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Control of plant stature by manipulation of day and night temperatures (DIF regimes)

PART 1

Controlled Environment Cabinet experiments

EXPERIMENTAL SECTION

INTRODUCTION

Public concern over environmental issues is putting increased pressure on growers to produce crops with fewer applications of chemicals. One consequence of this is an increasing interest in controlling plant height without using plant growth regulators. A promising approach is to use temperature regimes that discourage stem elongation. Previous research on a variety of plant species has indicated that the use of lowered day temperatures causes plants to be shorter stemmed [1,2]. In this context, the term DIF is used to describe the difference between the day temperature and the night temperature. In a negative DIF regime the day temperature is lower than the night temperature, while the more usual situation, in which the day temperature is higher than the night temperature, is a positive DIF regime: zero DIF indicates a constant temperature.

There are indications that the regulation of stem elongation is more sensitive to temperature at some times of day than at others. If this is so, it should be possible to shorten the duration of the low temperature period and, by applying it when the plant is most receptive, still get good control of height. This may permit height control at times of year when it is difficult to maintain low temperatures for the whole of the day. A regime that incorporates such a low temperature period is called a DROP regime.

The four experiments reported here were conducted in controlled environment cabinets at Littlehampton. The experiments were designed to study the effect of a range of DIF and DROP regimes on the growth of poinsettia, geranium and chrysanthemum and the results of these experiments were then utilised in further development work conducted in glasshouse compartments at Efford (reported in PART 2). Controlled environment cabinets were used so that temperatures could be maintained precisely for periods of four to five weeks, and so that temperature differences could be achieved rapidly and precisely, features that are not always possible to achieve in the glasshouse. Treatments were applied when stem elongation was likely to be rapid, so that maximum effects could be obtained. For poinsettia and chrysanthemum, the start of treatment coincided with the start of short days. After treatment, plants were grown on in a common glasshouse environment so that any after effects of the treatments could be assessed.

THE EXPERIMENTS

Materials and methods

1. Plant material

1.1 Poinsettia

1.1.1 Stock plant production. Rooted cuttings of poinsettia (*Euphorbia pulcherrima* cv Steffi) obtained from Hollyacre Plants Ltd., Toddington Lane, Littlehampton, were potted in 130mm diameter pots of Levington M2 compost to which Aaterra had been added at 40g/m^3. The plants were grown

in a glasshouse with a minimum temperature setting of 20°C venting at 22°C, and long-day conditions were maintained by using incandescent lamps to provide a night break from 2300 hours to 0100 hours at an illuminance of 300 lux.

1.1.2 Cutting production. Cuttings were taken from the stock plants as required, stuck in Jiffi 7's and rooted under mist in long days. When rooted, they were potted in M2 compost in 130mm pots. Some of the rooted cuttings obtained for stock plant production were used as starting material in the first experiment. Aaterra was added to the compost for the second experiment but not for the third and fourth.

1.1.3 Pre-treatment of plants. Prior to transfer to the cabinets, the plants were grown in a glasshouse at 20°C venting at 22°C. Long-day conditions were maintained either by using incandescent lamps (average illuminance of 300 lux) to provide a night break from 2300 hours to 0100 hours (Experiments 1 and 2), or by lighting with SON-T lamps (4000 lux) from midnight to 1600 hours (Experiments 3 and 4). The plants were stopped at about seven leaves. In the first experiment, all shoots were allowed to grow except that in the axil of the uppermost leaf. In subsequent experiments all axillary shoots but one, normally the shoot in the second leaf axil, were removed before the start of treatments. This was done in an attempt to improve uniformity of shoot growth.

1.2 Geranium

Plug-grown seedlings of geranium (*Pelargonium* x *hortorum* cv Century Scarlet) were obtained from Roundstone Nurseries, Roundstone Lane, Angmering. When large enough to handle, they were potted into M2 compost in 90 mm pots and were grown in a glasshouse with a minimum air temperature of 18°C venting at 21°C. The potted plants were placed in the cabinets when they had four expanded leaves (Experiments 1 and 2). Neither Cycocel nor any other growth regulator was applied to the plants.

1.3 Chrysanthemum

Rooted cuttings of chrysanthemum (*Dendranthema grandiflora* cv Bright Golden Anne) were obtained from Yoder Toddington Ltd, Toddington Lane, Littlehampton, and potted in M2 compost (Experiments 3 and 4). The pot size used for Experiment 3 (95mm square) proved too small to keep the plants adequately watered and so, 130mm diameter pots were used in Experiment 4. Prior to transfer to the cabinets, the plants were grown in a glasshouse with a temperature setting of 17°C venting at 22°C. Long days were provided by a night break using incandescent lamps (300 lux) from 2200 hours to 0200 hours each night. When the plants were established they were stopped at about seven leaves and allowed to carry either one (Experiment 3) or three axillary shoots (Experiment 4); all other axillary shoots were removed before the start of treatments.

2. Controlled environment cabinet protocol

Six 'Saxcil' controlled environment cabinets were used. Apart from temperature, all other environmental factors were kept the same in all six cabinets. 'Warm-white' fluorescent tubes provided an irradiance of 60 Wm^{-2} for 12 hours, equivalent to the daily integral of photosynthetically active radiation (PAR) received within a glasshouse at the equinox. Fifteen 30W tungsten lamps per cabinet were used to enhance the red and far-red regions of the spectrum so as to encourage stem elongation. The target VPD was 0.4 kPa. Carbon dioxide was enriched to 1000 vpm but a fault developed in the control system part way through Experiment 1, and so carbon dioxide levels were at ambient for the second half of Experiment 1 and for the whole of Experiment 2. Plants were watered by hand with Hoagland No1 solution [3] and the plant pots were placed on saucers of appropriate size to prevent nutrient draining into the base of the cabinet.

3. Treatments

In all four experiments, the duration of both the day and night periods was twelve hours. In Experiments 1 and 3, all treatments also had the same 24-hour mean temperature of 18°C but, in order to achieve this, night temperatures were different (Fig 1a). Four DIF treatments (+4, 0, -4, and -12 DIF) were tested in these two experiments, together with two DROP treatments in which a drop of 8°C was given either for the first six hours of the day period (-8E) or for the second six hours (-8L). The magnitude of the drop in temperature in both of these treatments was midway between that of the -4 and -12 DIF treatments, whereas the actual temperature achieved following the change (i.e. 12°C) was the same as in the -12 DIF treatment (Fig. 1a). Furthermore, the product of the change in temperature and the duration of the change in the -8E and -8L DROP treatments, i.e. 8°C x 6 h = 48 °h, was the same as in the -4 DIF treatment, i.e. 4°C x 12 h = 48 °h. Thus, the treatment effects could be compared in various ways to see which features were most important; the magnitude of the temperature drop, the timing of the temperature drop, the actual temperature achieved, or the product of the drop and its duration.

In Experiments 2 and 4, the treatments all had the same night temperature of 18°C (Fig. 1b) and so their 24-hour mean temperatures were different. The results of Experiments 1 and 3 indicated that poinsettia and chrysanthemum responded more to DROP treatments given in the first half of the day than the second. Consequently, all the treatments employed in Experiments 2 and 4 focussed on establishing the main features of the responses to lower temperatures in this period. Three DROP treatments (-2, -4, and -8°C) were given for six hours each day, starting at 'lights on', and formed a graded series of increasing magnitude of temperature drop. The response to these treatments was compared with that to a control treatment with no difference between day and night temperature (0 DIF). The -8E DROP treatment in these two experiments, therefore, used the same drop in temperature and the same timing of the change in temperature as the -8E DROP treatment of Experiments 1 and 3, although the actual temperatures employed were different (Fig. 1a and 1b). Differences in sensitivity to temperature changes in the two halves of the first six-hour period of the day were examined by giving an 8°C change for three hours beginning either at 'lights on' (-8EE) or three hours later (-8EL) (Fig. 1b). The magnitude of the change in temperature in these two treatments was the same as in the -8E DROP treatment but the duration was shorter and so the product of the change in temperature and its duration (i.e. 8°C x 3 h = 24 °h) was the same as in the -4E DROP treatment (i.e. 4°C x 6 h = 24 °h). As before, these

arrangements of treatments allowed comparisons to be made between the magnitude of the temperature drop, the timing of the temperature drop, and the product of the drop and its duration.

4. Post-treatment protocol

Sample plants were removed at intervals for recording and, at the end of an experiment, the remaining plants in each cabinet were removed and transferred to the glasshouse, where they were all kept in one common environment until they flowered. Poinsettias were watered with the appropriate ADAS recommended feed [4] and chrysanthemums with the Littlehampton standard feed which contained 200 ppm N, 150 ppm K, 30 ppm P and 0.5 ppm Fe. Air temperature varied with the time of year but was generally around 18°C. Carbon dioxide levels were not enriched. Poinsettia and chrysanthemum were kept in short days which, between 10 March and 30 September, were produced by covering the plants with black polythene sheet from 1700 to 0700 hours each night.

5. Records taken

5.1 Poinsettia

The length of the axillary shoot (in Experiment 1, the first three axillary shoots) was measured at weekly intervals from the start of treatment until flowering. Apical development was determined by dissection five weeks after the start of short days and recorded on an unpublished scale (0 = vegetative; 6 = cyathium fully enclosed by the integument) devised by Horridge. The fresh and dry weights of leaves and stems of the main stem and the axillary shoot, the leaf area and the chlorophyll and carotenoid content of the youngest fully expanded leaf [5,6,7] were recorded at the end of treatment. The date of anthesis (first emergence of anthers from the integument) of the primary cyathium was recorded, also the star diameter (diameter of the circle enclosing the largest bracts) at anthesis, numbers of green and coloured leaves and bracts, and the total number of cyathia formed 25 days after anthesis.

5.2 Geranium

The length of the main stem from the cotyledonary node to the apex was measured at weekly intervals from the start of treatment until flowering. The fresh and dry weights of main stem leaves and the main stem, the lengths of the petioles of selected fully-expanded leaves, and the leaf area were recorded at the end of treatment. The date of anthesis of the first flower was recorded.

5.3 Chrysanthemum

The length of the axillary shoot was measured at weekly intervals from the start of treatment until flowering. Development of the apical meristem was assessed two and four weeks after the start of short days using a scale devised by Cathey and Borthwick [8] (0 = vegetative; 2 = stem terminal forming capitulum; first bracts of the receptacle present; 10 = perianth primordia present on all florets) . The fresh and dry weights of leaves and stems of the main stem and the axillary shoot, the leaf area and the chlorophyll and carotenoid

content of the youngest fully expanded leaf were recorded at the end of treatment. The stage of flower development was recorded at weekly intervals from buds visible to flowers fully open using a scale (0 = vegetative apex; 4 = florets visible on the capitulum; 8 = flower open) devised by Cockshull and Hughes [9].

6. Experimental schedules

Full details of the experiment schedule are given in Appendix 1.

RESULTS AND DISCUSSION

1. Poinsettia

Poinsettia was the only species grown in all four experiments. The temperature treatments applied in Experiments 1 and 3 were identical and a separate set of identical treatments were applied in Experiments 2 and 4. Nevertheless, the separate pairs cannot be treated as replicates because the plants in Experiment 3 carried one side shoot, whereas those in Experiment 1 had many side shoots, and the treatments were applied for eight weeks in Experiment 2 and for five weeks in Experiment 4. Also, the plants used in Experiments 1 and 2 were somewhat larger and more vigorous than those used in Experiments 3 and 4. These differences in the plant material probably reflect differences in the time of year when they were propagated.

1.1 Shoot elongation

The effects of treatment on shoot length were relatively small up to the time the treatments ended, but they continued to be expressed following transfer to a common glasshouse environment and differences between treatments were more marked by the time the plants flowered . When night temperatures were altered to maintain the same 24-h average temperature (Experiments 1 and 3), a positive DIF (+4 DIF) always increased shoot length relative to the 0 DIF treatment. Plants in the -4 DIF treatment were always shorter than those in +4 DIF and were shorter than those in 0 DIF at the end of treatment in both experiments, and at flowering in Experiment 3 (Fig. 2, Plate 1). The -12 DIF treatment was no more effective than -4 DIF. Of the two DROP treatments, -8E DROP was more effective than -8L DROP at reducing plant height in Experiment 1 but not in Experiment 3. The reason for this discrepancy is unclear, for the same treatment was effective on chrysanthemum in both experiments (Fig. 11a and b), and the -8E DROP treatment of Experiments 2 and 4, which had the same change in temperature and timing of change, was very effective on poinsettia. The data suggest that the magnitude of the negative difference between day and night temperature, the duration of change, and the timing of the change in temperature are important in regulating stem length. However, an excessive negative difference, either of 12°C or to 12°C, can lessen the expected effect. Calculation of 'specific stem lengths' (stem length per mg dry weight) indicated that stems of plants grown with -12 DIF were also consistently 'weaker' than those of plants grown in other regimes. That is, for a given dry weight, stems were longer than for other treatments.

When night temperature was kept constant at 18°C (Experiments 2 and 4), increasing the magnitude of the drop in temperature in the first half of the day tended to produce increasingly shorter plants, especially by the time of flowering (Figs 3a and b, Plate 2). In these experiments, the first half of the day (E) was itself divided into two halves, i.e. EE (from 'lights on' for three hours) and EL (from three hours after 'lights on' to six hours after 'lights on' (Fig 1b)). An 8°C drop in temperature at 'lights on' (-8EE) was more effective than the same drop given three hours later (-8EL). Overall, the -8EE DROP treatment was usually as effective as -8 DROP, suggesting that the timing and the magnitude of the temperature reduction are more important than either the integral or the actual temperature achieved in DROP treatments that start at the beginning of the day. There were no consistent differences in stem strength over the various DROP treatments.

1.2 Leaf colour

The first visible effect of treatment in Experiments 1 and 3 was a yellowing of the young leaves of plants in the -12 DIF treatment. This became apparent within four days of the start of treatment and eventually the leaves became bright yellow in colour. Plants in the -4 DIF treatment were also paler than those in positive or zero DIF. The chlorophyll content of leaves that had expanded in the treatments was reduced by an order of magnitude in the -12 DIF plants (Fig. 4a) but was less consistently affected in other treatments. In Experiment 2, the chlorophyll content of leaves tended to reduce with increasing magnitude of DROP (Fig. 4b) but there was little consistent effect of treatment in Experiment 4. The changes in chlorophyll b concentration were generally similar to those of chlorophyll a. Carotenoid concentrations also showed a similar pattern of response but were reduced less by negative DIF and DROP treatments, so that the proportion of carotenoids to chlorophylls was increased. This effect was most marked at -12 DIF, hence the vivid yellow colour of the leaves in this treatment.

The normal green colour of the leaves was restored within a week of removing the plants from most DIF and DROP treatments, and they quickly became indistinguishable from the controls. Affected leaves from the -12 DIF treatment also become greener but continued to retain some paler patches.

1.3 Dry weight and leaf area

After five weeks of treatment, there was very little effect on the dry weight of the main stem, but the dry weights of the axillary shoots tended to reduce as the intensity of the DIF treatment increased from +4 to 0 to -4 to -12 DIF (Fig. 5a). As discussed above, the -12 DIF treatment also had the least chlorophyll in its leaves and its pale leaves might be expected, therefore, to absorb less light. The DROP treatments in all experiments had less consistent effects, although plants from the -8E DROP treatment in Experiments 2 and 4 always had axillary shoots with the lowest leaf dry weights (Fig. 5b). This treatment also had the lowest leaf area (Fig. 6b), possibly because the 24-hour mean temperature was reduced. Total leaf area also tended to decrease with intensity of DIF treatment (Fig. 6a) but not in the -12 DIF treatment, while the effects of DROP treatments were again inconsistent, except for the consistently smaller leaf area in the -8 DROP treatment (Fig 6b). It has been reported by others that it is undesirable to maintain -DIF and DROP treatments for longer than the first few weeks of short days as bract size is often adversely affected by low day temperature [12, 13]. Leaf area per unit dry weight was increased by about 50% in the -12 DIF treatment, relative to the 0 DIF and +4 DIF treatments, indicating thinner

leaves in the -12 DIF treatment. Leaf moisture content was increased by about 5% in this same treatment. There was only a slight effect of treatment on the partitioning of dry matter between leaves and stems, but both DIF and DROP treatments tended to increase the percentage of dry matter in the stems (Table 1). This even occurred in the -12 DIF regime in Experiment 3 but not in Experiment 1.

1.4 Flowering

The temperature treatments applied in Experiments 1 and 3 had a marked effect on flowering of poinsettia, even though they all provided the same 24-hour mean temperature. Apical dissection after 5 weeks of treatment showed that plants in the -4 DIF treatment, were at a more advanced stage of floral development than those in the 0 and +4 DIF treatments (Table 2). This effect was maintained following transfer to a common environment (Plate 1). Plants in the -8E and -8L DROP treatments also showed flowering advance. At the completion of treatment, only some of the -12 DIF plants had begun to initiate cyathia primordia. The slower floral initiation in the +4 and -12 DIF treatments produced a small increase in leaf number below the bract whorl. In both experiments, the -4 DIF and -8E DROP treatments were the first to reach anthesis of the primary cyathium (Fig. 7a), together with the -8L DROP treatment in Experiment 3, though it was slightly delayed in Experiment 1. All three of these treatments shared one common feature - a night temperature of 20°C. Plants from the +4 DIF treatment were the last to flower in both experiments, and they received a night temperature of 16°C. The plants in -12 DIF were delayed, even relative to +4 DIF at the 5 week stage, but they reached anthesis ahead of this treatment. The night temperature in -12 DIF was 24°C, which, in combination with a day temperature of only 12°C, may have delayed initiation but accelerated subsequent development.

The plants in Experiment 1 started to develop red colour in their leaves before the end of treatment (as did the plants in Experiments 2 and 4) but colouring of the leaves was delayed in Experiment 3 and did not occur until well after removal from the cabinets. Colour developed first in treatments where floral development was most advanced after 5 weeks, but by anthesis, treatments in which the floral development was slower could have a greater number of coloured leaves, as shown by the leaf colour index (Table 2). The total number of cyathia per plant, recorded 25 days after anthesis, tended to be higher in treatments that flowered later. Some plants of the +4 DIF treatment developed an abnormal star structure, with some or all of the secondary cyathia being surrounded by three bracts as if they were primary cyathia. This caused bracts to protrude into the middle of a spread cyathia-cluster, spoiling the appearance of the star. In total, 77% of stars were affected in this way in Experiment 1 and 30% in Experiment 3.

The DROP treatments applied in Experiments 2 and 4 had smaller effects on flowering, probably because the night temperature in all treatments was the same, i.e. 18°C (Table 3, Fig. 7b). However, flower development of the -8EE DROP treated plants was more advanced than that of the -8EL DROP treated plants at the 5 week stage and, indeed, was more advanced than any other treatment, and plants of this treatment were the first to reach anthesis. Thus, in addition to the effect of night temperature on flowering, there appeared to be a flower promoting effect resulting from dropping the temperature by 8°C for three hours at the start of the day. No treatments consistently affected the diameter of the star at anthesis.

There were large differences between experiments in the number of SD to anthesis in the control treatment at a constant temperature of 18°C (0 DIF). This treatment was common to all experiments, yet plants in Experiments 1 and 4 took about 70 short days to reach anthesis while those in Experiments 2 and 3 took about 85 short days to reach the same stage. The probable explanation was the prevailing environment in the glasshouse, as plants from Experiments 1 and 4 were transferred there in July and May, and those from Experiments 2 and 3 in October and February.

2. Geranium

2.1 Appearance and colour

At the end of the treatment period, plants grown in the -12 DIF regime looked paler and a little more compact than those grown in other treatments, but leaf chlorophyll content was not estimated. Leaf colour differences disappeared within a week of removal to the glasshouse.

2.2 Shoot and petiole lengths

The effects of DIF and DROP treatments on geranium were small, partly because of the very short stem on the plant in the early stages of growth. Shoot length was very little affected by negative DIF treatments, showing only a small reduction at -12 DIF (Fig. 8a). The -8 DROP treatment reduced stem length a little but otherwise, DROP treatments were not very effective (Fig. 8b). The petioles of certain leaves were progressively shorter in the treatment sequence from +4 to 0 to -4 to -12 DIF (Fig. 9), and the effects of the -8E and -8L DROP treatments approached that of -4 DIF. The effects of DROP treatments on petiole length, indicating progressive reduction with increase in magnitude, are shown in Plate 3. The petioles of geranium are relatively long, by comparison with the stem and so, any shortening of their length gives the plants a more compact appearance.

2.3 Dry weight and leaf area

There was very little effect of DROP treatments on plant dry weight and leaf area, but these two parameters were slightly reduced by the -12 DIF treatment (Fig. 10). Leaf thickness was reduced slightly by negative DIF treatments but not by DROP treatments. Partitioning of the dry matter was unaffected by the treatments applied, except -12 DIF, which increased the stem fraction at the expense of the leaves. The number of expanded leaves present at the end of treatment was slightly increased by negative DIF treatments but was reduced by DROP treatments in Experiment 2, possibly as a result of the lower 24-hour mean temperature.

2.4 Flowering

No growth regulators were used as this might have interfered with any effects of the treatments applied. As a result, however, the plants were very variable in flowering. Some of those used in Experiment 1 were already reproductive when they were transferred to the the cabinets for treatment. In Experiment 2, plants grown in the -8E DROP regime flowered four days later than those grown in the 0 DROP regime, probably due to the lower 24-hour mean temperature.

3. Chrysanthemum

3.1 Shoot length

The length of axillary shoots of chrysanthemum at the end of treatment was progressively reduced in the sequence of treatments passing from +4 to 0 to -4 DIF in Experiment 3 (Fig. 11a, Plate 4). The -12 DIF treatment was only slightly more effective than the -4 DIF treatment in this experiment and calculations of specific stem length (see page 10) showed that, as for poinsettia, stems of plants grown in the -12 DIF regime were consistently weaker than those of plants grown in other regimes. All treatments received the same 24-h average temperature of 18°C in Experiment 3, so as not to affect flowering. Despite this precaution, the combination of a 12°C day temperature with a 24°C night temperature (-12 DIF) did delay flower initiation (Fig 12a) and flower development (Fig.12b, Plate 5). It is probably for this reason that plants from the -12 DIF treatment had the longest shoots at flowering (Fig. 11a). Flower initiation and development were also delayed a little in the -4 DIF and -8L DROP treatments (Fig. 12a and b, Plate 5) and their stems were slightly longer than those from 0 DIF at flowering (Fig. 11a). Final shoot length in the -8E treatment was similar to that in 0 DIF, as was its flowering.

When the night temperature was held constant at 18°C (Experiment 4), increasing the magnitude of the DROP treatment from 0 through -2 and -4 to -8E DROP, produced shorter shoots at the end of treatment (Plate 6), though the graded nature of the responses was less evident at flowering (Fig. 11b). There were no obvious effects on stem strength. Flowering was slightly delayed in the -8E DROP treatment, perhaps because of its lower average 24-h temperature (Fig 13). Of the two shorter duration DROP treatments (-8EE and -8EL), -8EL DROP produced the shorter shoots at end of treatment and at flowering. Furthermore, flowering was not as delayed in this treatment as in -8EE. These data suggest that the most sensitive period for retarding the stem growth of chrysanthemum by a drop in temperature is not immediately after dawn but lies between three and six hours after dawn.

The treatments were applied only for the first four weeks of short days, and so considerable shoot elongation occurred after the end of treatment. Unlike poinsettia, however, the initial effects on stem length were lost in the period after treatment in Experiment 3 and became much less marked in Experiment 4. These differences in the stem elongation response of the two species were probably related to differences in their flowering responses to temperature.

3.2 Leaf colour

Young leaves of plants grown in -4 and -12 DIF regimes appeared slightly paler than those grown in +4 and 0 DIF and the chlorophyll a content of leaves of chrysanthemum was reduced in the former treatments, especially in -12 DIF (Fig. 14a, Plate 4). The effect was not so great as in poinsettia, the leaves of which turned bright yellow in -12 DIF. The DROP treatments in Experiments 3 and 4 had no effect on chlorophyll concentration (Fig. 14a and b). Any colour differences that were produced by the treatments, quickly disappeared once the plants were returned to the glasshouse.

3.3 Dry weight and leaf area

The dry weights and leaf area of the main stem were unaffected by the treatments applied in either experiment, except for a slight reduction in the main stem dry weight in -12 DIF. Neither the dry weights of the leaves of the axillary shoot nor their areas were systematically affected by the treatments applied in Experiment 3 (Fig. 15a), in which average 24-h temperatures were the same. Increasing magnitude of DROP, however, reduced axillary shoot leaf area and, to a lesser extent, leaf weight in Experiment 4 (Fig. 15b), perhaps because these treatments also produced lower average 24-h temperatures. Of the shorter DROP treatments, -8EL had more effect on leaf area and weight than -8EE The dry weights of the stems of axillary shoots were reduced by the -4 and -12 DIF treatments in Experiment 3 (Fig. 16a) and by increasing magnitude of the DROP treatments in Experiment 4 (Fig. 16b). The -8EE DROP treatment had less effect than -8EL. The dry weight of the secondary axillary shoots was reduced by both the -4 and -12 DIF treatments of Experiment 3 (Fig. 17a) and by -8E DROP in both experiments (Fig. 17a and b), though not by the short -8EE DROP treatment. The percent dry matter of the leaves was slightly reduced (2%) by the -12 DIF treatment and leaves were slightly thinner than in other treatments. The partitioning of dry matter between the leaves and the stem tended to favour the leaves in negative DIF treatments and in the DROP treatments of Experiment 4 (Table 4).

3.4 Flowering

In Experiment 3, the temperature treatments all provided the same 24-h mean temperature. Even so, flower development in the -4 and -12 DIF treatments was delayed relative to the +4 and 0 DIF treatments, both in the early and late stages of flower development (Fig. 12a and b, Plate 5). Two more leaves were formed before the flower in the -12 DIF treament, indicating that at least part of the delay was caused by a delay in flower initiation. Leaf number was not affected in other negative DIF treatments nor in the two DROP treatments, which suggests that these treatments affected only flower development. Flowering was delayed in -8L but not -8E (Plate 5) suggesting that a cooler period occurring in the second half of the day, such as occurred in -8L and all negative DIF treatments, delayed flower development.

The 24-h average temperature varied with treatment in Experiment 4, and those treatments with 24-h average temperatures that were lower than that of 0 DIF did flower later (Fig. 13). The delay was progressively greater, as the difference in average 24-h temperature increased from 0 DIF through -2 and -4 to -8E DROP. The delay with -8EE (a drop of 8°C for three hours) was similar to that with -8E DROP but was greater than with -8EL or -4E DROP with which it shared the same 24-h mean temperature. Clearly, 24-h mean temperature alone is not the only factor controlling flower development and the timing of any change in temperature is also important. The data from both experiments suggest that low temperature in the first three hours of the day or in some part of the last six hours of the day, has a greater effect on flowering than would be expected as a result of its effect on the 24-h average temperature.

4. General

4.1 Stem length

Although all three species were responsive to DIF and DROP regimes, the nature and magnitude of response differed, as did the persistence of effects once the plants had been removed from treatment. In general, poinsettia and chrysanthemum were more responsive than geranium, and effects persisted longer in poinsettia than in chrysanthemum.

When 24-h average temperatures were maintained at 18°C (Experiments 1 and 3), there was a clear and progressive reduction in stem length, evident at the end of the treatment phase, in poinsettia and chrysanthemum, for treatments +4 DIF, through 0 DIF, to -4 DIF (Figs. 2a and 11a; Plates 1 and 4). Geranium reacted similarly with regard to petiole length (Fig. 9) but not to stem length (Fig. 8a). In both experiments, plants were transferred to a common environment after treatment, and considerable further growth took place during this time. Nevertheless, the effects of DIF on stem length persisted in poinsettia and even appeared to increase in magnitude (Fig. 2b). In contrast, stem length differences in chrysanthemum gradually disappeared and, at harvest, plants given -4 DIF actually had the longest stems (Fig. 11a). It would appear that for chrysanthemum, DIF treatments need to be continued for most of the life of the crop to ensure that height reductions are shown at the marketing stage.

The most extreme negative DIF treatment, -12 DIF, proved ineffective in reducing stem length of poinsettia at the completion of treatment (Fig. 2a), although it further reduced petiole length in geranium (Fig. 9) and was at least as effective as -4 DIF in reducing stem length in chrysanthemum (Fig. 11a). However, this effect on chrysanthemum was not maintained after transfer to the common environments, and plants given this treatment were by far the tallest at final harvest (Fig. 11a). Only for geranium was a beneficial effect of -12 DIF maintained to the marketing stage.

In general, DROP treatments in Experiments 1 and 3 proved effective in all three species in reducing stem length (petiole length in geranium) (Figs. 2a, 9 and 11a; Plates 2, 3 and 6). Reductions were greatest when DROP was given during the first half of the day rather than the second in poinsettia (Experiment 1 but not Experiment 3) and chrysanthemum, but there was no such difference for geranium. In Experiments 2 and 4, where DROP treatments were given during the first half of the day and where the night temperature (but not the mean 24-h temperature) was maintained at 18°C, increasing the magnitude of the DROP consistently increased its effectiveness in shortening stem length both of poinsettia and chrysanthemum (Figs. 3a and 11b). With poinsettia, the effects were most evident at flowering (Fig. 3b), while with chrysanthemum they were most evident at the end of treatment. When shorter durations of DROP were given, treatment in the first three hours of the day (-8EE) was more effective than treatment during the second three hours of the day (-8EL) for poinsettia (Fig. 3a). The reverse was true for chrysanthemum (Fig. 11b).

4.2 Flowering

There were clear differences between poinsettia and chrysanthemum in flowering responses to the various DIF and DROP regimes. In general, the progress of flowering of chrysanthemum was regulated by the 24-h mean temperature while that of poinsettia was not. It has been found by others that the rate of flowering of poinsettia is accelerated by

increasing night temperatures up to about 21°C, but that at higher temperatures still it is delayed [10,11]. Consequently, both positive DIF regimes with low night temperatures, and negative DIF regimes with night temperatures in excess of 21°C, would be expected to delay flowering. This proved to be the case, for example, in treatments +4 DIF and -12 DIF (Experiments 1 and 3) (Fig. 7a) where, in both cases, an increased number of leaves was recorded below the bract whorl. Plate 1 shows the advance of flowering associated with -4 DIF contrasted with +4 DIF. Over and above this night temperature effect, there appeared to be a flower promoting effect in poinsettia due to low temperatures given in the first three hours of the day (-8EE DROP in Experiments 2 and 4) (Fig. 7b). Others have also found flowering of poinsettia to be accelerated by decreasing day temperatures down to at least 14°C [12].

Although it has been shown to be generally the case that chrysanthemum flowers in response to the average 24-h mean temperature [1], it appeared that low temperature during the second half of the day slowed the rate of flower development. Thus in Experiment 3, delays were associated with the -4 DIF, -12 DIF and -8L DROP treatments (Fig. 12b, Plate 5). Delay was greatest in the -12 DIF treatment, and an increased number of leaves on the axillary shoots of plants in this treatment indicated that a delay in flower initiation contributed to the overall effect. Flowering delay in treatment -8E DROP (Experiment 4) (Fig. 13) was probably a consequence of the reduced 24-h mean temperature. It is less clear why delay was also associated with treatment -8EE DROP, and noteworthy that this treatment gave the fastest flowering in poinsettia!

4.3 Stem length/flowering interaction

It seems probable that differences between poinsettia and chrysanthemum in stem elongation responses to DIF and DROP, were, to some extent, a consequence of the inherent differences in flowering responses between the two species. Flower initiation stops the production of new leaves and internodes, and internodes normally stop elongation by the time that the flowers reach anthesis. Consequently, delays in flower initiation tend to be associated with increased numbers of internodes and delays in flower development tend to be associated with a longer duration of internode extension and, therefore, longer internodes.

In the case of poinsettia, flowering delays in treatments +4 DIF and -12 DIF (Experiments 1 and 3) were associated with particularly long stems at final harvest (Figs. 7a and 2b) and the flower promoting effect of treatment -8EE DROP (Experiments 2 and 4) was associated with short stems at final harvest (Figs. 7b and 3b). In chrysanthemum, flowering delays in treatments -4 DIF, -12 DIF and -8L DROP (Experiment 3) were associated with long stems at final harvest (Figs. 12b and 11a). These stem elongation effects were generally less apparent at the end of cabinet treatment since it is only in the later stages of plant growth that effects due to flower promotion or inhibition show up.

4.4 Chlorophyll content

Negative DIF and DROP treatments were accompanied by a reduction in the chlorophyll and carotenoid content of the leaves of all three species used. This reduction occurred mainly in young leaves that expanded in the treatments, which suggests that the synthesis of chlorophyll was affected by treatment. On the other hand, extreme treatments such as -12 DIF, gave leaf yellowing symptoms on poinsettia within days of the start of

treatment, suggesting that chlorophyll destruction could also be involved. The synthesis of chlorophyll was not permanently impaired for, with the exception of poinsettias from the -12 DIF regime, the affected leaves greened up normally when removed from treatment. The implication of this is that DIF and DROP treatments should, ideally, be ended one or two weeks before harvest in order not to reduce final plant quality. The reduced chlorophyll content can be expected to impair light interception and may, thus have contributed to the production of the smaller dry weights associated with some negative DIF and DROP treatments, especially -12 DIF. However, lower average temperatures also reduce leaf expansion and probably had an influence in some cases.

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APPENDIX 1.

EXPERIMENT SCHEDULES

Experiment 1.

16-17/5	Poinsettia cuttings potted
24/5	Poinsettias stopped
5-6/6	Geraniums potted
12/6	Uniform plants of both species selected
13/6	20 measured plants of each species placed in each cabinet; start of treatments
2/7	Fault with carbon dioxide enrichment system
16/7	Destructive harvest of geraniums; 5 plants per treatment
	Chlorophyll content of poinsettia leaves measured; 3 plants per treatment
17/7	Destructive harvest of poinsettias; 5 plants per treatment
18/7	10 plants per species per treatment transferred to glasshouse
13/8	Final records of geranium
27/9	Final records of poinsettia

Experiment 2.

2/8	Poinsettia cuttings potted
12-13/8	Poinsettias stopped
3/9	Geraniums potted
3-4/9	Uniform poinsettia plants selected, shoots reduced to one
5/9	20 measured poinsettias placed in each cabinet
9/9	Uniform geranium plants selected
10/9	20 measured geraniums placed in each cabinet
10/10	Chlorophyll content of poinsettia leaves measured; 5 plants per treatment
29/10	Destructive harvest of geraniums; 5 plants per treatment
	10 remaining plants per treatment transferred to glasshouse
31/10	Destructive harvest of poinsettias; 5 plants per treatment
	10 remaining plants per treatment transferred to glasshouse
16/12	Final records of geranium
23/12	Final records of poinsettia

Experiment 3

13/12	Poinsettia cuttings potted
3/1	Chrysanthemum cuttings potted
	Poinsettias stopped
13/1	Chrysanthemums stopped
21-22/1	Uniform poinsettia plants selected, shoots reduced to one
23/1	20 measured poinsettias placed in each cabinet
27/1	Uniform chrysanthemum plants selected, shoots reduced to one
28/1	20 measured chrysanthemums placed in each cabinet
24/2	Chlorophyll content of chrysanthemum leaves measured; 5 plants per treatment
25/2	Destructive harvest of chrysanthemums; 5 plants per treatment
	10 remaining plants per treatment transferred to glasshouse

27/2	Destructive harvest of poinsettias; 5 plants per treatment 10 remaining plants per treatment transferred to glasshouse
28/2	Chlorophyll content of poinsettia leaves measured; 5 plants per treatment
14/4	Final records of chrysanthemum
18/5	Final records of poinsettia

Experiment 4

20-21/2	Poinsettia cuttings potted
4/3	Chrysanthemum cuttings potted
13/3	Poinsettias and chrysanthemums stopped
25/3	Uniform chrysanthemum plants selected, shoots reduced to three
26/3	20 measured chrysanthemums placed in each cabinet
30/3	Uniform poinsettia plants selected, shoots reduced to one
31/3	20 measured poinsettias placed in each cabinet
23/4	Destructive harvest of chrysanthemums; 5 plants per treatment 10 remaining plants per treatment transferred to glasshouse
24/4	Chlorophyll content of chrysanthemum leaves measured on remaining 5 plants per treatment
5/5	Destructive harvest of poinsettias; 5 plants per treatment 10 of remaining plants per treatment transferred to glasshouse
6/5	Chlorophyll content of poinsettia leaves measured on 5 plants per treatment
28/5	Final records of chrysanthemum
3/7	Final records of poinsettia

APPENDIX 2

TABLES AND FIGURES

Table 1. The proportion of the dry matter of axillary shoots of poinsettia that was present in the stem (stem weight ratio). a) Same 24-h average temperature; b) Same night temperature

	Treatment					
a) same average temperature						
	+4 DIF	0 DIF	-4 DIF	-12 DIF	-8E DROP	-8L DROP
Expt. 1	0.197	0.196	0.223	0.184	0.201	0.197
Expt. 3	0.202	0.251	0.275	0.297	0.227	0.256
b) same night temperature						
	0 DIF	-2E DROP	-4E DROP	-8E DROP	-8EE DROP	-8EL DROP
Expt. 2	0.264	0.267	0.289	0.297	0.270	0.308
Expt. 4	0.191	0.165	0.186	0.218	0.192	0.192

Table 2. The effect of DIF and DROP treatments with a 24-hour mean temperature of 18°C on flower and star development of poinsettia (Experiments 1 and 3)

		Treatment					
	Expt	+4 DIF	0 DIF	-4 DIF	-12 DIF	-8E DROP	-8L DROP
Apical stage after 5 weeks	1	1.2	1.8	3.2	0.2	5.0	3.8
	3	1.5	2.1	4.1	0.7	4.1	3.8
Total leaf number	1	12.1	10.6	10.0	13.1	10.1	10.9
	3	13.6	10.7	10.2	11.7	10.3	10.7
Leaf colour index* before anthesis	1	7.3	13.5	13.5	19.9	18.1	14.2
	3	3.3	5.9	10.1	7.1	9.2	10.2
Leaf colour index after anthesis	1	8.7	12.4	11.9	21.4	16.7	11.6
	3	15.3	7.9	9.7	12.0	9.1	10.2
Total cyathia number	1	32.5	24.4	25.4	24.1	21.0	26.5
	3	33.8	33.7	29.5	28.4	31.0	31.1

* 'Leaf colour index' is a measure of the area of red pigment in the leaves (not the bracts) which are recorded in five colour categories and scored as follows:

1	Fully green	score 0
2	Less than one third of leaf red	score 1
3	One to two thirds of leaf red	score 2
4	More than two thirds of leaf red	score 3
5	Fully red	score 4

Table 3. The effect of DROP treatments with a night temperature of 18°C on flower and star development of poinsettia (Experiments 2 and 4).

		Treatment					
	Expt	0 DIF	-2E DROP	-4E DROP	-8E DROP	-8EE DROP	-8EL DROP
Apical stage after 5 weeks	2	5.0	5.4	5.0	4.8	5.6	4.8
	4	4.8	4.6	5.2	4.9	5.5	4.8
Total leaf number	2	11.5	11.8	11.9	11.2	11.0	11.9
	4	11.9	11.6	11.3	9.7	10.8	11.5
Leaf colour index* before anthesis	2	13.4	16.6	19.2	20.6	22.6	19.8
	4	13.7	11.8	14.9	15.5	18.0	13.9
Leaf colour index* after anthesis	2	13.2	15.8	19.5	22.7	22.6	18.1
	4	12.0	10.1	14.0	13.0	16.2	13.1
Total cyathia number	2	25.1	22.2	21.9	17.0	15.7	23.8
	4	39.5	40.7	33.9	31.9	32.6	37.3

* see Table 2.

Table 4. The proportion of the dry matter of the axillary shoots of chrysanthemum that was present in the stem (stem weight ratio). a) at same 24-h average temperature; b) at same night temperature.

	Treatments					
a) same 24-h average temperature						
	+4 DIF	0 DIF	-4 DIF	-12 DIF	-8E DROP	-8L DROP
Expt. 3	0.324	0.325	0.290	0.280	0.313	0.330
b) same night temperature						
	0 DIF	-2E DROP	-4E DROP	-8E DROP	-8EE DROP	-8EL DROP
Expt. 4	0.338	0.324	0.300	0.285	0.324	0.319

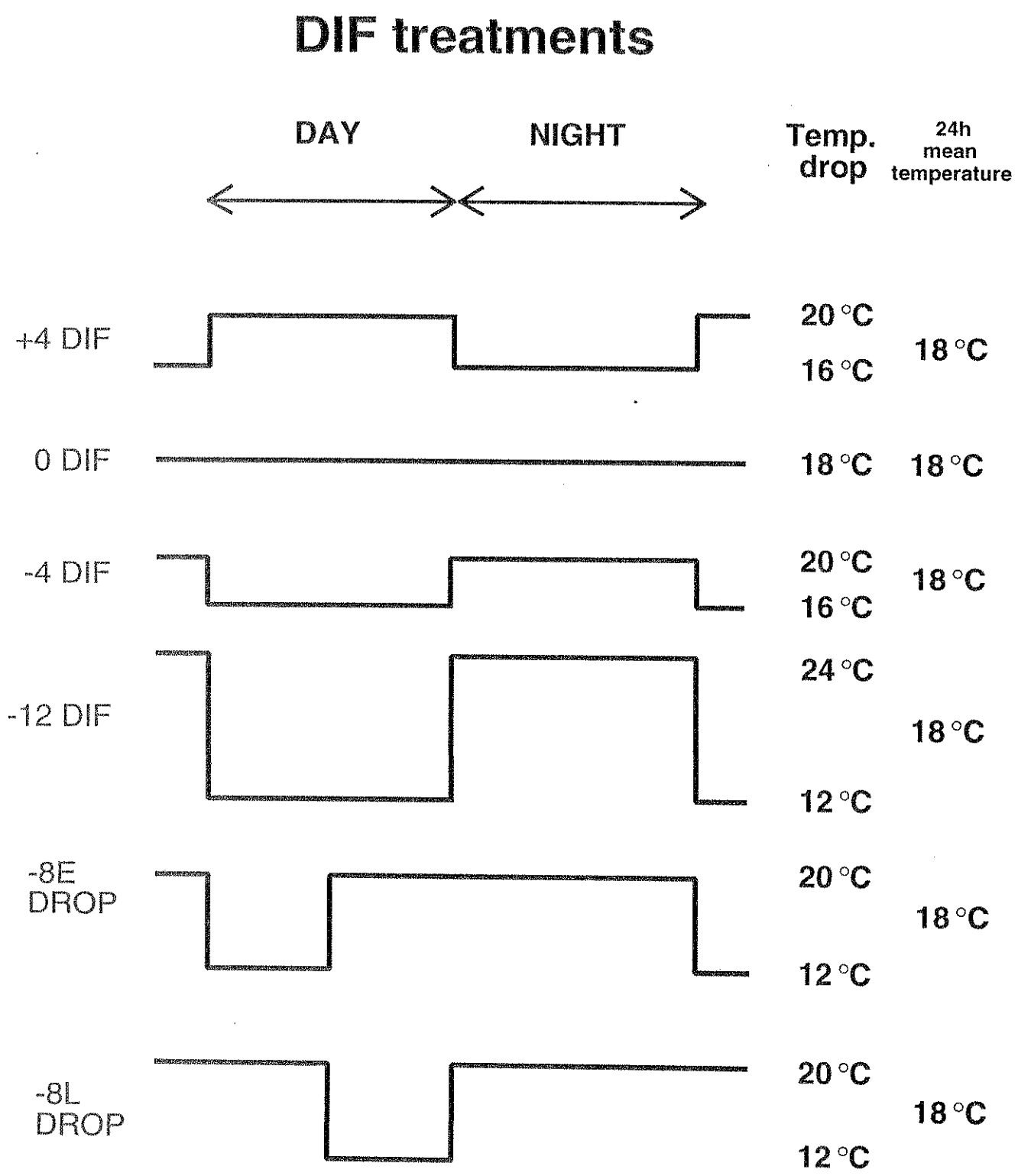


Fig. 1a. Treatments applied in Experiments 1 and 3.

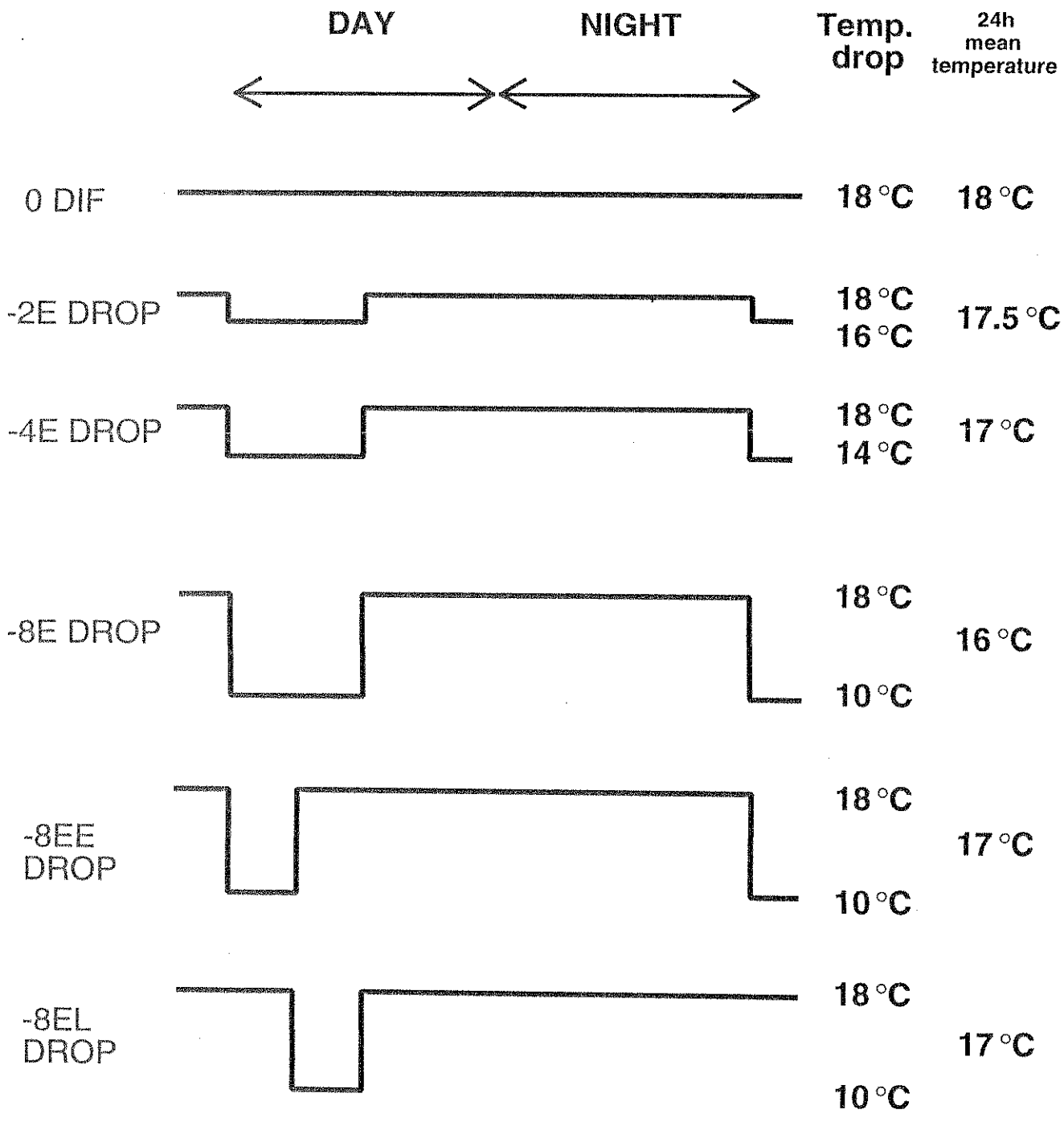
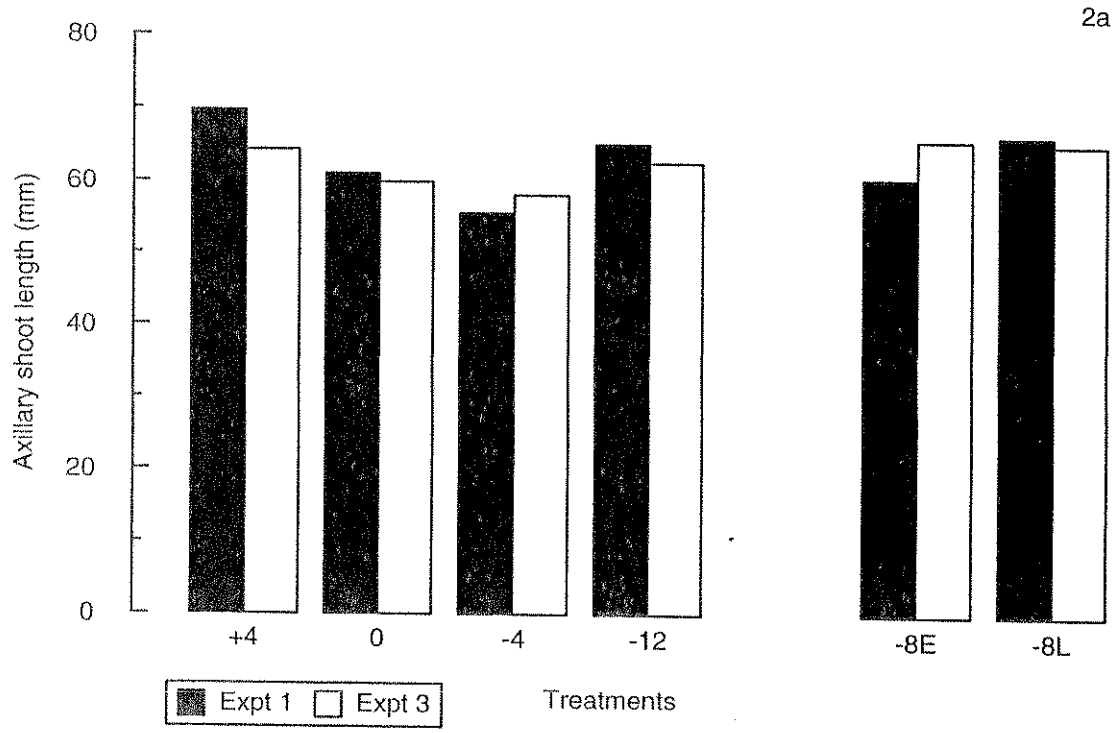


Fig. 1b. Treatments applied in Experiments 2 and 4.



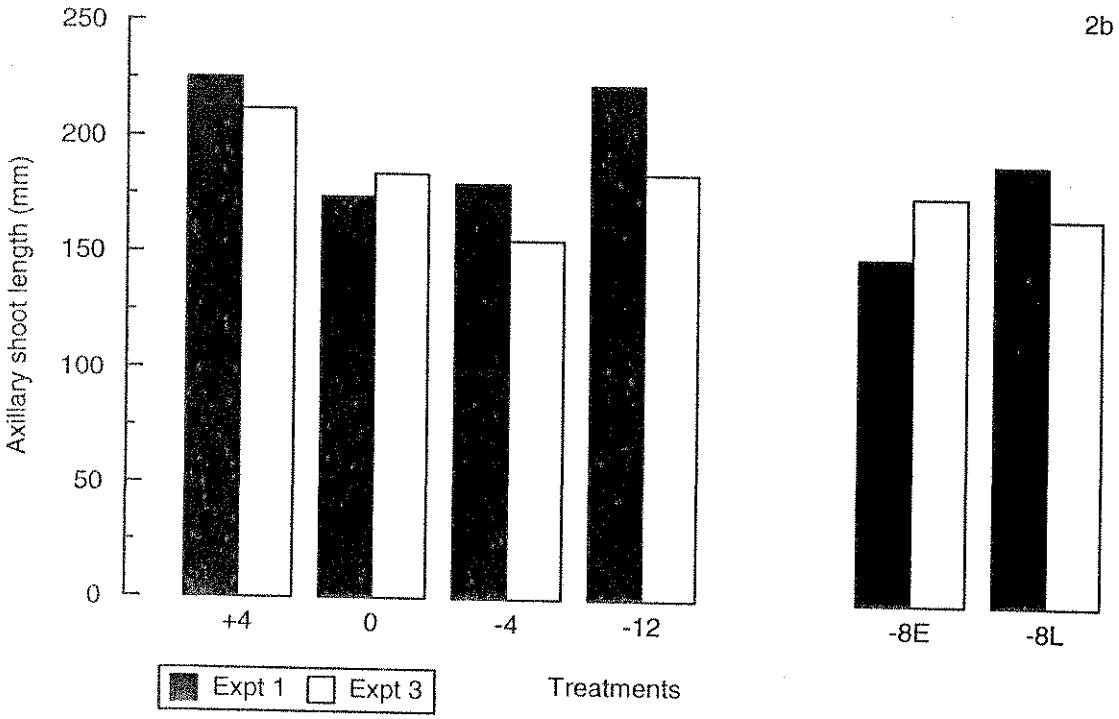
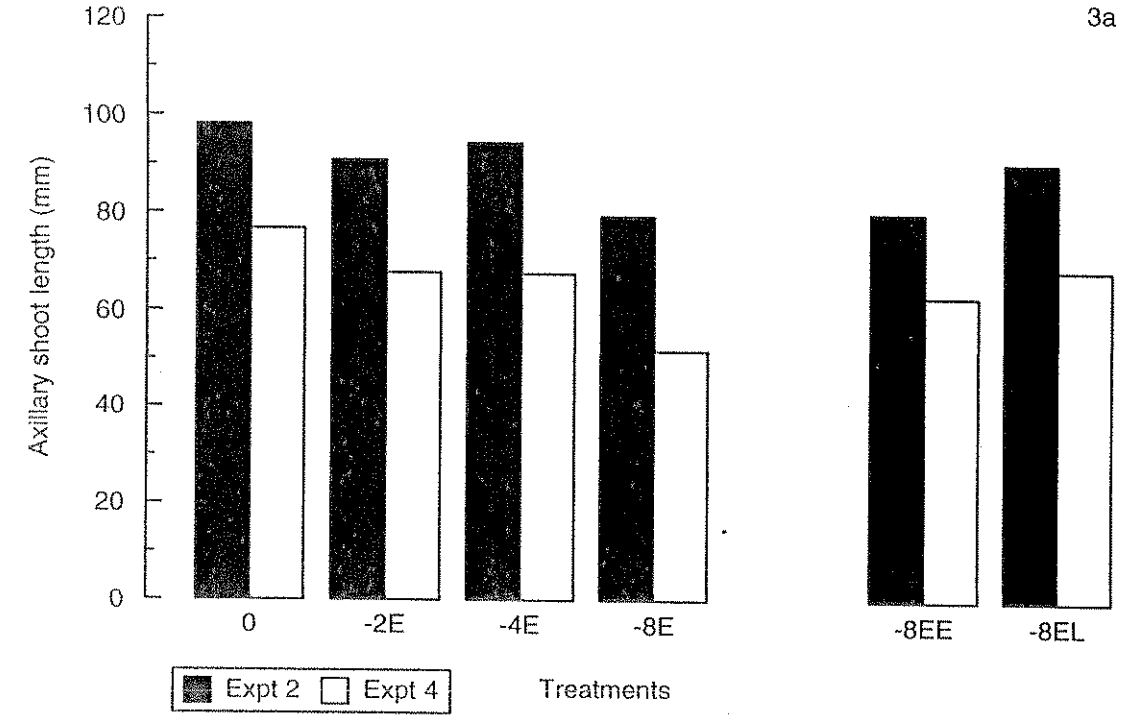


Fig. 2. The effect of DIF and DROP treatments having a 24-hour mean temperature of 18°C on extension growth (mm) of poinsettia (Experiments 1 and 3); a) after five weeks of treatment, b) at flowering.



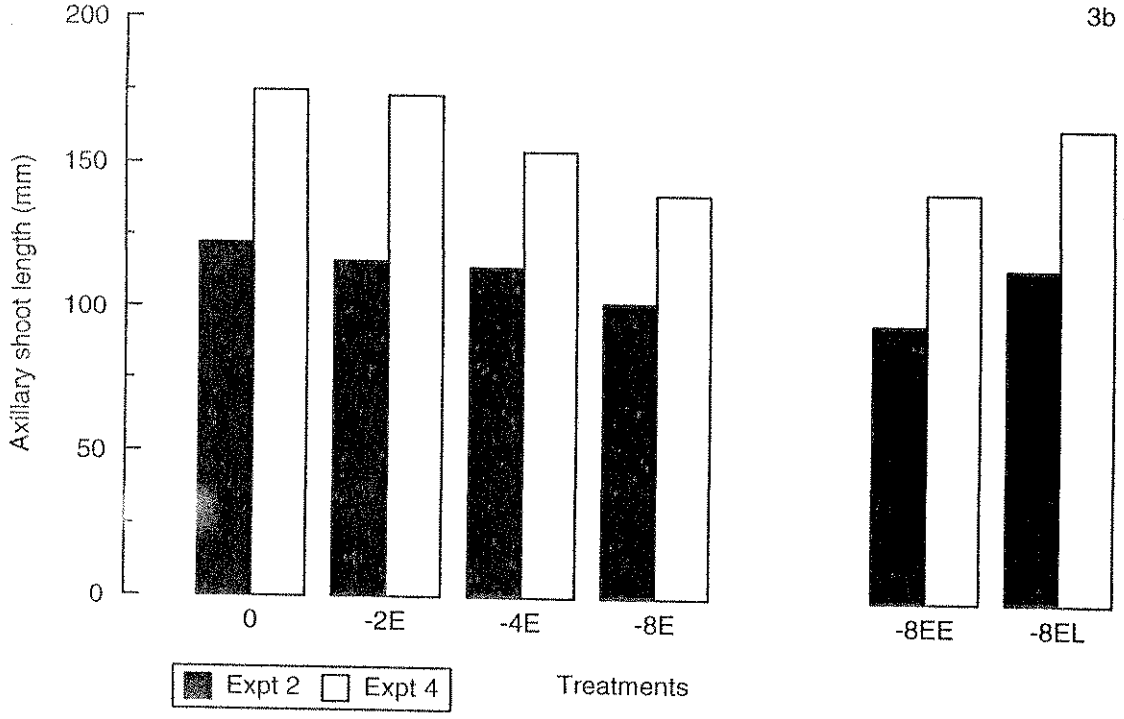
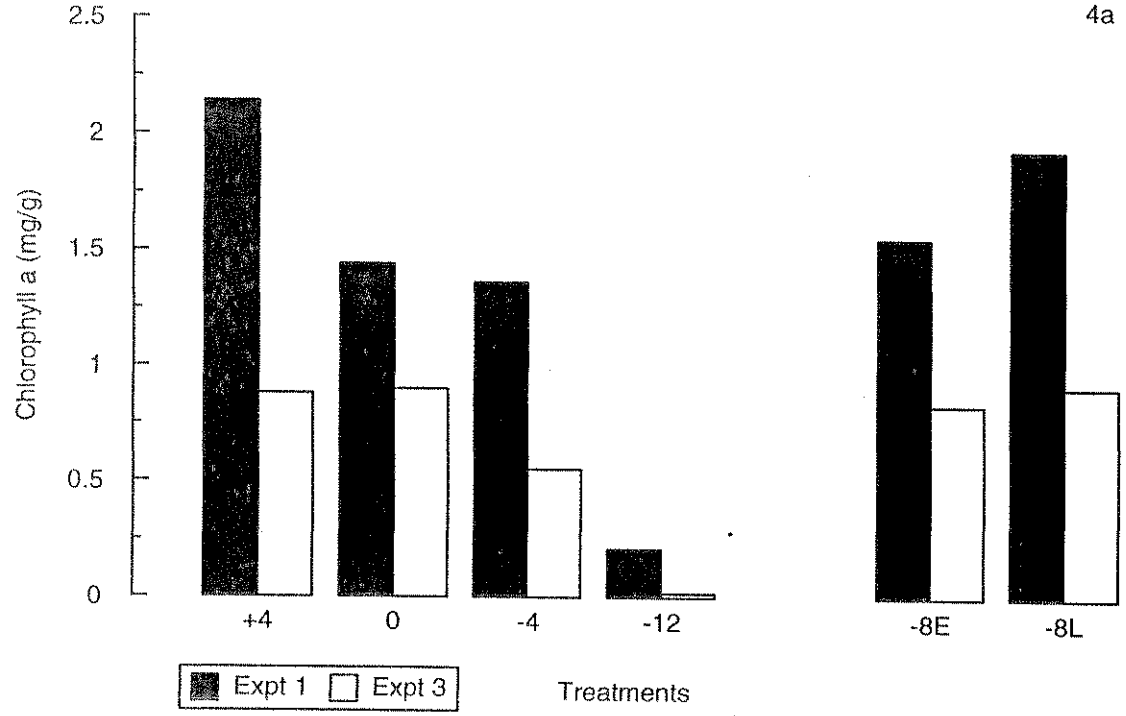


Fig. 3. The effect of DROP treatments having a night temperature of 18°C on extension growth (mm) of poinsettia (Experiments 2 and 4); a) after five weeks of treatment, b) at flowering.



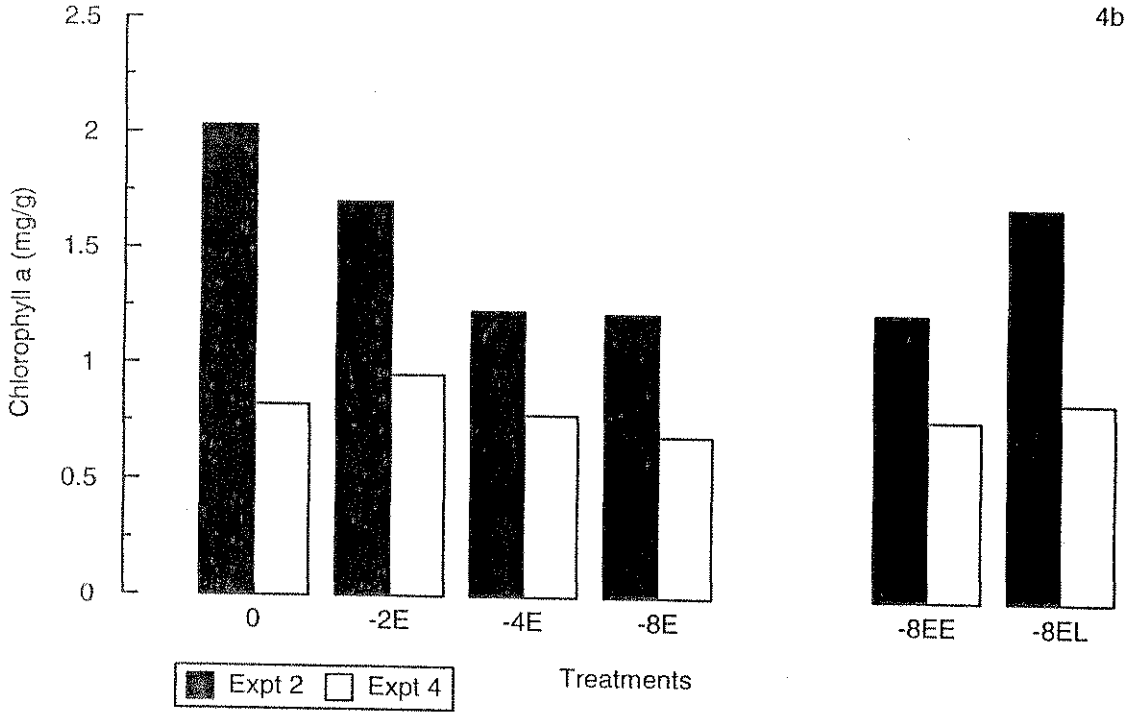


Fig. 4. The chlorophyll a concentration (mg/g fresh weight) in poinsettia leaves; a) grown in treatments with a 24-hour mean temperature of 18°C (Experiments 1 and 3), b) grown in treatments with a night temperature of 18°C (Experiments 2 and 4).

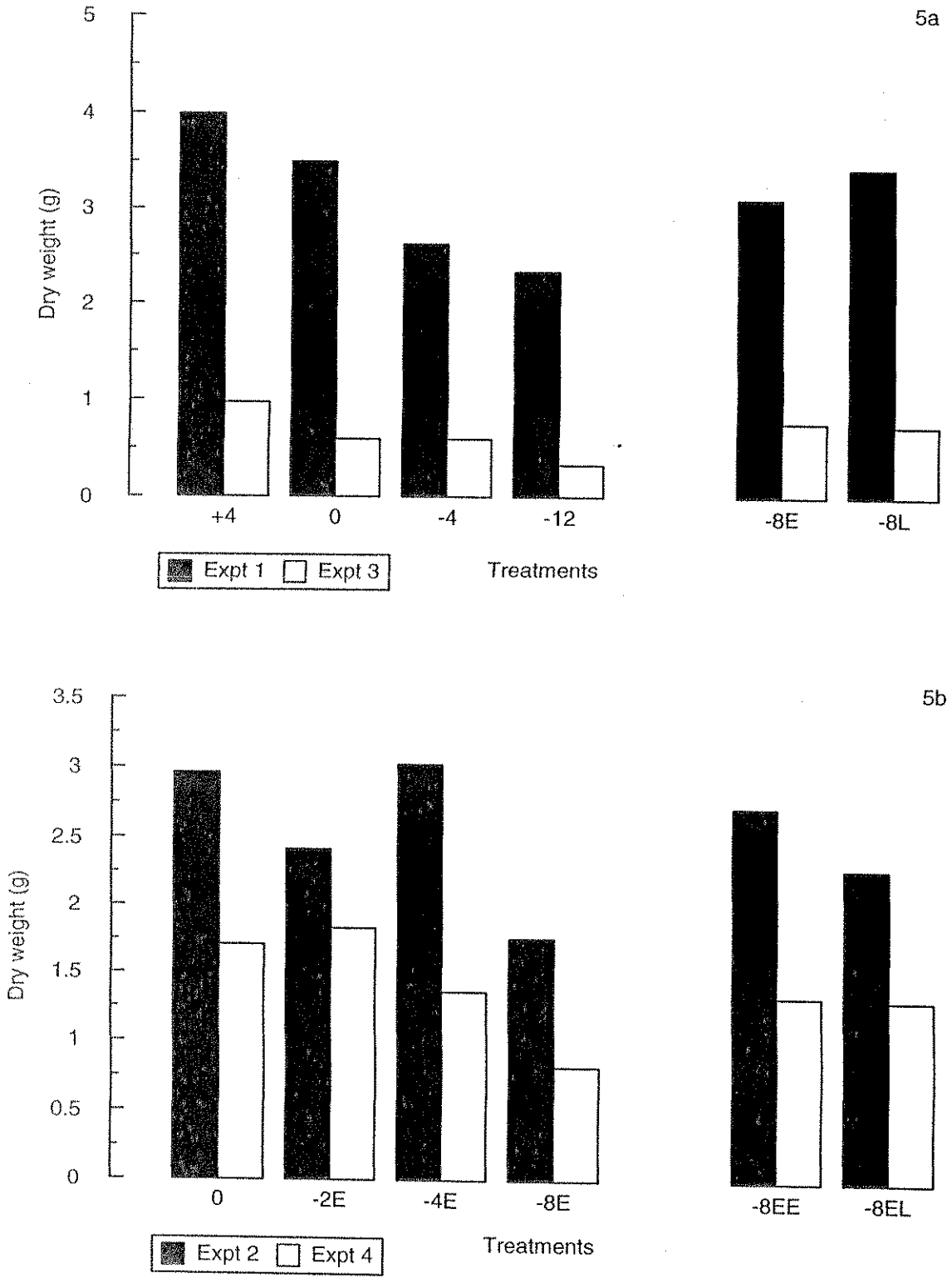
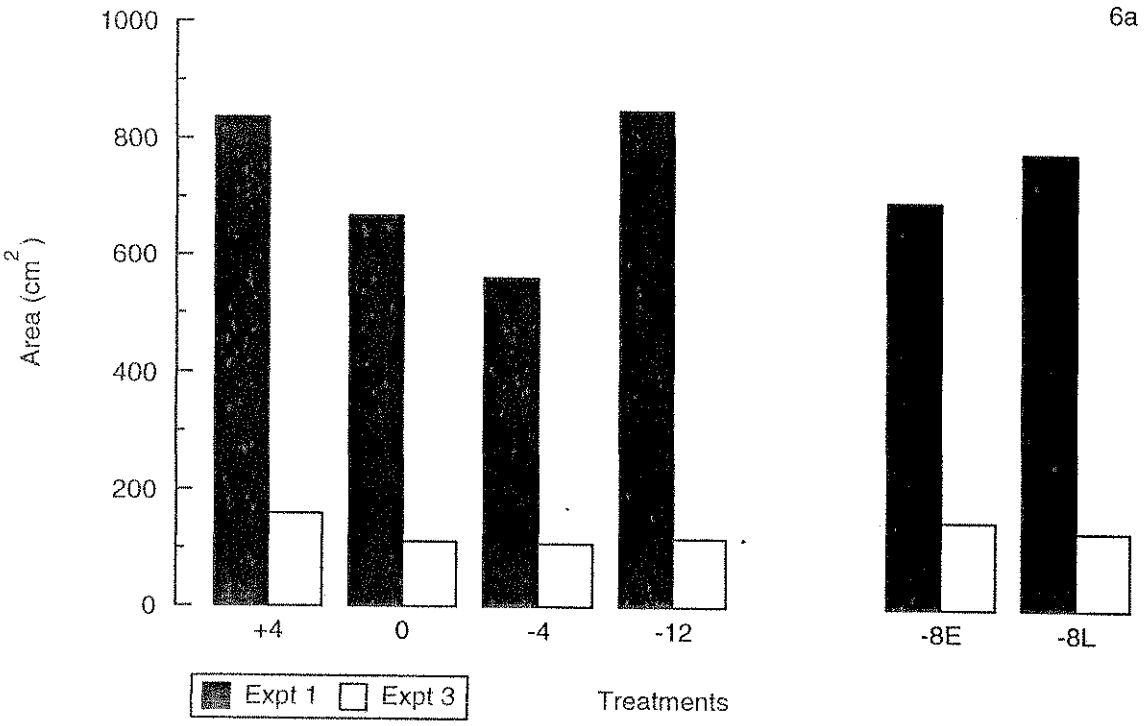


Fig. 5. The dry weight (g) of the leaves of axillary shoots of poinsettia; a) grown in treatments with a 24-hour mean temperature of 18°C (Experiments 1 and 3), b) grown in treatments with a night temperature of 18°C (Experiments 2 and 4).



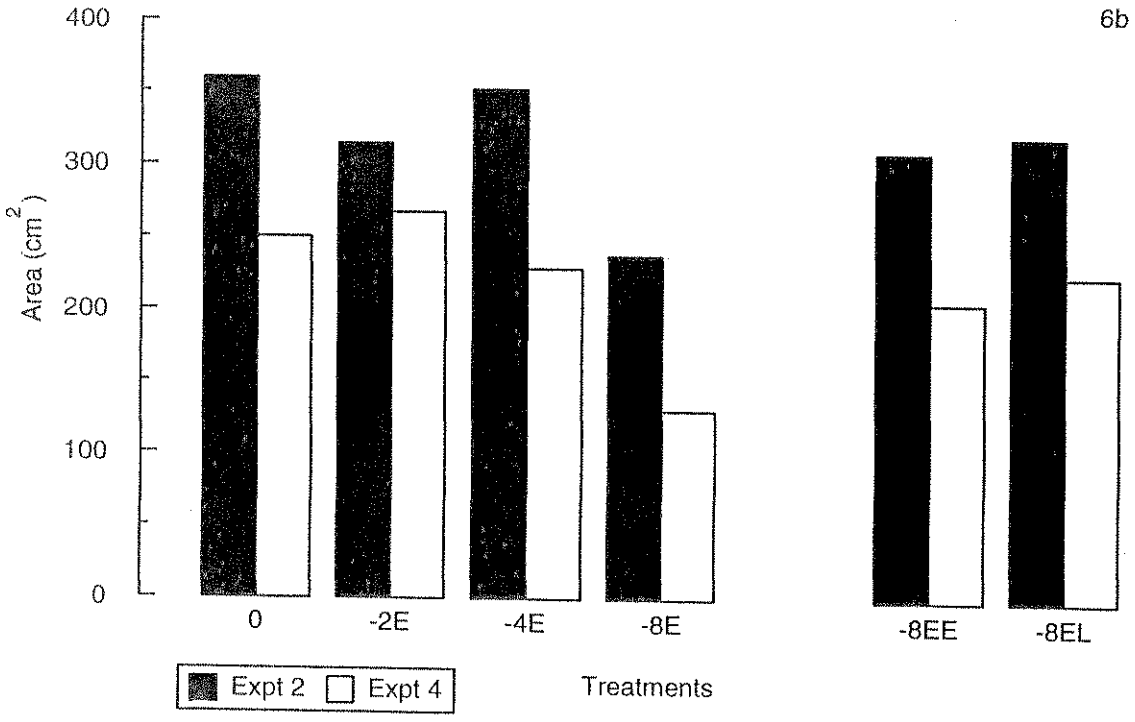
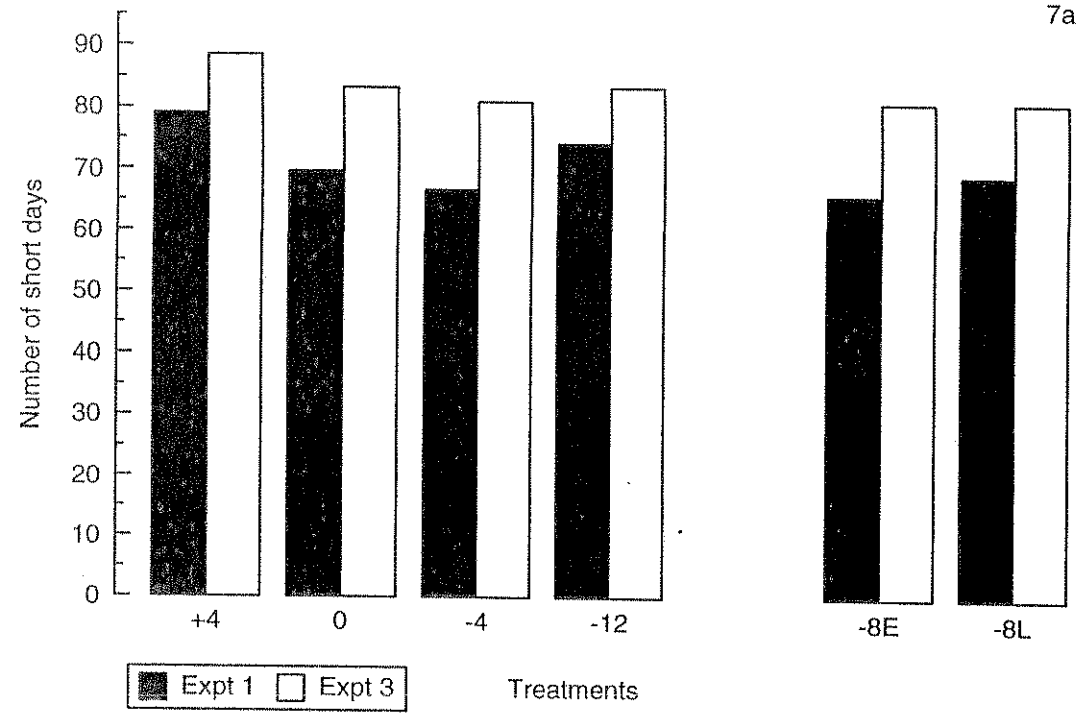


Fig. 6. The area (cm^2) of the leaves of axillary shoots of poinsettia; a) grown in treatments with a 24-hour mean temperature of 18°C (Experiments 1 and 3), b) grown in treatments with a night temperature of 18°C (Experiments 2 and 4).



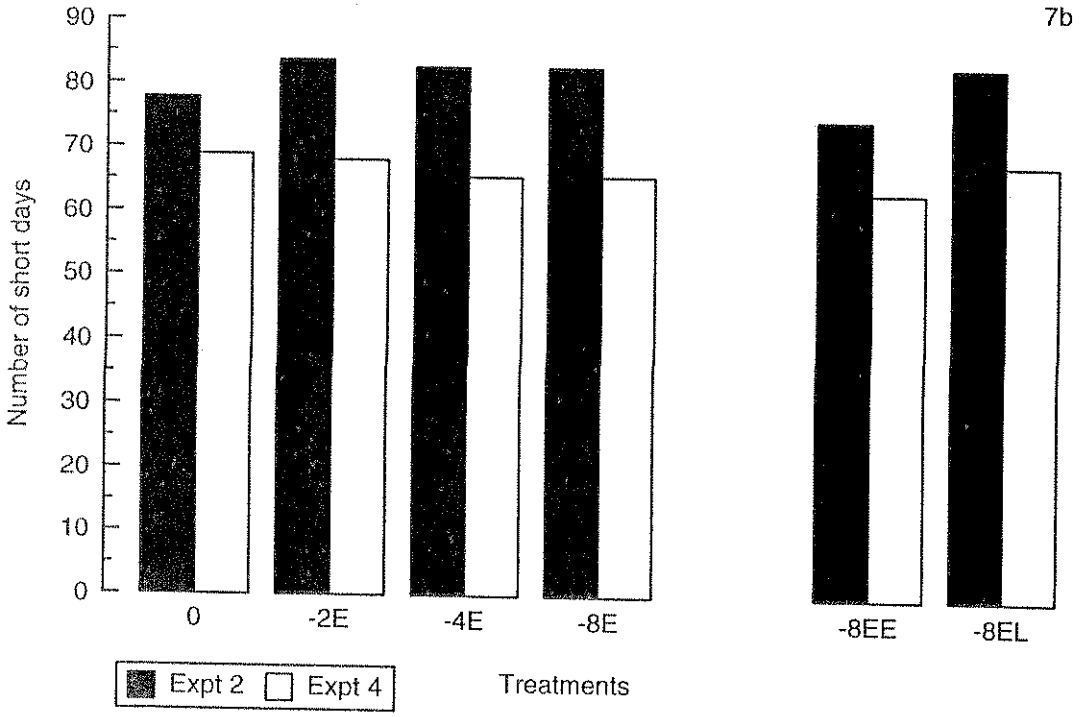


Fig. 7. The number of short days to anthesis of the primary cyathium of poinsettia; a) treatments having a 24-hour mean temperature of 18°C (Experiments 1 and 3), b) treatments having a night temperature of 18°C (Experiments 2 and 4).

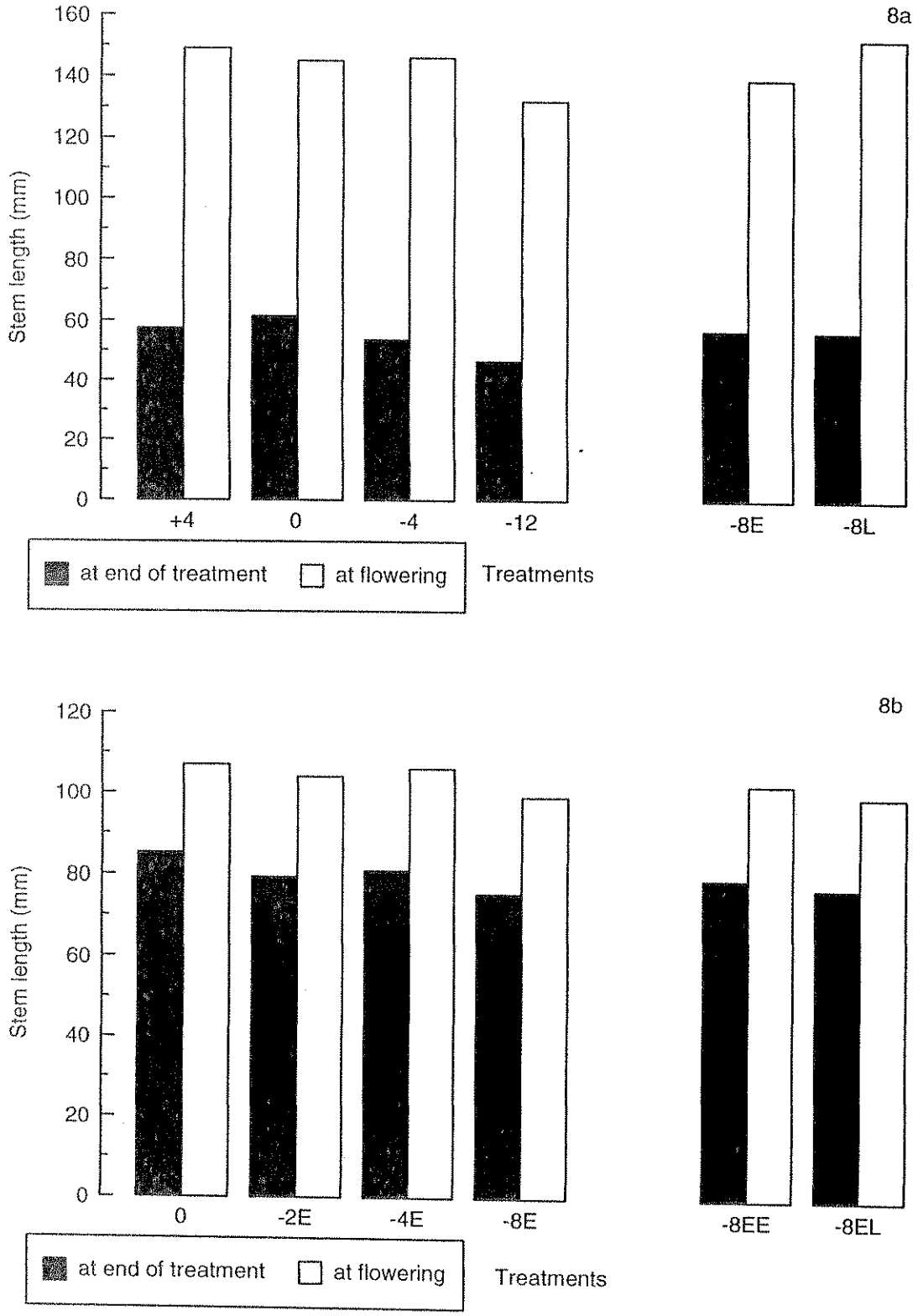


Fig. 8. Lengths (mm) of main stems of geranium plants; a) grown in treatments with a 24-hour mean temperature of 18°C (Experiment 1), b) grown in treatments with a night temperature of 18°C (Experiment 2).

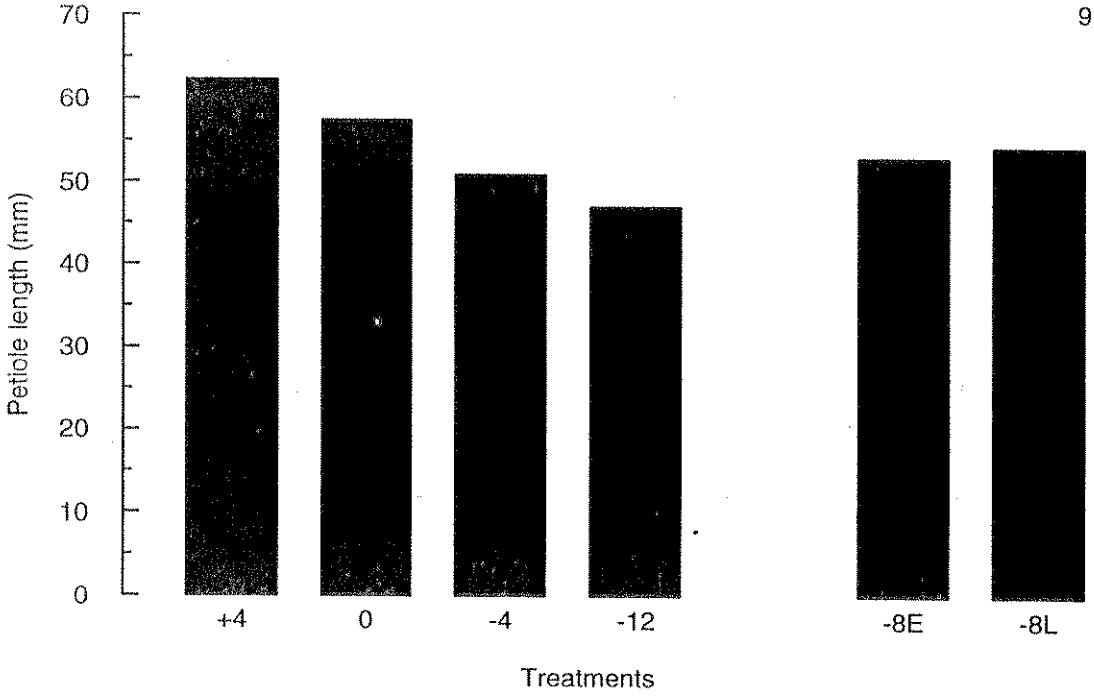


Fig. 9. Average petiole length (mm) of selected leaves of geranium plants after 5 weeks of growth in treatments with a 24-hour mean temperature of 18°C (Experiment 1).

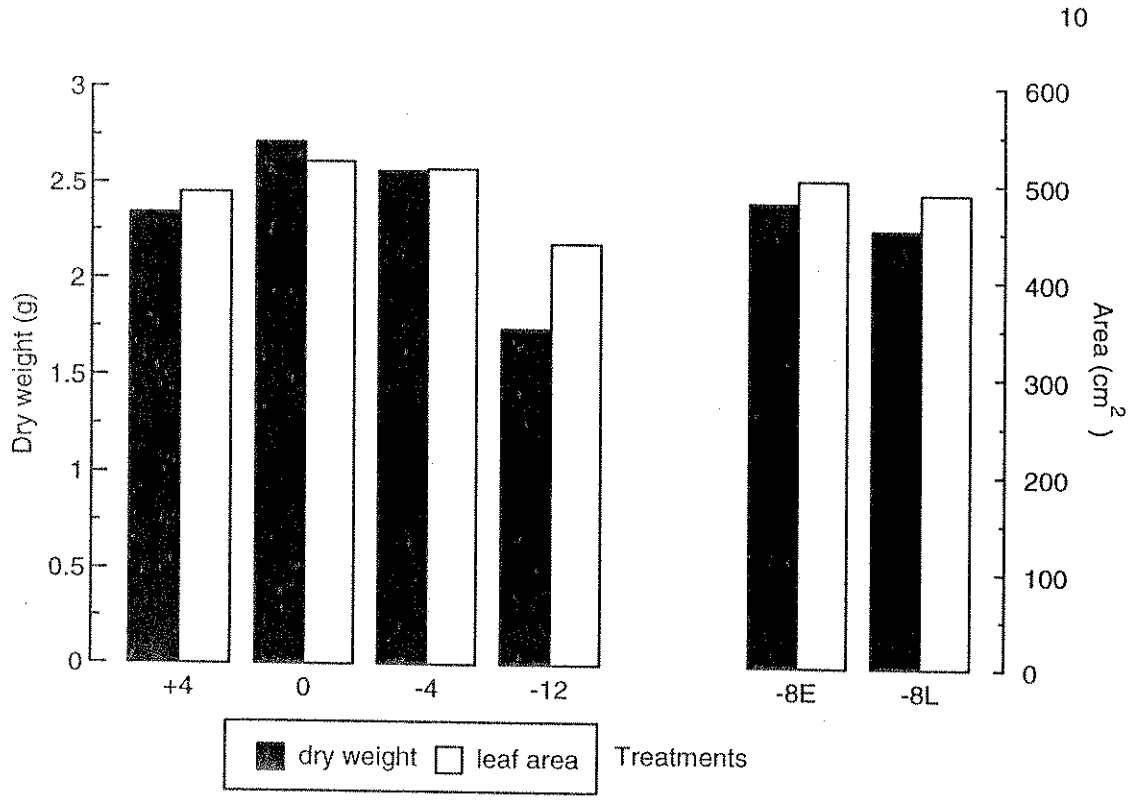


Fig. 10. The effect of treatments with a 24-hour mean temperature of 18°C on the leaf dry weight (g) and area (cm^2) of geranium (Experiment 1).

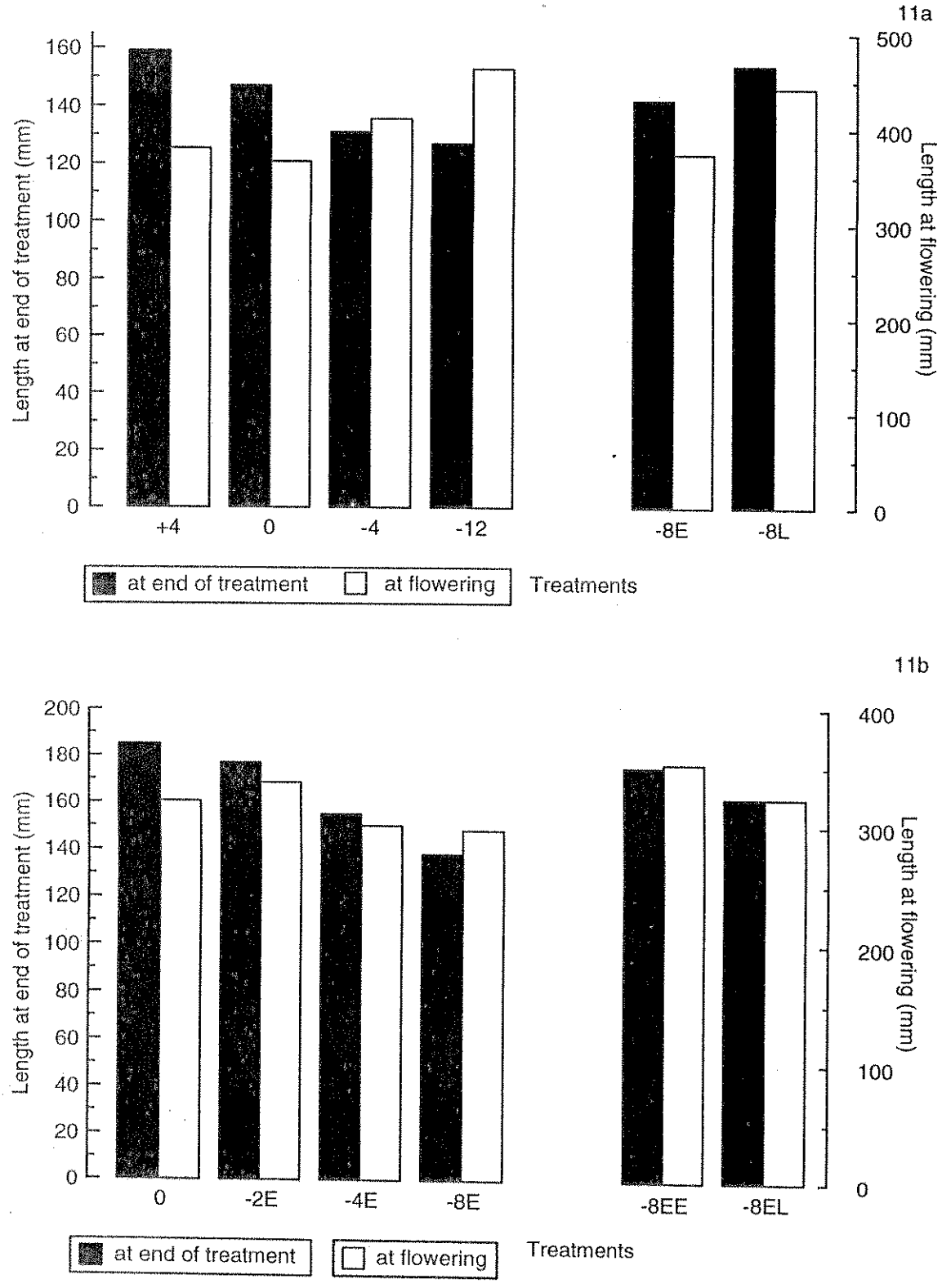
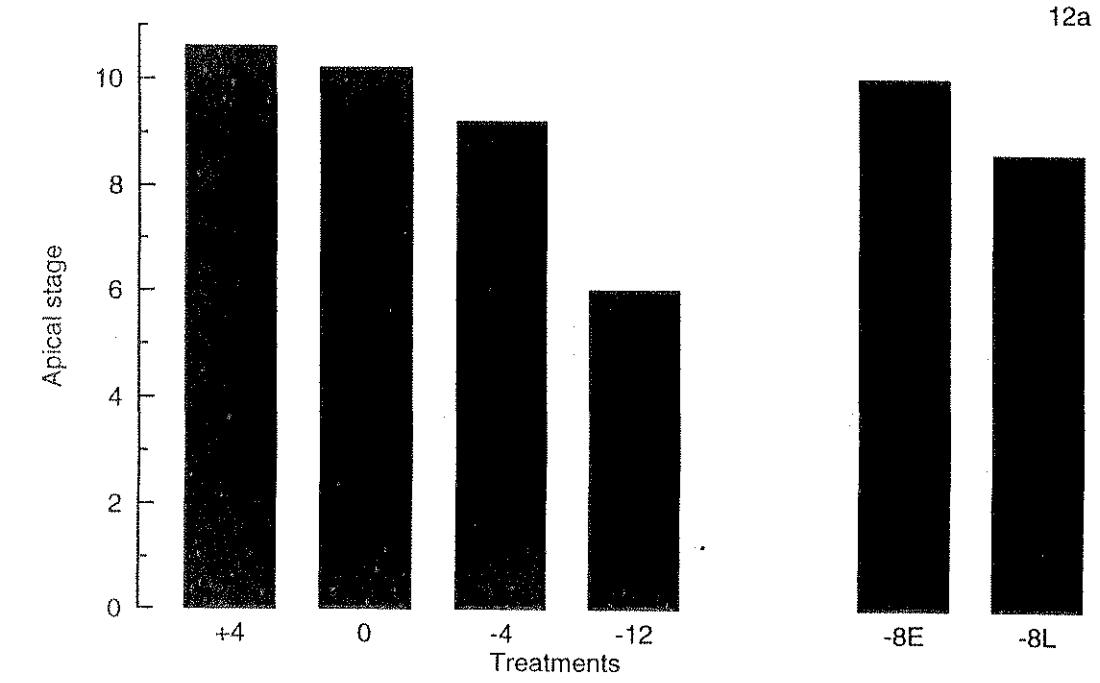


Fig. 11. Axillary shoot length (mm) of chrysanthemum plants; a) grown in treatments with a 24-hour mean temperature of 18°C (Experiment 3), b) grown in treatments with a night temperature of 18°C (Experiment 4).



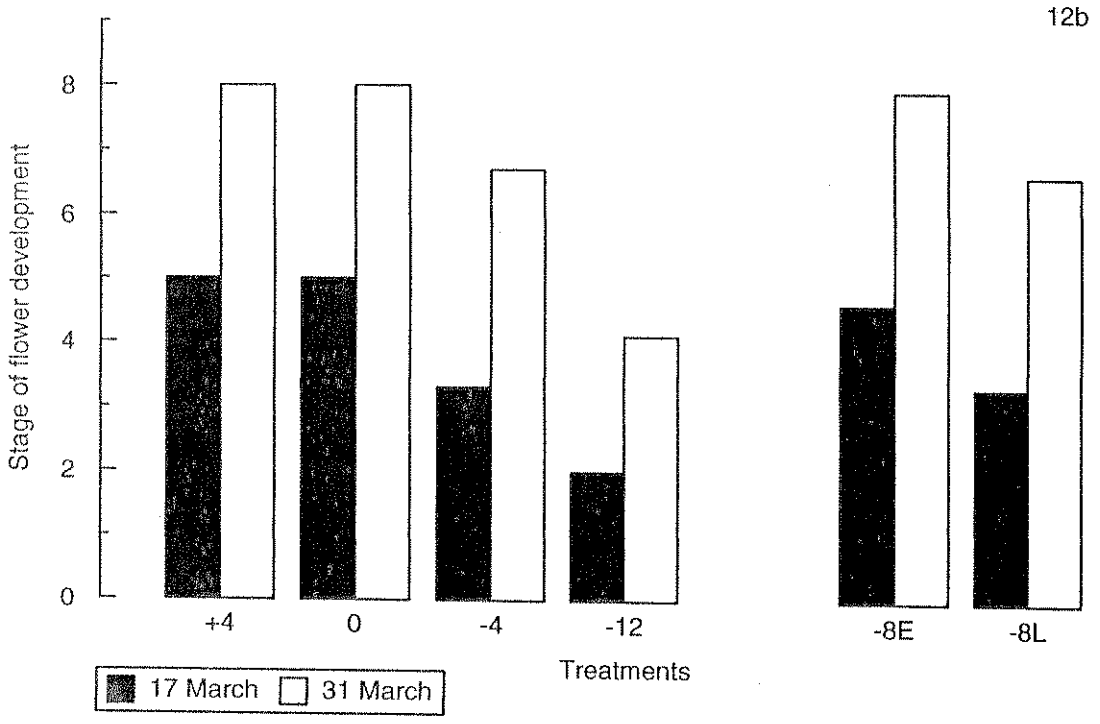


Fig. 12. Flower development of chrysanthemum plants grown in treatments with a 24-hour mean temperature of 18°C (Experiment 3); a) apical stage during treatment, b) flower stage after treatment.

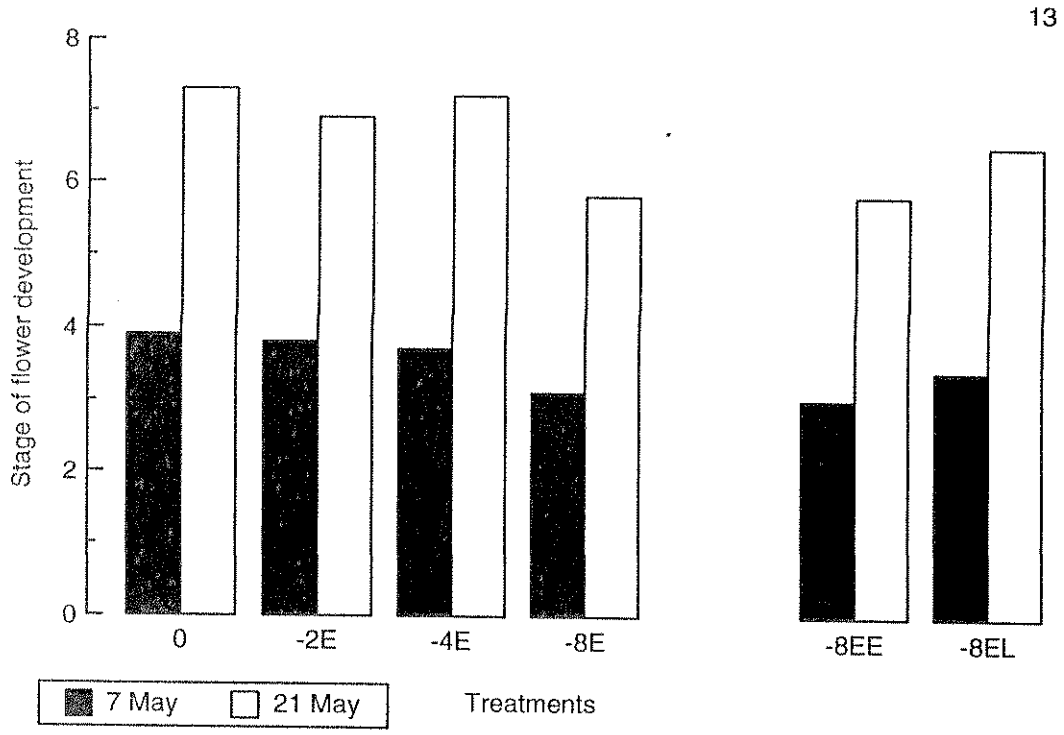


Fig. 13. Flower development of chrysanthemum plants grown in treatments with a night temperature of 18°C but recorded after transfer to a common environment (Experiment 4).

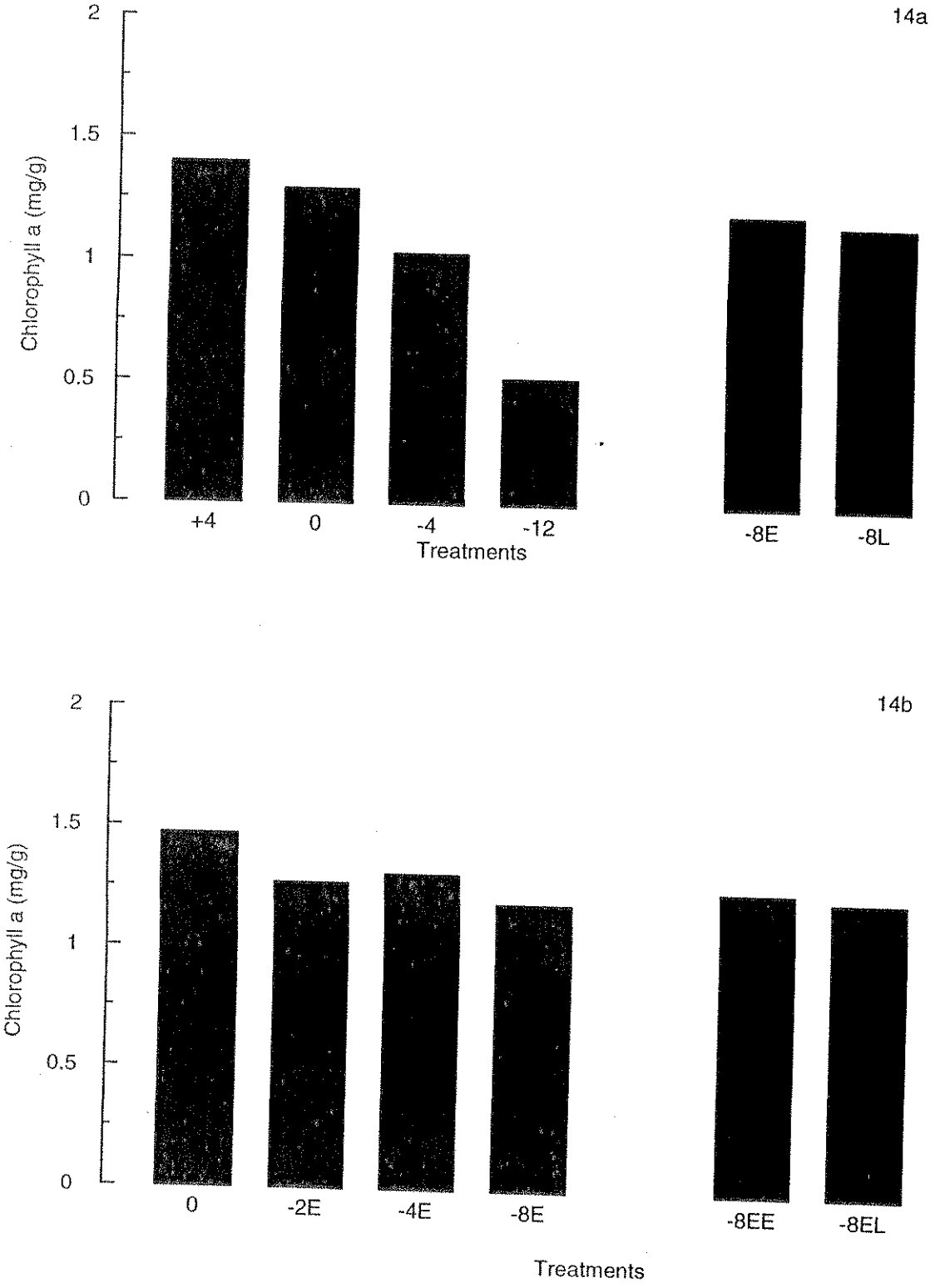


Fig. 14. The chlorophyll a concentration (mg/g fresh weight) in chrysanthemum leaf tissue; a) grown in treatments with a 24-hour mean temperature of 18°C (Experiment 3), b) grown in treatments with a night temperature of 18°C (Experiment 4).

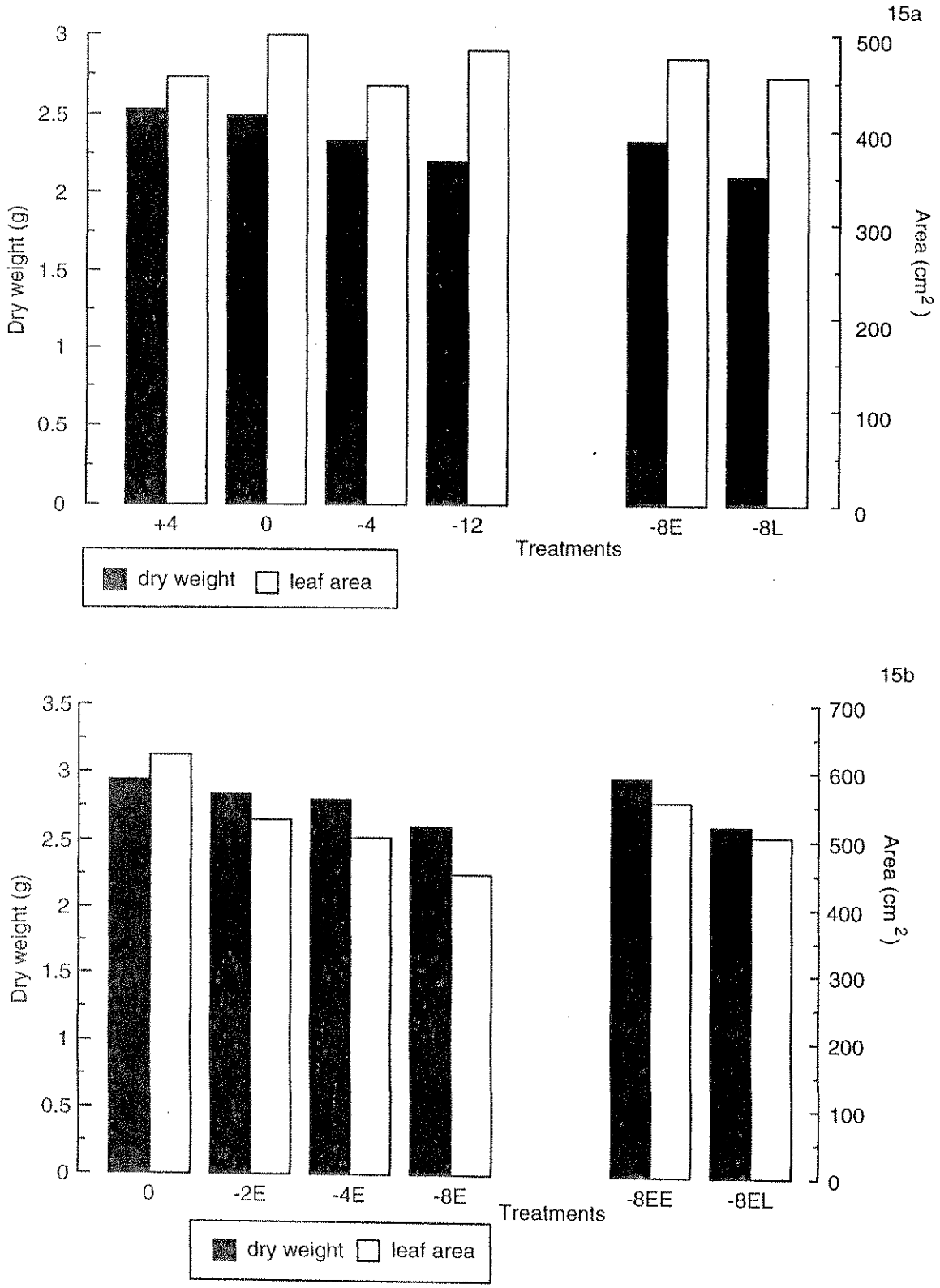


Fig. 15. Dry weight (g) and area (cm^2) of the leaves of the axillary shoots of chrysanthemum; a) grown in treatments with a 24-hour mean temperature of 18°C (Experiment 3), b) grown in treatments with a night temperature of 18°C (Experiment 4).

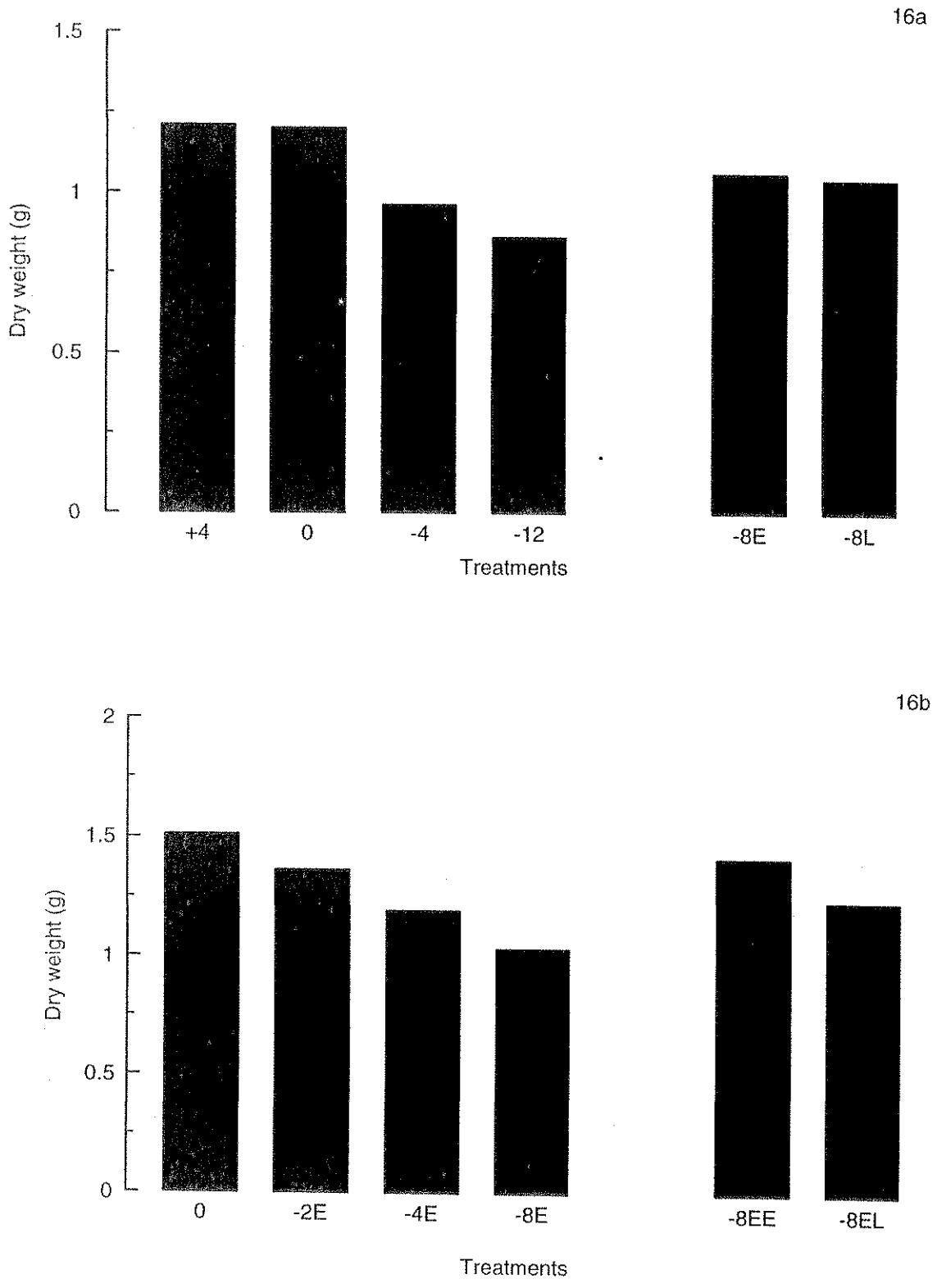
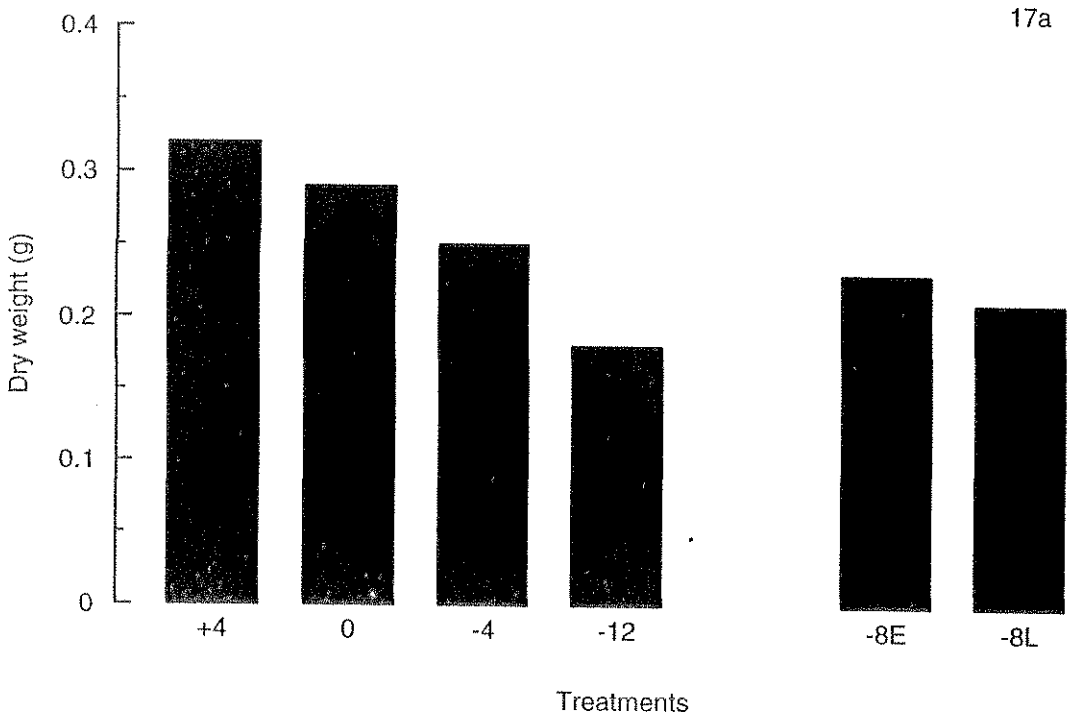


Fig. 16. Dry weight (g) of the stems of the axillary shoots of chrysanthemum plants; a) grown in treatments with a 24-hour mean temperature of 18°C (Experiment 3), b) grown in treatments with a night temperature of 18°C (Experiment 4).



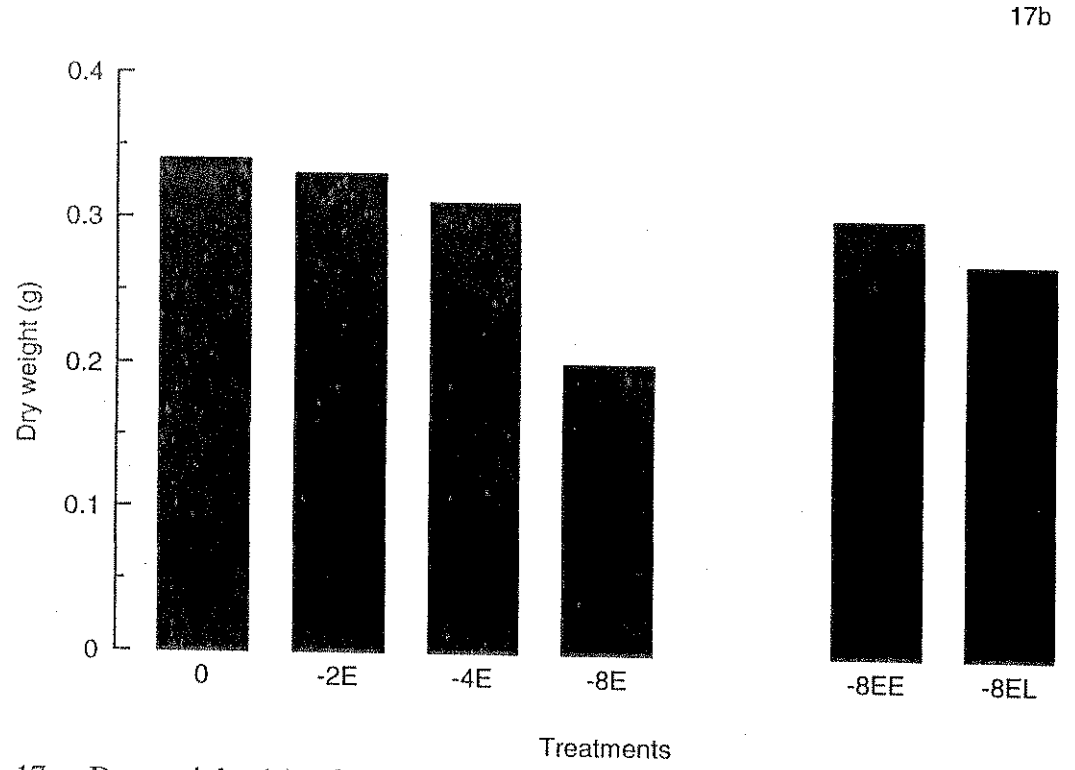


Fig. 17. Dry weight (g) of secondary axillary shoots of chrysanthemum; a) grown in treatments with a 24-hour mean temperature of 18°C (Experiment 3), b) grown in treatments with a night temperature of 18°C (Experiment 4).

APPENDIX 3

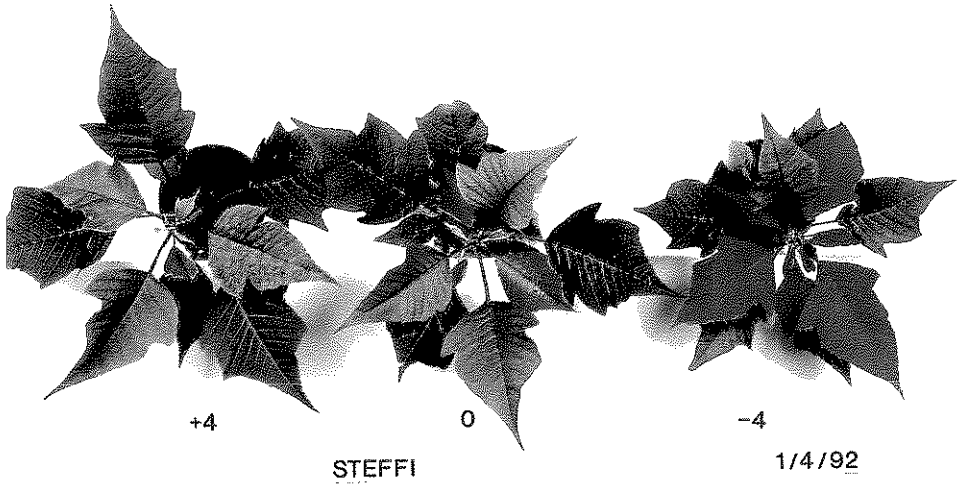


Plate 1. Poinsettia: progressive reduction in plant stature and advance in flowering from the application of +4 DIF through 0 DIF to -4 DIF (Experiment 3).

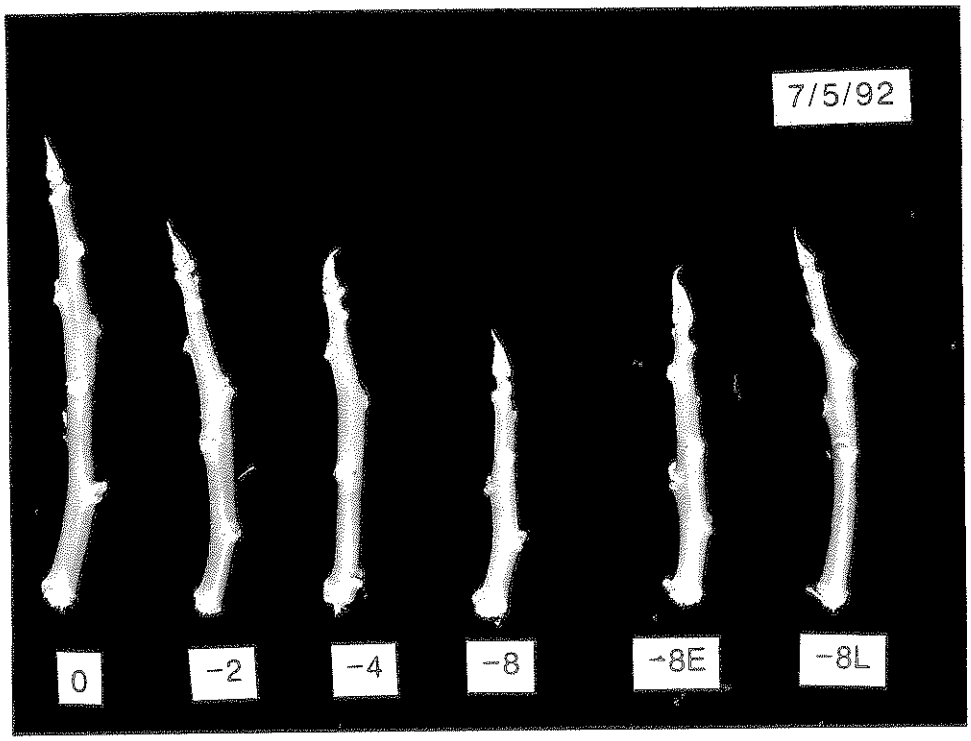


Plate 2. Poinsettia: effects of DROP treatments on axillary shoot elongation (shoots defoliated to aid visibility (Experiment 4). For 8E read 8EE and for 8L read 8EL.

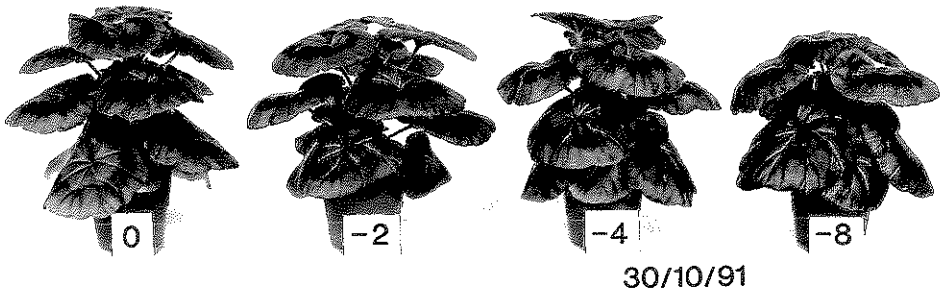


Plate 3. Geranium: effects of DROP treatments on petiole elongation (Experiment 2).

Plate 4. Chrysanthemum: progressive reduction in axillary shoot length and in leaf chlorophyll content from the application of +4 DIF, through 0 DIF and -4 DIF, to -12 DIF (Experiment 3).

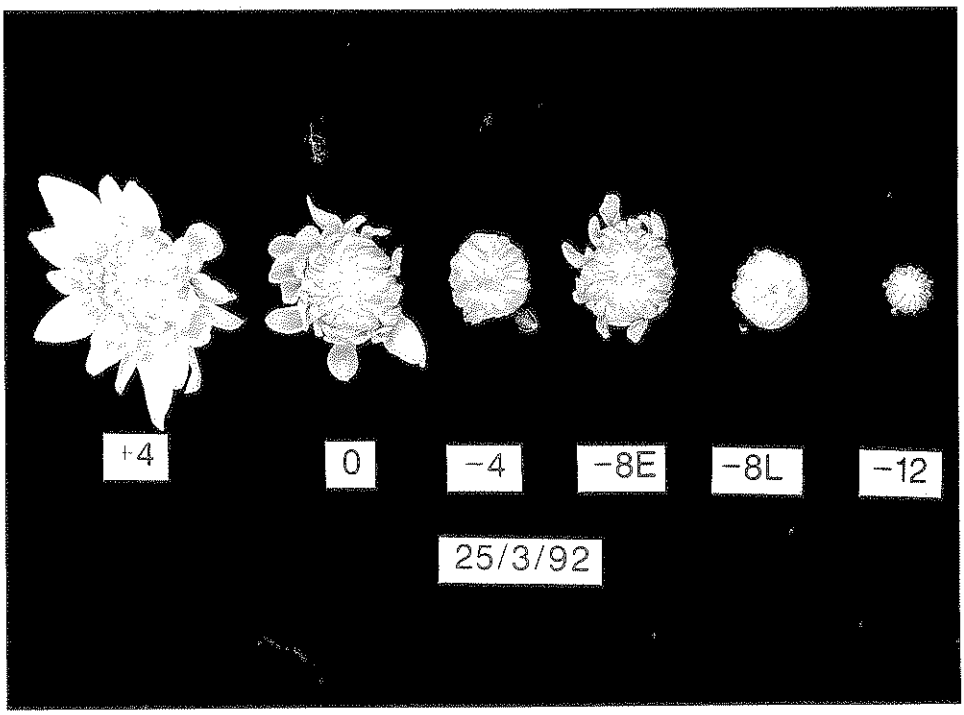


Plate 5. Chrysanthemum: effects of DIF and DROP treatments on flower development (Experiment 3).

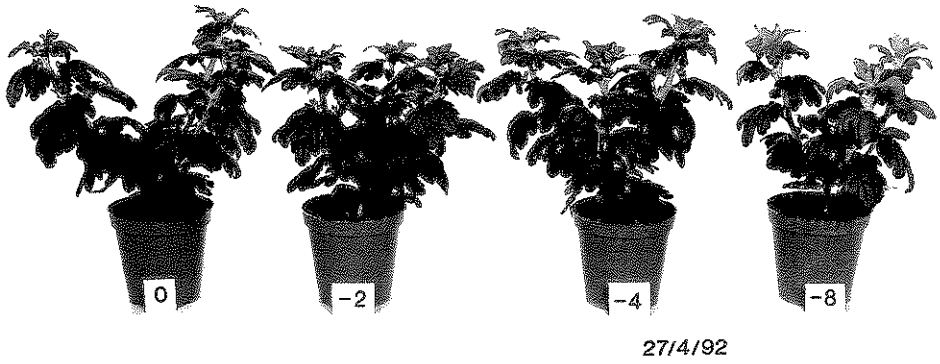


Plate 6. Chrysanthemum: effects of DROP treatments on axillary shoot elongation (Experiment 4).