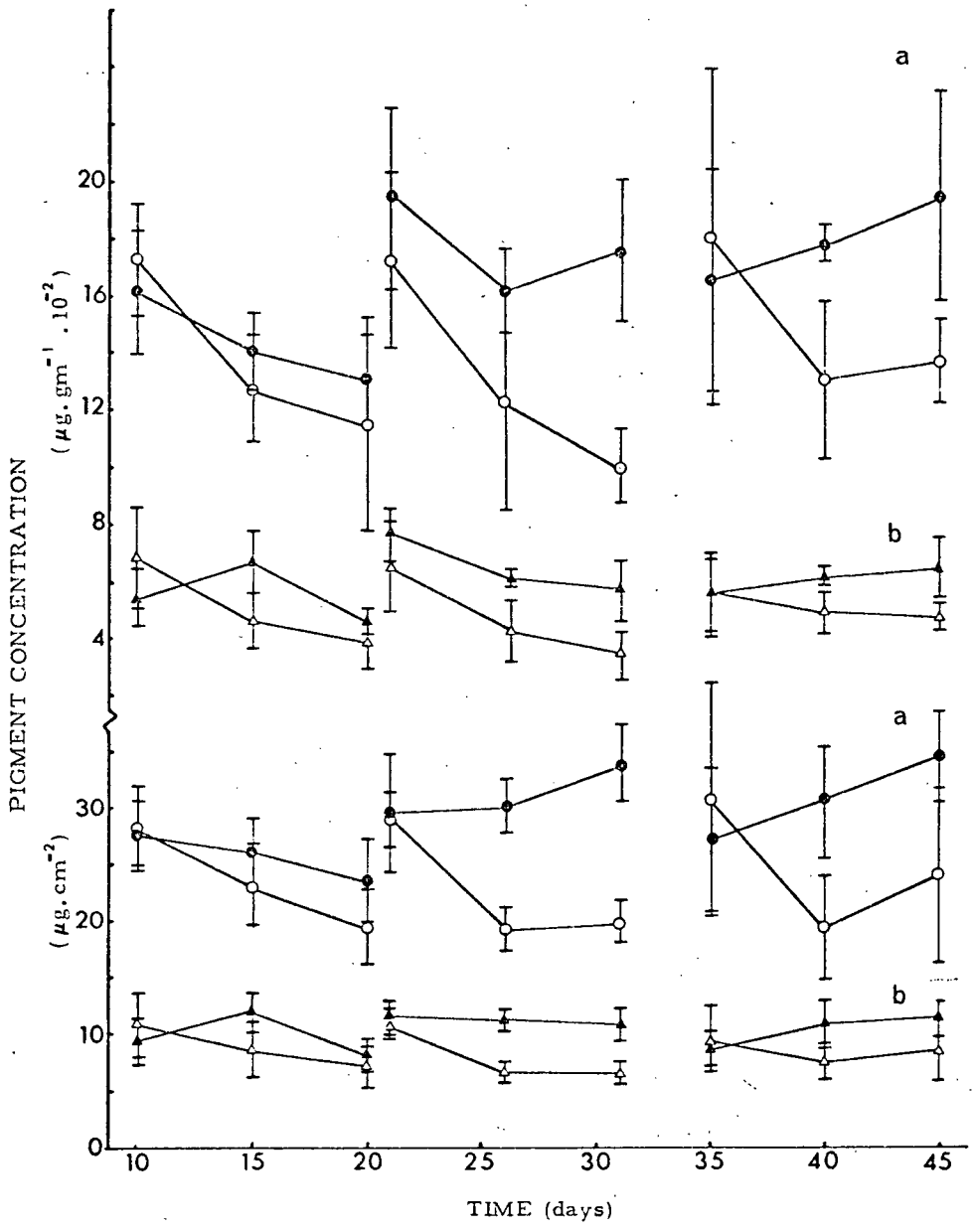

PIGMENT CONCENTRATION
(μg.gm⁻¹.10⁻²)
(μg.cm⁻²)
a
b
a
b
0
4
8
10
12
16
20
20
30
10
15
20
25
30
35
40
45
TIME (days)

Figure 2-10

Total chlorophyll (a) and total carotenoid (b) concentrations in 15, 26 and 40 day-old Gomphrena plants subsequently exposed to warm or cold conditions for 0, 5 or 10 days.

●▲ Warm temperature

○△ Cold temperature

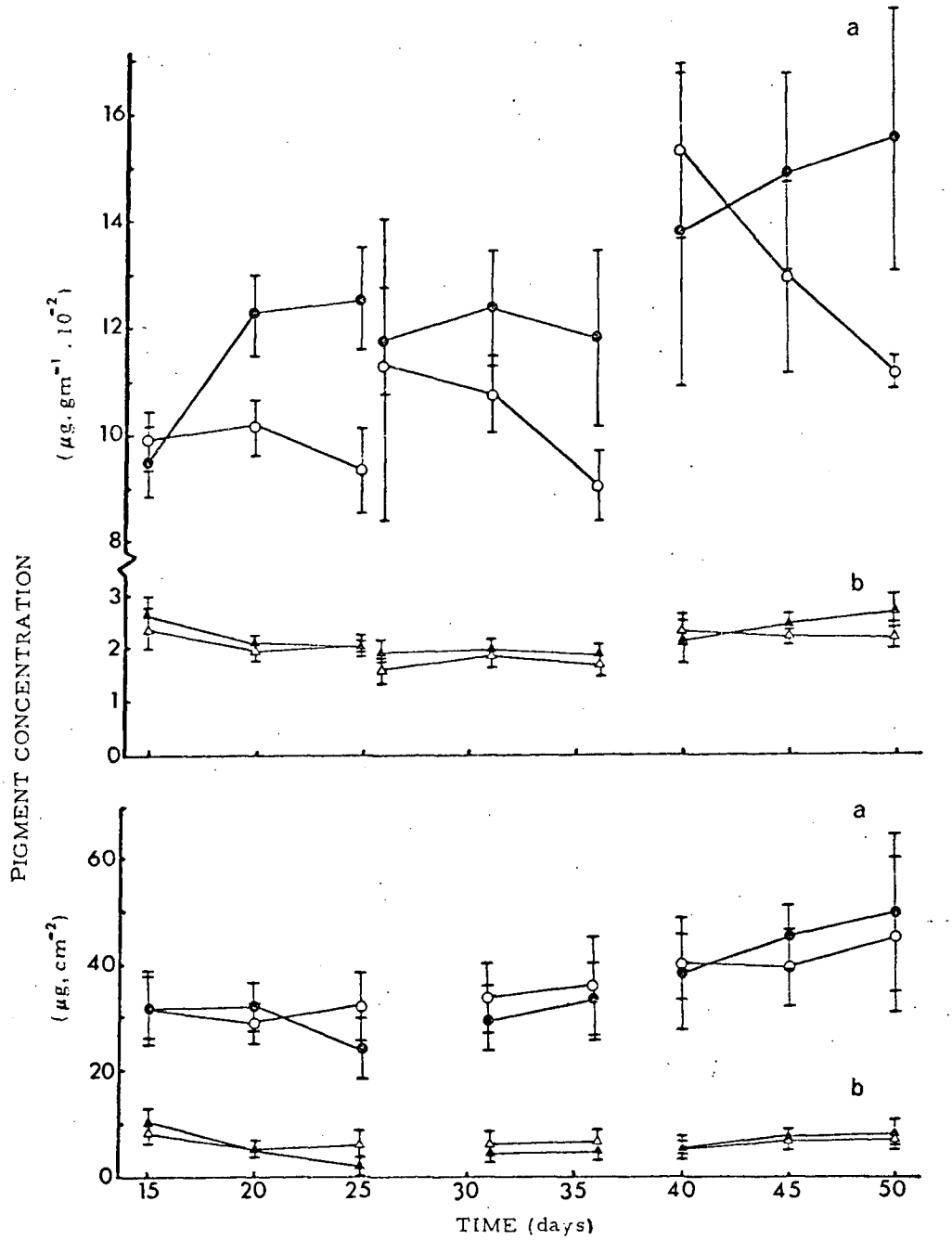
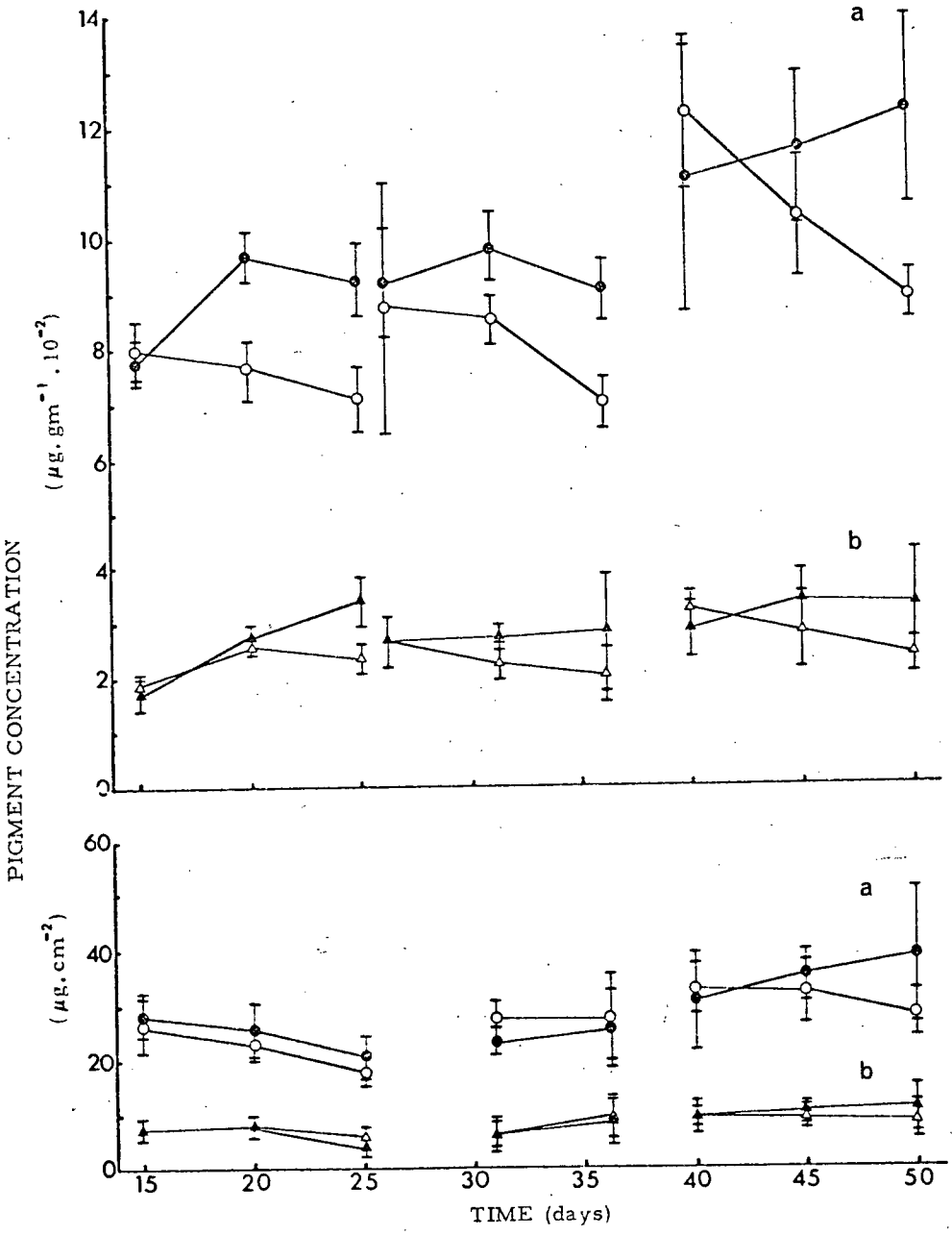

a
b
a
b
16
14
12
10
8
3
2
1
0
60
40
20
0
15
20
25
30
35
40
45
50
TIME (days)
PIGMENT CONCENTRATION
(μg.gm⁻¹.10⁻²)
(μg.cm⁻²)

Figure 2-11

Chlorophyll a (a) and chlorophyll b (b) concentrations in 15, 26 and 40 day-old Gomphrena plants subsequently exposed to warm or cold conditions for 0, 5 or 10 days.

●▲ Warm temperature

○▲ Cold temperature


PIGMENT CONCENTRATION
(μg.gm⁻¹.10⁻²)
(μg.cm⁻²)
a
b
a
b
TIME (days)
0
2
4
6
8
10
12
14
20
40
60
15
20
25
30
35
40
45
50

which contained slightly higher levels. No significant response to cold was evident in any case.

Whatever the mode of expression, the total carotenoid concentration did not change significantly in response to increasing age or cold temperature.

DISCUSSION

In summary, low, non-freezing temperatures caused significant effects on starch and chlorophyll accumulation, but not on carotenoid levels. The intensity of the effects varied according to species, developmental stage, and time of exposure to cold.

All of the species examined showed significantly more starch in the cold condition on at least one occasion during this experiment. Starch is generally known to accumulate to higher levels in the Dicotyledoneae than in Monocotyledoneae (Smith, 1969; Gates and Simpson 1966) and the present results are in agreement with this relationship. An explanation for the absence, or presence only in trace amounts, of starch in the Monocotyledoneae may lie in the presence of large amounts of fructose and polyfructosans, which also accumulate in greater amounts under cold temperature (Schmidt and Blaser, 1969). It is also known that within the grasses, tropical species tend to accumulate starch, while the temperate ones largely accumulate fructosans. The present results correlate well with these findings since Zea accumulated significantly higher levels of starch than did Triticum.

Because the optimum temperature for photosynthesis is lower for temperate species than for tropical ones, Evans (1964) has suggested that in most cases photosynthesis would be more reduced by cold conditions in tropical

species than in temperate plants. As a result, starch accumulation would be unlikely to occur in tropical species at low temperature. However, Evans also pointed out that the growth of tropical species may be greatly reduced by cold conditions, while cold temperatures, particularly at night, sometimes increase the growth of temperate species. From the effects of cold temperature on growth alone, it would be plausible to suggest that starch accumulation would occur in those plants whose growth is more restricted by cold temperature, namely the tropical species. Therefore, both the photosynthetic production of carbohydrates and their utilization in growth must be taken into account. It would be expected that starch will tend to accumulate whenever growth is reduced by cold to a greater extent than photosynthesis.

Since all of the species tested exhibited higher levels of starch in cold conditions than in warm, the results obtained do not permit a ready distinction between temperate and tropical species on the basis of starch accumulation at low temperature. In the two Monocotyledoneae studied, Zea appeared to be more sensitive to cold at the youngest age tested than was Triticum, while in the oldest plants tested the reverse occurred. In any case, when cold treatment resulted in significant differences in starch concentration, the magnitudes of the differences (g starch/g dry weight) were much larger in Zea than in Triticum. Cold temperature caused a greater reduction in height of Zea than Triticum, and this may partly explain the greater accumulations of starch in Zea. On the other hand, the per cent increase in starch concentration at low temperature was smaller in Zea than in Triticum. This may be accounted for by the very low starch concentration in Triticum at the warm temperature.

The Dicotyledoneae studied also differed with respect to the effect of temperature on starch accumulation. Gomphrena showed extremely large increases in starch concentration in cold conditions in the two ages tested, while Phaseolus exhibited relatively small significant increases in the young and intermediate ages only. It should be noted that since Gomphrena has a relatively long life cycle, only the early stages in its development were examined in this experiment. As in the case of the Monocotyledoneae, the tropical species (Gomphrena) showed a larger percentage reduction in growth in the cold condition (26.29%) than did the temperate Phaseolus (12.91%).

Figure 2-3 indicates that some of the starch concentrations observed with Gomphrena and Phaseolus exceeded 1 g per g dry weight. Two reasons can be advanced to explain this unusual result. First of all, the iodine-starch complex for Gomphrena and Phaseolus may be more effective in absorbing 620 nm light than was the potato starch-iodine standard. To avoid this, the method of starch measurement could be improved by extracting starch from each species and using these extracts to prepare a standard starch curve for each species. This change in method, however, would still not avoid the possibility that the structure of starch and its ability to complex with iodine may be affected by the temperature of starch synthesis. Secondly, the high starch concentrations in Gomphrena and Phaseolus made it necessary to dilute the plant extracts before spectrophotometric readings could be taken. It is possible that these dilutions may have introduced some error into the starch determinations.

The results show clearly that the effect of low, non-freezing temperature on chlorophyll concentration was also dependent on species, developmental

stage and duration of treatment. In some instances, cold temperature had no significant effect on pigment levels, but whenever a significant response was observed, it involved a decrease in the concentration of chlorophylls. In addition, the effects of cold on total chlorophyll concentration can be attributed to the parallel responses of both chlorophyll a and chlorophyll b.

In the Monocotyledoneae studied, Zea exhibited a significant reduction in chlorophyll concentration under cold conditions. This result is in agreement with the findings of several other studies (Alberda, 1969; Friend, 1966; Millerd and McWilliam, 1968; McWilliam and Naylor, 1967) but most of these previous studies used only very young seedlings. Triticum, on the other hand, exhibited no significant response in chlorophyll concentration to cold temperature treatment. This too is in agreement with previous indications (Friend, 1961; Friend, 1966). These differences in pigment response to low temperature were correlated with differences in growth. Cold temperature treatment caused a much larger reduction in growth and increase in starch concentration in Zea than in Triticum, and it seems reasonable to suggest that lower growth rate of Zea reflected lower CO_2 fixation caused by pigment limitations to photosynthesis. This may not be the only reason, however. Short-term gas exchange studies have shown that the optimum temperature for photosynthesis in Zea is about 39° C while for Triticum it is about 25° C (Jolliffe, 1970). The lower growth rate of Zea may therefore also be due to the fact that at cold temperature it is much farther from optimum conditions than Triticum.

In the Dicotyledoneae used, both Phaseolus and Gomphrena exhibited significant reductions of chlorophyll concentration in the cold. In Gomphrena,

cold treatment caused a greater reduction in growth but a smaller reduction in chlorophyll concentration than in Phaseolus. Thus the effects of cold were quite different from the grasses. It is possible that this difference is only apparent, for with Phaseolus measurements were made during much of its life cycle, while in Gomphrena, which has a relatively long life cycle, only the early developmental stages were observed.

In the case of Gomphrena the pigment results were different according to the mode of expression used. Chlorophyll accumulation was significantly less in some of the cold treatments when the results were expressed on a fresh weight basis, but no significant response was evident when leaf area was the basis of expression. In other words, under cold temperature, the ratio of chlorophyll concentration to fresh weight declined while the ratio of chlorophyll concentration to leaf area remained constant. Therefore, differences such as these can be attributed to the effects of age and cold treatment on the ratio of leaf fresh weight to leaf area.

In the three species which exhibited significant reductions in chlorophyll concentration in the cold, the extent of the reduction varied according to plant age and the duration of treatment. While in the case of Gomphrena similar reductions in chlorophyll concentration per g fresh weight were found at all ages studied, the greatest reduction in Zea occurred in the two youngest ages, and in Phaseolus the intermediate age, which just preceded flower development, was the most sensitive. In all three cases, the reductions in chlorophyll level coincided with periods of development marked by the initiation and expansion of many young leaves. Significant effects of the duration of cold treatment were evident in only 3 cases. The two youngest sets of Zea, and the intermediate Gomphrena plants showed

reductions in total chlorophyll concentration between 5 and 10 days of treatment which were significant at the 5 per cent level.

To some extent, the effect of low, non-freezing temperature on starch concentration appear to be inversely related to its effect on chlorophyll concentration. For example, in Zea the two youngest ages exhibited significant increases in starch concentration and decreases in chlorophyll concentration under cold treatment. Similar changes occurred in Gomphrena. In Phaseolus, however, an increase in starch concentration and a reduction in chlorophyll concentration occurred only in the intermediate age. In Triticum, there was only a small increase in starch concentration in the cold condition, while the chlorophyll concentration was not significantly affected.

It must also be pointed out that the interpretation of the changes in pigment concentration is difficult in those cases where large changes in the starch concentration occurred. For example, in Gomphrena the change in starch concentration during the cold treatments was of the order of one gram per gram dry weight. That is, the dry weight of Gomphrena had little starch at the start of the cold treatment, but was mostly starch after 10 days of cold. It would be expected that this large increase in starch concentration would be reflected by decreases in the proportional concentrations of other components of the dry matter of the plant. The often inverse relationship between the changes in starch and chlorophyll concentrations in the species tested was pointed out in the previous paragraph and may be based on this sort of effect. Examination of the starch results indicates that increases in starch concentration may have been sufficiently large to contribute to the decreases in chlorophyll

concentration observed in Gomphrena and in the intermediate ages of Phaseolus and Zea. There does not seem to be any convenient way of directly expressing the starch and chlorophyll results relative to each other. Perhaps, if these studies are continued, the results could be made relative to protein content, cell number, or some other possibly independent index.

In these experiments, carotenoid concentration was unaffected by cold or age. This may indicate that low temperatures do not influence the postulated role of carotenoids in preventing chlorophyll photooxidation. Confirmation of this aspect must await studies of the interactions of individual carotenoid pigments with cold temperature and chlorophyll concentration.

In conclusion, depending on species, age, and duration of treatment, low non-freezing temperature caused high starch or low chlorophyll concentrations to occur, or in some cases the cold had no significant effect. The effects of cold on starch and chlorophylls in the tropical species used were often greater than in the temperate species. This was particularly true when the youngest ages only are considered, but this relationship was sometimes reversed in the oldest plants. Therefore, general correlations between low temperature effects and photosynthetic metabolism or higher systematic categories are not evident from these results. Starch accumulation at low temperature may be the result of an excess of photosynthesis over growth, while low temperature may reduce chlorophyll accumulation by inhibiting its synthesis or promoting its breakdown.

3. The Effects of Temperature and Kinetin on the Accumulation of Chlorophyll in Etiolated *Zea* leaves

INTRODUCTION

The preceding experiment was limited in scope by the relatively long time required for the starch and pigment analyses. As an alternative to the extractive determination of pigment concentration, a procedure was developed for the rapid indication of pigment concentration during the greening of etiolated leaves. This procedure was based on the photometric determination of the transmission of 665 nm light through the leaves. Fully green leaves could not be used since leaf light transmission is only slightly affected by moderate changes in pigment concentration in green leaves.

This method was applied to a study of the greening process in etiolated *Zea* leaves in both warm and cold temperatures. *Zea* was chosen since the previous experiment and other studies (Alberda, 1969; Friend 1966; McWilliam and Naylor, 1967; Millerd and McWilliam, 1968) had identified the sensitivity of pigment leaves to cold temperature in young leaves. A series of tests was carried out to determine the kinetics of pigment accumulation in etiolated leaves during the first 24 hours of illumination at warm or cold temperatures. To establish the relationship between light transmission and chlorophyll concentration, chlorophyll extractions were carried out to complement the transmission measurements. Finally, the possibility of using kinetin to overcome the inhibitory effect of cold temperature on chlorophyll accumulation was tested.

MATERIALS AND METHODS

Zea mays L. var. Pioneer plants were grown in the same way as before except that no illumination was supplied so the plants remained etiolated. When the first leaf was about 4 cm long, the plants were exposed to continuous light in the Percival growth chambers. Following the start of illumination, the plants were exposed to different periods of treatment in warm (25 - 28° C) or cold (7 - 11° C) temperature conditions.

The apparatus for measuring the absorption of light by the greening leaves included a white light source which provided approximately 1.6 x 10^4 ergs cm^{-2} sec^{-1} at the surface of the leaf. The light beam was intercepted by a red filter (Balzers B-40, 665 nm, with a band width of 11 nm) before it reached the leaf. A light detector (PIN photodiode-Hewlett Packard HP 5082-4220) was placed on the other side of the leaf to detect the transmitted light. The light sensor was connected to an amplifier from which the readings were taken. When measuring the light transmitted through the leaf, the leaf which developed first was cut and placed between two microscope slides, and these were then placed between the light source and the light sensor in such a way that the beam was incident at approximately the centre of the leaf. Ten measurements from ten different leaves were made under each set of conditions. The leaf thickness was measured after the transmission reading was finished. For this purpose a micrometer which had a Vernier mechanism allowing measurements to ten thousanths of an inch was used. Chlorophyll determinations were carried out as previously described and six replicate determinations were made for each condition.

For the kinetin experiments, 6-furfurylaminopurine (A grade M.W. 2152 from CalBiochem) in a concentration of 20 mg/l was sprayed on to the plants at the beginning of the experiment until the leaves were wet.

RESULTS AND DISCUSSION

Figure 3-1 shows the time course of leaf light transmission after etiolated Zea leaves were exposed to 11000 lux illumination in warm and cold temperatures. After a two hour lag period, which was also observed in subsequent tests, the transmission of light by the warm leaves declined rapidly with time. A logarithmic regression curve with the equation shown on Figure 3-1 was fitted to the data. To account for the two hour lag period, the first point (time = o) was omitted from the regression. The high degree of correlation between the regression and the data (r^2 = 0.94) confirmed the exponential nature of the decrease in leaf light transmission. If it is assumed that the leaf posesses a constant thickness and that it behaves as a pure pigment solution, then according to Lambert's law the exponential decrease in transmission could be the result of a linear increase in pigment concentration. As will be seen later, a linear increase in chlorophyll concentration was actually observed during the initial 24 hours of greening.

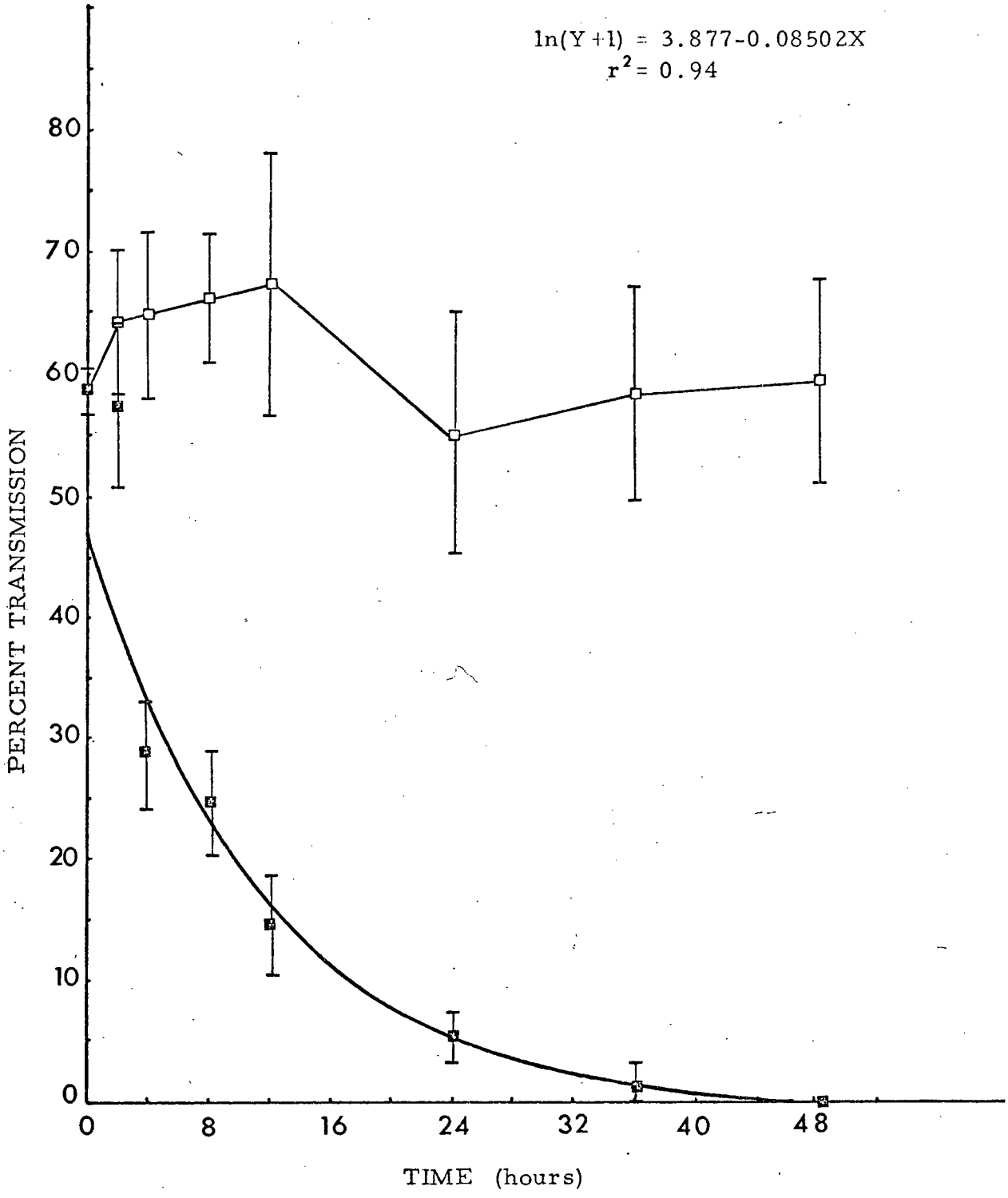
For the leaves which were exposed to cold conditions, however, the light transmission values remained constant. The sample variation for the cold treated plants was relatively large. It was observed that on exposure to cold, some of the leaves suffered partial bleaching of their initially pale yellow colour, and this may have resulted in some high light transmission readings.

Figure 3-1

Changes in the per cent transmission of light through leaves during the initial 48 hours of greening of etiolated Zea seedlings exposed to warm or cold conditions.

■ Warm temperature

□ Cold temperature


ln(Y+1) = 3.877-0.08502X
r² = 0.94
PERCENT TRANSMISSION
0
10
20
30
40
50
60
70
80
0
8
16
24
32
40
48
TIME (hours)

Since leaf thickness, and therefore leaf light transmission might have been influenced by temperature, the thickness of each leaf was recorded. Figure 3-2 demonstrates that temperature and treatment time had no significant effect on leaf thickness during the period of study. Therefore, the observed variations in leaf light transmission during this experiment were not the result of changes in leaf thickness.

Figure 3-3 shows the results obtained from chlorophyll extractions carried out at different times during the warm temperature treatment. Only the values for chlorophyll a are shown, because it is the first chlorophyll formed during greening and it represents up to 90% of total chlorophyll. The cold treated plants did not accumulate any significant quantity of chlorophyll. In the plants exposed to warm temperature, there was a continuous increase in chlorophyll a concentration with time, and this increase could be described by the linear regression equations given in Figure 3-3. A better correlation was obtained if the chlorophyll a concentration was expressed on a per gram fresh weight basis rather than on a leaf area basis.

In addition, Figure 3-4 shows the relationship between chlorophyll a concentration and leaf light transmission. For both modes of expression of chlorophyll a concentration, a correlation coefficient of 0.99 was obtained, indicating that variations in leaf light transmission are almost entirely attributable to changes in chlorophyll a concentration. Therefore, this relationship can be used to predict leaf chlorophyll concentration from measurements of leaf light transmission during greening.

Figure 3-5 summarizes the results of a series of measurements of light transmission by etiolated Zea leaves which were illuminated and kept

Figure 3-2

Variations in leaf thickness during the initial 48 hours of greening of etiolated Zea seedlings exposed to warm or cold conditions.

■ Warm temperature

□ Cold temperature

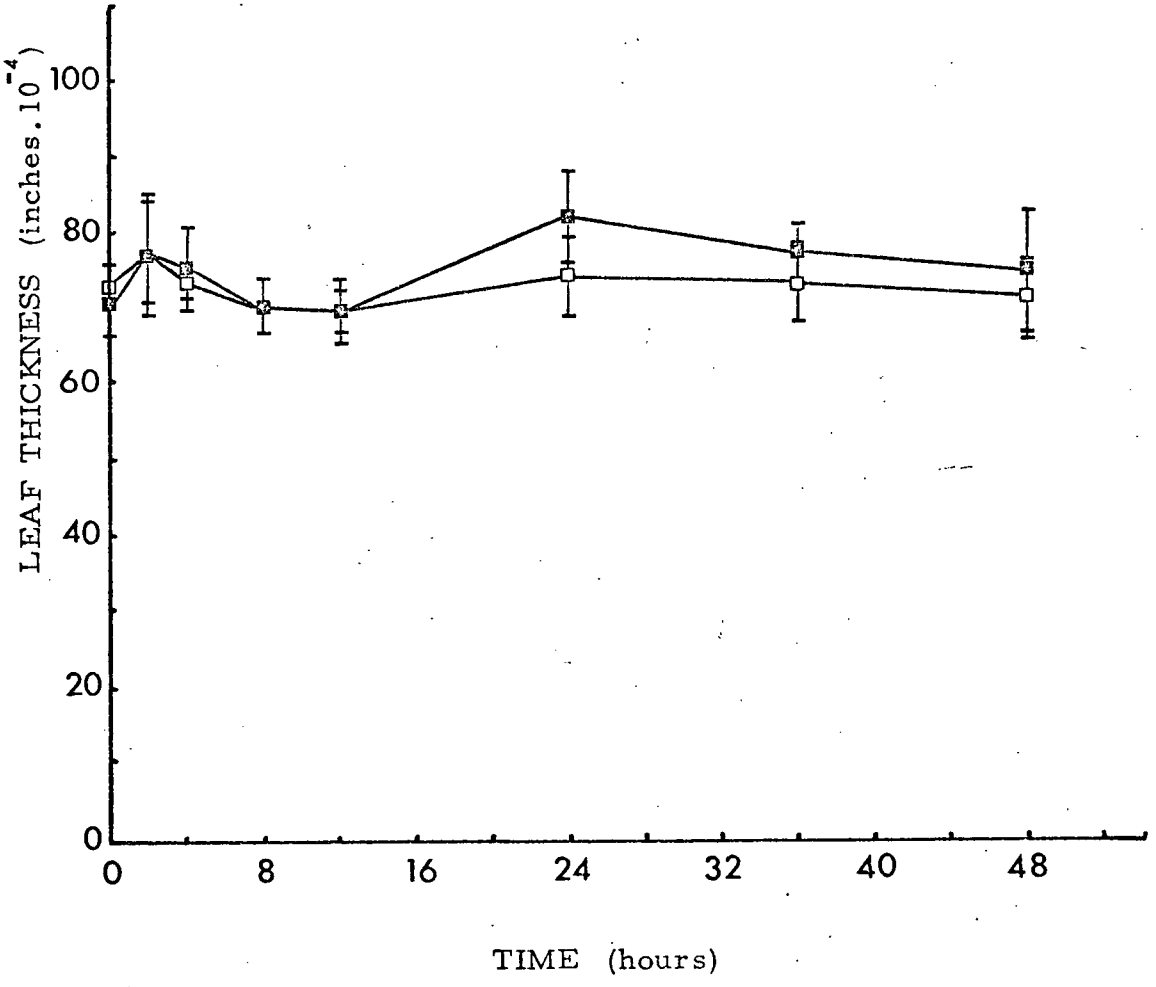

LEAF THICKNESS (inches.10^{-4})
100
80
60
40
20
0
0
8
16
24
32
40
48
TIME (hours)

Figure 3-3

Changes in chlorophyll a concentration during the initial 48 hours of greening of etiolated Zea seedlings in warm conditions.

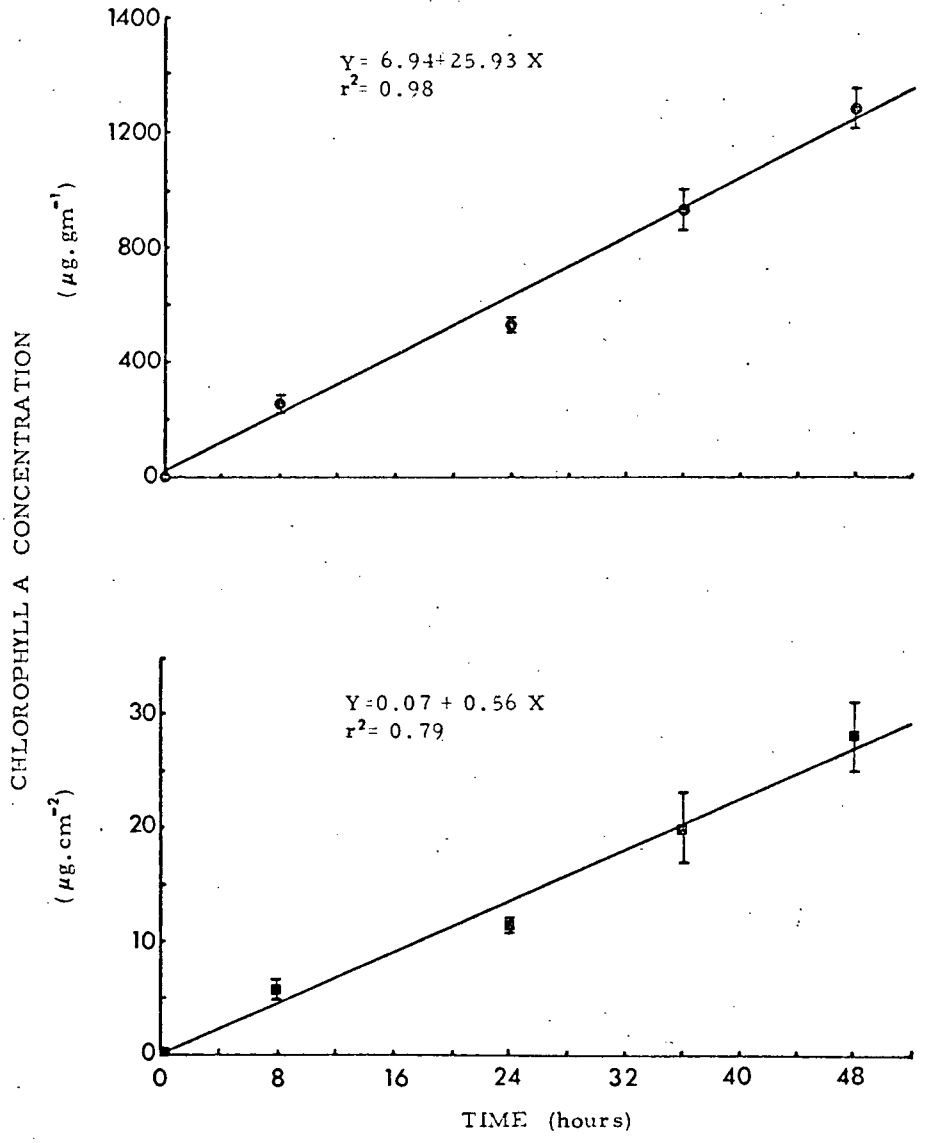

Y= 6.94+25.93 X
r²= 0.98
1400
1200
800
400
0
(μg.gm⁻¹)
Y=0.07 + 0.56 X
r²= 0.79
30
20
10
0
(μg.cm⁻²)
CHLOROPHYLL A CONCENTRATION
0
8
16
24
32
40
48
TIME (hours)

Figure 3-4

Relationship between the per cent transmission of light through leaves and the leaf chlorophyll a concentration during the greening of etiolated Zea seedlings in warm conditions.

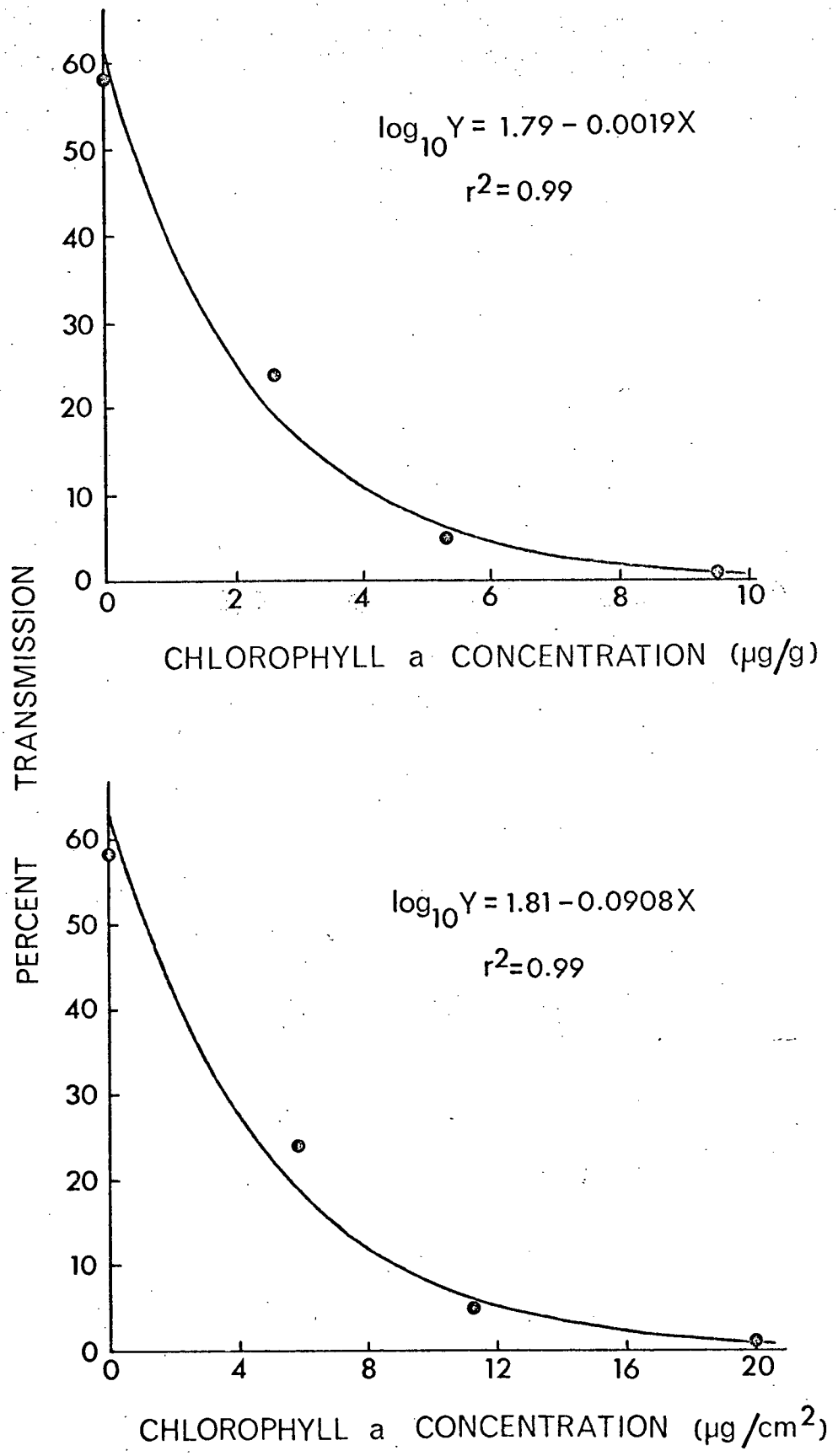

$\log_{10}Y = 1.79 - 0.0019X$
$r^2 = 0.99$
PERCENT TRANSMISSION
CHLOROPHYLL a CONCENTRATION (µg/g)
$\log_{10}Y = 1.81 - 0.0908X$
$r^2 = 0.99$
CHLOROPHYLL a CONCENTRATION (µg/cm^2)

Figure 3-5

Changes in the per cent transmission of light through leaves during the initial 24 hours of greening of etiolated Zea seedlings.

● Warm temperature for entire 24 hours

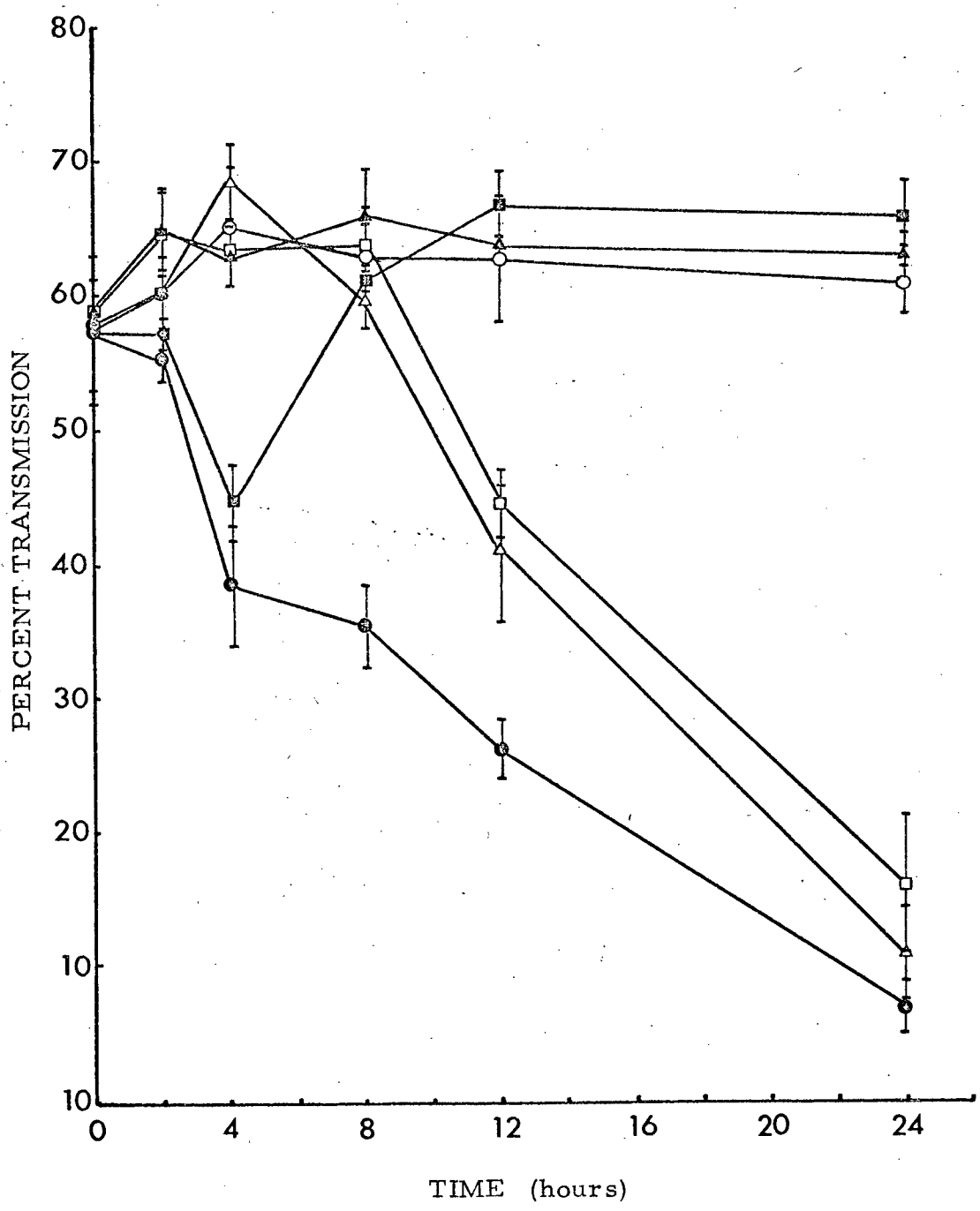
○ Cold temperature for entire 24 hours

▲ Warm temperature for initial 2 hours, remainder cold

■ Warm temperature for initial 4 hours, remainder cold

△ Cold temperature for initial 2 hours, remainder warm

□ Cold temperature for initial 4 hours, remainder warm


80
70
60
50
40
30
20
10
10
0
4
8
12
16
20
24
PERCENT TRANSMISSION
TIME (hours)

for different periods of time in warm or cold conditions. As before, the plants which were kept in the cold for the entire period showed no significant change in leaf light transmission. In the warm condition, there again appeared to be a lag of about two hours before the leaf light transmission values decreased significantly. Plants which were exposed to warm temperature for two hours and then transferred to the cold did not exhibit any significant change in leaf light transmission. The presence of an initial lag before the onset of chlorophyll accumulation is in agreement with earlier reports (Virgin, 1955; Kirk, et al 1967). Plants which were exposed to warm temperature for four hours showed distinctly lower values of leaf light transmission, indicating that the accumulation of chlorophyll had started by that time. However, when plants were then transferred to the cold temperature, leaf light transmission quickly increased until after eight hours of illumination it was equivalent to the values exhibited by leaves which had been in continuous cold treatment. This result indicates that the pigment synthesized during the warm temperature treatment was destroyed, or was synthesized at a slower rate than its destruction, in the cold. Previous studies have also indicated a rapid destruction of chlorophyll under cold temperature in corn (MacWilliam and Naylor, 1967; Klein, 1960) in wheat (Virgin, 1956 and 1958) and in barley (Augustinussen and Madsen, 1965; Virgin, 1955). On the other hand, when the plants were exposed for two or four hours of cold treatment at the beginning of illumination, no significant decline in leaf light transmission was evident until they had subsequently been in warm conditions for eight or ten hours respectively. Therefore the initial lag in chlorophyll accumulation in warm temperature was extended by

preceding cold treatment. Nevertheless, once chlorophyll began to accumulate following the initial cold treatment, the kinetics of accumulation resembled those observed with the plants which were exposed to warm temperature throughout. These results may indicate that the initial cold treatment may have retarded that step of chloroplast development which is coincident with the end of the lag phase. The early steps of chloroplast formation are known to occur during the lag phase (Kirk, 1967). At the end of the lag phase, chloroplast structure undergoes specific changes following which chlorophyll accumulation parallels chloroplast development. It is possible that the initial cold treatment may prevent these structural changes in chloroplasts by causing the accumulation of inhibitory substances or by some other mechanism. Once the structural changes have occurred and the lag phase is ended, chlorophyll accumulation seems to be independent of the initial cold treatment.

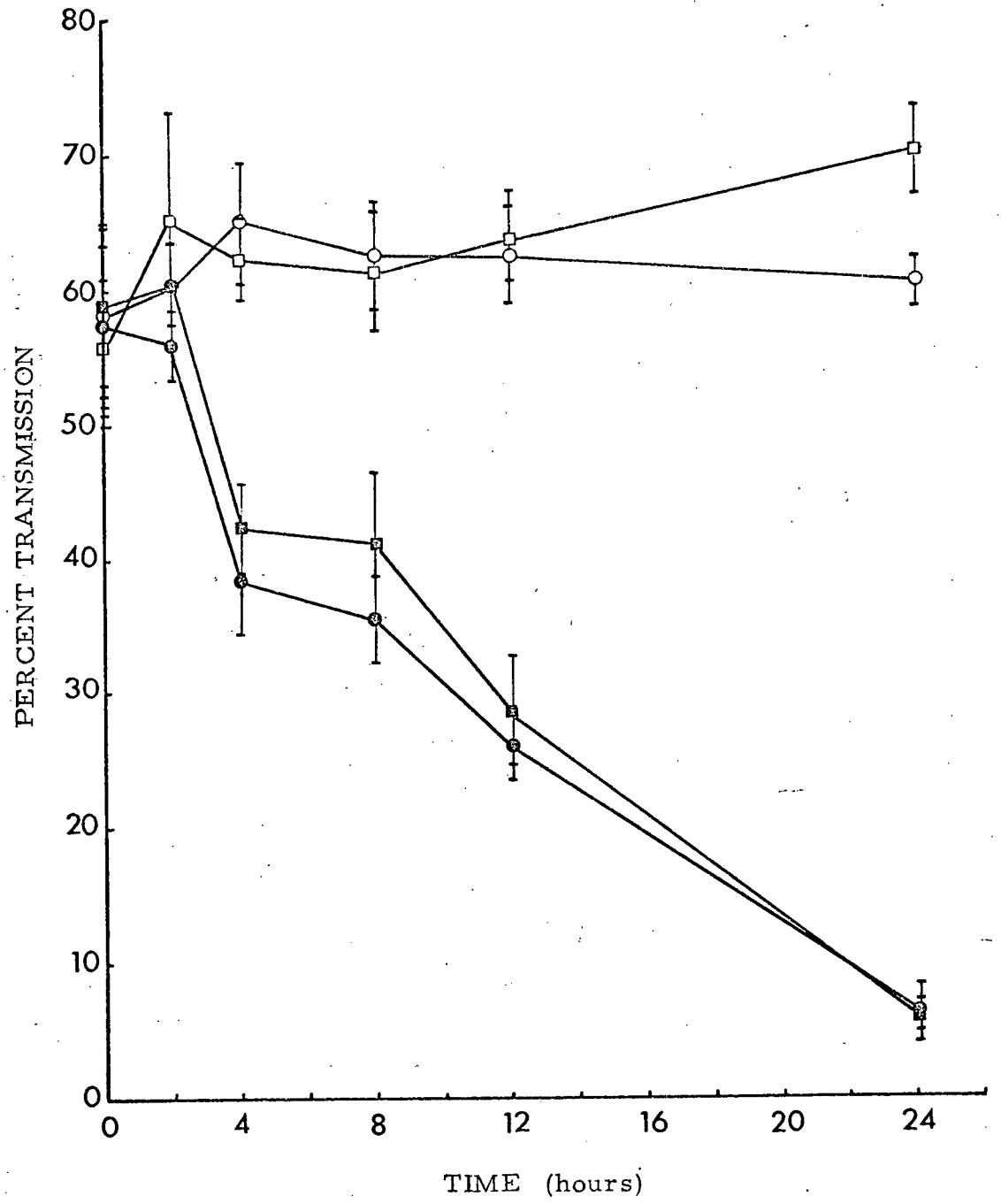
Figure 3-6 shows that the application of kinetin did not significantly alter leaf light transmission in either the warm or the cold condition. Thus, kinetin did not overcome the inhibitory effect of cold temperature on greening, even though kinetin is known to suppress the loss of chlorophyll from darkened disks of mature green Xanthium leaves (Osborne and McCalla, 1961).

In summary, the technique of measuring leaf light transmission is a convenient and rapid method of determining chlorophyll concentration in greening leaves. It is particularly useful in studies of the effects of various factors on the kinetics of chlorophyll accumulation, and in future it might be adapted to continuous measurements on the same leaf. In the present study, this technique has been used to show that cold temperature extends the lag phase in chlorophyll accumulation, and causes a net decrease in chlorophyll concentration which is not prevented by kinetin treatment.

Figure 3-6

Effect of kinetin on the changes in per cent transmission of light through leaves during the initial 24 hours of greening of etiolated Zea leaves exposed to warm or cold conditions.

- ● Warm temperature
- ■ Warm temperature plus kinetin
- ○ Cold temperature
- □ Cold temperature plus kinetin


80
70
60
50
40
30
20
10
0
PERCENT TRANSMISSION
0
4
8
12
16
20
24
TIME (hours)

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5. APPENDIX I

Vertical gradient in light intensity in the Percival growth chamber.

The light intensity in the warm growth chamber was measured with a Gossen Trilux footcandle meter located at 35 cm from the front walls and 80 cm from the lateral ones, at a distance from the pots as indicated below. The figures are average from three replicates. The following results were obtained:

Distance from the top of the pot (cm)	Light Intensity (lux)
0	7,300
10	8,200
20	9,000
30	9,900
40	10,800
50	12,100
60	19,400
70*	23,700

*Approximately 17 cm below the light barrier.

These measurements are included to better define the environmental conditions during the experiments. It must be noted that the light intensities to which the plants were exposed varied with the different species since there were marked variations in shoot structure among the species and from one developmental stage to another.

6. APPENDIX II

Variations in Chlorophyll Concentration within Plant Leaves.

It was suspected that variations in chlorophyll concentrations within the leaves of the test species could contribute to sample error. To assess this possibility, the leaves from each of the four test species were collected from 3-day-old plants which had been grown in the warm condition. The leaves were cut laterally into sections and the chlorophyll concentrations in 0.5 g samples from these sections were determined.

For Gomphrena and Phaseolus, the variations in chlorophyll content between different leaf segments were small and were less than the differences between different leaves of the same species. The variations within leaves of Zea and Triticum were large, however, as is indicated by the following data:

Zea

Leaf Segment	Chlorophyll Content (µg/gm fresh weight) Leaf 1	Leaf 2	Leaf 3
1	230	265	230
2	534	547	530
3	828	891	820
4	1179	1059	1348
5	1442	1441	1453
6	1329	1356	1322
7	1290	1640	1322
8	1063	2079	1097
Leaf Total	7898	9281	7923
Average	987	1160	990

Triticum

Leaf Segment	Chlorophyll Content (µg/gm fresh weight) Leaf 1	Leaf 2	Leaf 3
1	2853	2967	2736
2	2838	2925	2700
3	2701	2721	2654
Leaf Total	8394	8614	8090
Average	2798	2871	2696

On the basis of these results, it was decided to use entire leaves where possible, and if this was not possible, to use leaf segments cut from the central portion of the leaf (intermediate between the leaf base and tip).

7. APPENDIX III

Determination of the Effects of Time, Temperature, and Light on the Absorbance of Chlorophyll Extracts.

When chlorophyll sections are exposed to light in the presence of oxygen, they can be irreversibly bleached. Degassed solutions or solutions kept in darkness at room temperature do not change their absorbance. Consequently both oxygen and light are required for bleaching to occur (Jen, 1970; Bruinsma, 1963). Because the present experiments included four species and a large number of samples per day, tests were done to find what storage conditions were adequate for the extracted chlorophyll samples. Chlorophyll solutions were prepared from 0.5 gm fresh weight of leaves for all four species. After extraction, the chlorophyll solutions were diluted to 100 ml in volumetric flasks and kept under the conditions shown in the table below. Absorbance measurements were made just after extraction, after 15, 30, 60 minutes, and then hourly until 8 hours after extraction. Further readings were then made daily for one month. Three replicates were made for each condition. The results are shown in the following table.

STORAGE CONDITION	TIME AFTER EXTRACTION	BLEACHING %
Room temperature and light	24 hours	20 - 30%
Room temperature and dark	1 week	0
	2 weeks	10 - 20%
4° C and light	1 month	0
4° C and dark	1 month	0

From the results obtained, it was concluded that extraction of the samples, which took less than ten minutes, could be carried out at room temperature if the volumetric flasks receiving the solution were covered with aluminium foil. For storage, the samples were therefore kept cold to prevent bleaching.