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FLOWER FORMATION IN CAMPANULA MEDIUM

by

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CONTENTS

1. Introduction

Investigations on the physiology of flower formation in *Campanula medium* have been carried out by the present writer since 1947. The results have been published very fragmentarily only and mainly in the dutch language. The scope of the present publication is to present the total results. Although not all problems studied have been solved in details, the research has been stopped for several reasons. *C. medium* is genetically not homogeneous with regard to its reactions towards flower inducing factors and although by inbreeding a definite improvement has been obtained, a simultaneous deterioration took place. In the course of the investigations the growth of the plants, obtained by inbreeding, became often rather poor and resulted in premature death without diseases as a causal factor. Also, experiments with *C. medium* take relatively much space and are time consuming. Nevertheless, several quite convincing results were obtained which characterize *C. medium* as a very special case that has not been described in literature yet. Only *Scabiosa succisa (Succisa pratensis)* as recently studied by Chouard (1) has similar reactions towards low temperature and day length, however not studied as extensively as *C. medium*. Therefore, a summarizing publication seems to be justified.

2. Materials and methods

Originally seed was obtained from a commercial seed firm. Later on seeds were used which were obtained by repeated selfing, as indicated already above.

In order to understand the methods used, it is necessary to start from the results of some preliminary experiments. These demonstrated that plants grown in long day at ordinary greenhouse temperature always stay in a vegetative rosette stage and never flower. On the other hand, the same conditions are very suited for a realization of flower formation accompanying stem elongation, after the action of flower inducing factors which may be either low temperature or short day. Therefore, the general principle has been to grow plants from seed in long day at normal temperature, to apply different cold or short day treatments with plants of different ages, and to grow the plants after these treatments again in long day at normal temperature. In certain experiments other photoperiods than long day only were used as after-treatment. This will be indicated.

Notes were taken on the percentages of plants with elongating stem which, unless otherwise stated, was always followed by flower formation and flowering. Also, notes were taken on the numbers of days during the after-treatment for the first signs of stem elongation to occur and sometimes also on the numbers of days for the first flower to open. Rather extensive counts of leaf numbers in several stages of development in several experiments were made. However, these numbers turned out to be so variable that they were useless in interpreting the results and therefore they will be left out of consideration.

"High temperature" or "warm" means ordinary greenhouse temperature, averaging 20 °C, however with sometimes considerable variations due to suddenly changing weather conditions, but never reaching vernalizing effects. "Low temperature" or "cold" means a constant temperature of 5 °C in a cold room.

"Short day" (SD) is 8 hours of natural light – in the winter season sometimes intensified with strong artificial light – and 16 hours of darkness. "Long day"

(LD) is 8 hours of darkness and 16 hours of light, obtained by extending the natural day length, if necessary, by rather weak light of incandescent lamps; sometimes, according to season, high intensity light was used to intensify the natural light.

During the cold treatments the plants received artificial light only, supplied by fluorescent lamps with an intensity averaging 250 $\mu W/sec/cm^2$, which proved to be sufficient for the plants, not only to keep them alive, but also to keep them growing, be it of course slowly. Illumination during 8 hours or during 16 hours, hence both SD or LD, were used during the cold treatments of the first experiments. Since it turned out that no clear difference in effect between SD and LD at low temperature existed, later on only SD was used, sometimes as 12 hours light per day.

3. Experimental results

3.1. *General orientation*

We shall start in summarizing part of the results of two large scale experiments, *A* and *B*, which immediately confront us with the main factors influencing flower formation, viz. age, cold, day length. In following sections details about each of these factors will be discussed.

A. In the first experiment plants of 5, 13, 21, 29 or 37 w. (= weeks) old were treated during 4, 7 or 10 w. with cold LD, cold SD or warm SD. This makes a total of 45 treatments, each with finally 12 plants. The sowing of the experimental plants and the beginning of the treatments were so timed that all treatments ended simultaneously, so that during the after-treatment in warm LD all plants had identical circumstances. The results, expressed as percentages of flowering plants per treatment, are summarized in fig. 1.

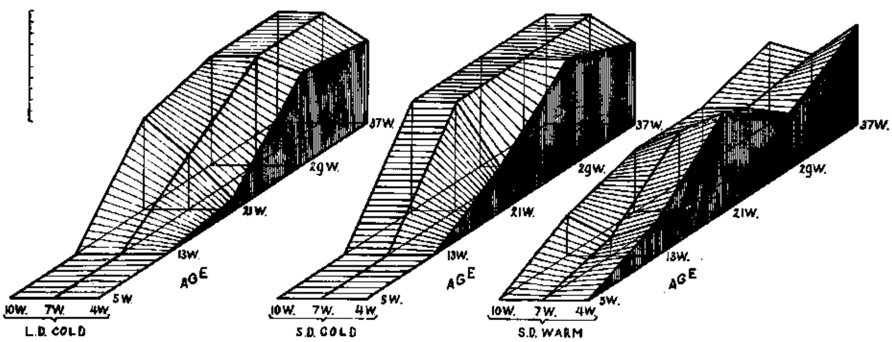

FIG. 1. The influence of age, treatments: cold LD, cold SD or warm SD, and duration of treatment: 4, 7 or 10 weeks, upon the flowering of *C. medium*, expressed as percentages of flowering plants.

In the left part of fig. 1 (cold LD) we see that plants of 5 or 13 w. old do not yet react to cold LD of the durations used. They are in a juvenile phase. Plants of 21 w. old or older react, but the younger the plants are, the more cold they need.

The middle part of fig. 1 (cold SD) gives very much the same picture, although in some cases there seems to be a stronger reaction, e.g. in the 21 w. age-group and in the 29 w. age-group with a treatment during 4 weeks. This might suggest that cold SD has a stronger effect than cold LD, but the differences are statistically uncertain.

The picture in the right part of fig. 1 (warm SD) is rather different from the other two. First of all we see the existence of a juvenile phase (no reaction of 5 w. old plants), but this lasts shorter than with regard to both cold treatments: the reaction already starts with 13 w. old plants. For the rest the shape of the curved figure is decidedly less regular than in the other two cases. The impression is made that the reaction to warm SD is not as regular as to cold and this was confirmed in later experiments.

A very interesting conclusion is reached, when we compare the reactions of the 13 w. old plants towards the different treatments. There is a reaction – be it a rather weak one – to warm SD, but not to cold SD. This points to an absence of a photoperiodic SD-reaction at 5°C which must be below the minimum temperature for such a reaction. Furthermore, plants of 13 w. old do no yet react to cold, because they are in the juvenile phase for cold. Therefore, the combination of cold and SD is inactive with 13 w. old plants, because they are in the juvenile phase for cold, while no photoperiodic reaction takes place at 5°C. Similar conclusions were obtained in later experiments.

B. The second experiment was a modification of the first one with more variation in the ages, shorter intervals between the durations of the treatments and omitting cold LD. Plants of 10, 12, 14, ... 28 w. old were treated during 4, 6 or 8 w. with cold SD or warm SD. Hence there were 60 treatments, each with 10 plants. Again, the after-treatment with warm LD started on the same day for all treatments. For the results see fig. 2.

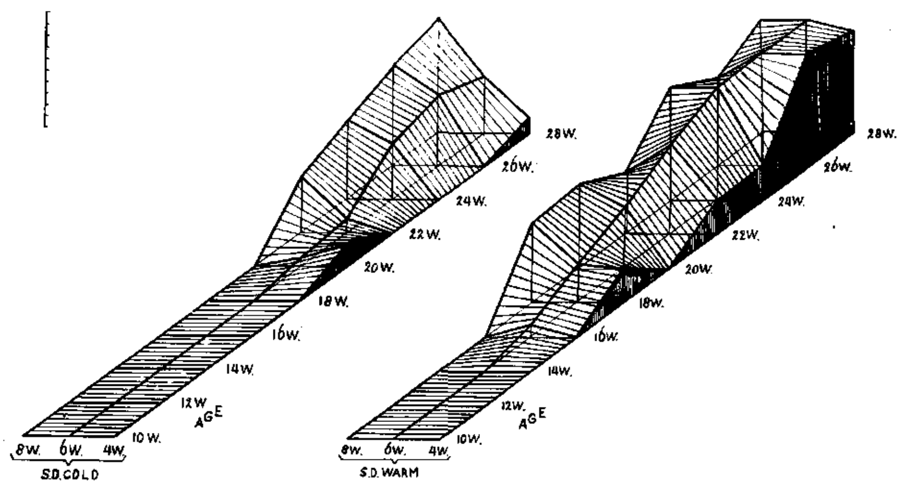

FIG. 2. The influence of age, treatments: cold SD or warm SD, and duration of treatment: 4, 6 or 8 weeks, upon the flowering of *C. medium*, expressed as percentages of flowering plants.

As a whole the results of this second experiment are very similar to those of the first one: *a.* the juvenile phase towards cold SD (left part of fig. 2) lasts longer than towards warm SD (right part of fig. 2); *b.* the right figure is less regular than the left one; *c.* plants of 16 w. or 18 w. old are in the transitory stage of reacting to warm SD, but not yet to cold SD. A discrepancy between fig. 1 and fig. 2 seems to be that in the first case plants of 13 w. old are no longer in the juvenile phase for warm SD, while in the latter case plants of 14 w. old are still in this phase, while with 16 w. old plants the reaction is very weak. It is self-evident, however, that the length of the juvenile phase is not fixed, but is subject to modification.

Several details of the above experiments will be discussed in the following, together with the results of other experiments.

3.2. *Vernalization*

Attempts to vernalize germinated seeds have not met with success. This is in accordance with the existence of a juvenile phase for cold as already demonstrated in 3.1. Hence vernalization in *Campanula medium* is always plant vernalization. We shall discuss some details of this plant vernalization.

3.2.1. The relation between age of plants and effect of duration of vernalization. – Tables 1 and 2 present data on this relation from two representative experiments. In all other experiments the conclusions were similar.

TABLE 1. Age of plants and duration of cold treatment. Column 1: age of plants in weeks at beginning of cold treatment. Column 2: duration of treatment in weeks. Columns 3 and 4: %% of bolting plants after cold LD and cold SD respectively. Columns 5 and 6: average numbers of days from end of treatment to beginning of visible bolting in warm LD after cold LD and cold SD respectively.

1	2	3	4	5	6
Age	Duration	% bolting		Bolting in days	
		cold LD	cold SD	cold LD	cold SD
5–13 w.	4–10 w.	0	–	0	–
21 w.	4 w.	9	38	61	47
	7 w.	50	100	26	27
	10 w.	83	100	24	18
29 w.	4 w.	83	100	36	40
	7 w.	100	100	26	26
	10 w.	100	100	20	21
37 w.	4 w.	75	75	40	34
	7 w.	100	100	24	21
	10 w.	100	100	20	18

In the first row of table 1 we see that plants of 5 or 13 w. old were in the juvenile phase. Furthermore, column 3 demonstrates that within one age-group the percentage of bolting plants was higher the longer the treatment lasted. The period for bolting, however, runs just opposite: column 5; this is illustrated in photo 1 with plants of another experiment. The figures in column 4 and 6 have exactly the same trend. By comparing columns 3 and 4 it seems as if cold SD gives higher %% of bolting, especially in the 21 w. age-group. The data in columns 5 and 6 do not always confirm this impression. The differences were statistically uncertain and in several later experiments have not been found. Therefore, there is no reason to assume that day length has an effect at 5°C.

TABLE 2. Age of plants and duration of cold treatment. Column 1: age of plants in weeks at beginning of cold treatment. Column 2: duration of treatment in weeks. Column 3: %% bolting plants after cold (during 12 hours). Column 4: average numbers of days from end of treatment to beginning of visible bolting.

1	2	3	4
Age	Duration	% Bolting	Bolting in days
4–8 w.	8–20 w.	0	–
12 w.	8 w.	0	–
	10 w.	0	–
	12 w.	0	–
	16 w.	100	42
	20 w.	100	30
16 w.	8 w.	0	–
	10 w.	0	–
	12 w.	17	9
	16 w.	100	13
	20 w.	100	6
20 w.	8 w.	0	–
	10 w.	0	–
	12 w.	100	13
	16 w.	100	7
	20 w.	100	3

The data in table 2 tend to confirm those of table 1. The most interesting figures are those in column 3 for the durations of treatment of 12 w. (see column 2). We see the percentage of bolting rise from 0% via 17% (16 w. old plants) to 100% (20 w. old plants).

We conclude from tables 1 and 2 that within certain limits the older the plants are, the less cold they need which finds its expression in a higher % of bolting and a smaller number of days to bolting. This has certain implications with regard to the length of the juvenile phase. Apart from the modifiable nature of this length, it is connected with the amount of cold. It would follow from table 2 that plants of 12 w. old are juvenile, when we do not vernalize them longer than for 12 w. With 16 or 20 w. vernalization there is a complete reaction. Therefore the question presents itself whether an "absolute" juvenile phase exists, in other words: what happens to very young plants when they are exposed to a prolonged cold treatment? We shall answer this question in the next section.

3.2.2. The effect of a prolonged cold treatment of very young plants. – Already in table 2 the negative effect of vernalizations up to 20 weeks with plants of 4 or 8 weeks initial age has been dealt with. In another experiment 5-week old plants were vernalized both in LD and in SD during 4, 7, 10, 16, 22, 28 or 34 weeks. None of these plants has flowered in warm LD, given after the vernalization, although some remained alive and were observed during 18 months! These results point to the existence of an absolute juvenile phase. Also, this juvenile phase is not completed during a prolonged vernalization, in other words: at 5°C the juvenile phase is fixed.

The growth of the plants during the prolonged cold treatment was quite remarkable in so far as they did not form a rosette, but a slowly elongating stem. After the cold treatment, hence during the warm LD-after-treatment, a rosette was formed on the top of the elongated stem. Photo's 2 and 3 illustrate this phenomenon for cold LD-treatment. The plants in cold SD did not differ in habit.

3.2.3. The effect of a prolonged cold treatment of adult plants. – Adult plants behave similarly to juvenile plants during a very long cold treatment in so far as stem elongation occurs always. A principle difference is that adult plants also start to form their flowers *during the cold treatments.* In a first experiment rather complete flower buds could be observed microscopically, but no further development took place. In later experiments no complete flower buds were found, but always initial stages, of course after a considerable lapse of time.

We shall illustrate the problem in question by an experiment which started with plants of 12, 16, 20 or 24 weeks old. Of each age-group 91 plants were put at 5 °C. The results are summarized in table 3.

TABLE 3. The behaviour of plants of 4 different ages when put at 5 °C. Initial number of plants: 91 per age-group.

Observations \ Age	12 w.	16 w.	20 w.	24 w.
1. plants died	33	8	19	22
2. remaining number	48	73	62	59
3. bolted	48	73	62	59
4. after... days average	182	164	156	131
5. average stem-length of 20 plants in cm after 192 days .	10.5	14.9	20.0	30.2

In row 1 of table 3 it seems as if the youngest plants (12 w.) stood the cold less well than the others. A similar observation was made in other experiments. However, there is no general correlation between initial age and cold-resistance. Rows 2 and 3 show that all surviving plants have bolted, according to row 4 after less days as the initial age was older. Row 5 shows that the older plants have grown faster than the younger ones. Photos 4 and 5 picture the extreme cases. This difference in rapidity of bolting must be ascribed to a larger amount of assimilating leaf-surface and perhaps to a certain amount of reserve substances in the older plants. It is remarkable that a considerable difference in stem-length between initially young and older plants has remained in all experiments, the longest of which has been continued for over 400 days. Detailed measurements were beyond the scope of this research.

After 185 days of vernalization 20 plants from every age-group were transferred from the cold to warm LD. The results are summarized in table 4.

TABLE 4. Observations on plants from table 3 during warm LD following 185 days at 5 °C. Initial number of plants: 20 per age group.

Observations \ Age	12 w.	16 w.	20 w.	24 w.
1. plants died	15	5	5	9
2. remaining number	5	15	15	11
3. flowering	5	15	15	11
4. after... days average	57	56	54	54

Again, relatively many plants of the 12 w. age-group died prematurely. Furthermore, all remaining plants have flowered after a practically identical average number of days (for the first flower to open).

3.2.4. The relation between vernalization, and day length during the after-treatment. – Originally the conclusion was drawn that flower formation after vernalization only occurred in LD. For instance: Plants of 28 weeks old were vernalized during 4, 6 or 8 weeks and received LD or SD as an after-treatment. In LD 1.3, 50 or 100% have flowered (the average numbers of days to bolting being 59, 62 or 20 days respectively), in SD no flowering occurred. Later on it turned out that also in SD flowering can occur, provided the vernalization lasts relatively very long, while also the initial age of the plants is of importance. We shall illustrate these results by dealing with 3 different experimental series, indicated as *A*, *B* and *C*.

A. Plants of 26 weeks old were vernalized during slightly more than 9 months, during which period bolting had started. Thirty five plants were then put in warm LD, 35 in warm SD. Of the former group 3 plants died prematurely, of the latter 10. All the remaining plants, hence 32 in LD and 25 in SD, have flowered, however the LD-plants after 33 days average, the SD-plants after 64 days which is almost twice as much. Microscopical examination at the moment of transfer to LD and SD revealed that the plants had already started to form flower buds, but a differentiation into calyx and corolla had not taken place yet. In the next two experiments no flower bud formation had started during the vernalization.

B. Plants of 20 weeks old received cold treatments of different durations and after-treatments both in LD and in SD. The plants did not bolt yet even during the longest cold treatment of 16 weeks. The results are summarized in table 5.

TABLE 5. The effect of LD- or SD-after-treatment upon plants of originally 20 weeks old, exposed to cold during different periods. Ten plants per treatment.

1	2	3	4
Cold in weeks	After-treatment	% bolting	After ... days
6 w.	LD	14	32
	SD	0	–
8 w.	LD	67	34
	SD	0	–
10 w.	LD	100	23
	SD	0	–
12 w.	LD	100	16
	SD	100	23
14 w.	LD	100	5
	SD	100	11
16 w.	LD	100	6
	SD	100	9

First of all we see from column 3 of table 5 that bolting – followed by flowering – has occurred in SD, but only after a relatively long cold treatment. There is a jump from 0% to 100% bolting in SD between 10 and 12 weeks of vernalization, while the transitions in LD are more gradual. In column 4 we see that there is a tendency for quicker bolting the longer the cold treatment lasts, both in LD and in SD.

C. In this experiment not only the duration of the cold treatment was varied, but also the initial age of the plants at the beginning of the vernalization. As in former experiments the sowing was so timed that the after-treatments started simultaneously for all the plants. The results are given in table 6.

TABLE 6. The effect of LD- or SD-after-treatment upon plants of different initial ages, after cold treatments of different durations. Ten plants per treatment.

	1	2	3	4	5	6	7
Cold in weeks / Age in weeks	4–8 w.	12 w.		16 w.		20 w.	
	% bolting	% bolting	after ... d.	% bolting	after ... d.	% bolting	after ... d.
	in LD-after-treatment						
8–10 w.	0	0	–	0	–	0	–
12 w.	0	0	–	17	9	100	13
16 w.	0	100	42	100	13	100	7
20 w.	0	100	30	100	6	100	3
	in SD-after-treatment						
8–12 w.	0	0	–	0	–	0	–
16 w.	0	0	–	0	–	100	15
20 w.	0	0	–	0	–	100	13

The data in table 6 speak for themselves and tend to confirm several results which have been discussed in the foregoing. Three points should be mentioned. *a.* According to columns 2 and 4 plants of 16 or 20 weeks old flower completely in LD after 12 or 16 weeks cold, according to columns 3 and 5 much quicker after the longest cold treatment. Plants of the same ages and the same cold treatments do not flower in SD. This means that the age of the plants at the beginning of the cold treatment is a factor in the flower formation in SD. *b.* In LD-after-treatment plants with the initial ages of 8 or 10 weeks are in the juvenile stage, while those of 12 weeks are in a transitory stage. In SD-after-treatment, however, plants of 8, 10 *and* 12 weeks seem to be completely juvenile. Hence also the after-treatment may influence the classification of plants into juvenile or adult. This, of course, is not real and the conclusion is that classification into juvenile or adult should take place under optimal circumstances. It is clear that although in SD flower formation may take place, it is certainly not optimal. *c.* When we compare the results of tables 5 and 6 we see that in table 5 flower formation has started under circumstances in which in table 4 no such flower formation took place. This holds true for both LD and SD, e.g. in table 6 after 8 weeks of cold 67% bolting in LD, after 12 or 16 weeks of cold 100% bolting in SD, while in table 6 under the same circumstances with the same age-groups (20 weeks) no flower formation occurred. The explanation is that the after-treatment in the experiment from table 5 took place in the late spring, in the experiment from table 6 however in the late fall and winter, hence under much worse circumstances, especially with regard to light intensity.

3.2.5. The effect of intermittent temperatures. – Four groups of plants, 9 months old at the beginning of the experiment, received the following daily treatments: *a.* 0 hrs 5° – 24 hrs 13°; *b.* 8 hrs 5° – 16 hrs 13°; *c.* 16 hrs 5° – 8 hrs 13°; *d.* 24 hrs 5° – 0 hrs 13°. The treatments lasted 6 weeks. The experiment was done with few plants and the investigations along this line have not been continued. Nevertheless, since the results were quite clear, they will be briefly mentioned. As illustrated in photo 6, no bolting has occurred after 24 hrs 13°. All other treatments have finally resulted in complete bolting, but faster as more cold had been given.

3.3. *Short-long-day induction*

In the general orientation of 3.1 it has been demonstrated that *Campanula medium* forms flower buds after a SD-treatment, provided this SD is followed by LD. The whole process takes place without the vernalizing action of low temperature. We shall now discuss details of this short-long-day (SLD)-induction.

3.3.1. The relation between age of plants and the effect of duration of SD-treatment. – We have seen before (p. 4) that "the reaction to warm SD is not as regular as to cold". This followed both from fig. 1 and from fig. 2. We shall mention some further data in table 7 which have been derived from the experiment pictured in fig. 1 and which are directly comparable to the data in table 1 for the effect of cold.

TABLE 7. Age of plants and duration of SD-treatment. Column 1: age of plants in weeks at beginning of SD-treatment. Column 2: duration of treatment in weeks. Column 3: %% of bolting plants. Column 4: average numbers of days from end of SD-treatment to visible bolting in warm LD.

1	2	3	4
Age	Duration	% bolting	After ... days
5 w.	4–10w.	0	—
13 w.	4 w.	29	75
	7 w.	17	49
	10 w.	38	64
21 w.	4 w.	92	42
	7 w.	75	44
	10 w.	58	42
29 w.	4 w.	50	42
	7 w.	55	54
	10 w.	55	41
37 w.	4 w.	90	55
	7 w.	64	56
	10 w.	75	48

The figures in table 7 are rather irregular indeed and as such they are quite representative for the SD-effect. It seems that plants of 13 w. old are in a transition between juvenile and adult. For the rest there is no straight relation between age of plants and effect of duration of SD-treatment on bolting and rapidity of bolting. This is very unlike the results in table 1 with regard to cold.

3.3.2. The juvenile phase for SLD-induction. – The existence of a juvenile phase for the photoperiodical induction has already been demonstrated in figures 1 and 2, as discussed in 3.1. Further observations are given in table 8 which speaks for itself.

TABLE 8. The juvenile phase for photoperiodical induction. The %% of bolting and the average numbers of days for bolting in LD following 4 weeks of SD with plants of different initial ages. Ten plants per treatment.

1	2	3
Age in weeks	% bolting	After ... days
12 w.	0	–
16 w.	15	70
20 w.	26	61
24 w.	50	63
45 w.	80	80

It follows from column 2 in table 8 that in the present experiment plants of 12 w. old did not react to 4 w. of SD, hence were in the juvenile phase. With increasing initial ages the %% of bolting increase. On the contrary the average numbers of days for bolting in column 3 do not regularly decrease. This is opposite to the effect of increasing durations of vernalization, as discussed in 3.2.1.

3.3.3. The effect of a prolonged SD-treatment of very young plants. – The question arises what happens to very young plants when they are exposed to a prolonged SD-treatment. The data in table 9 answer this question.

TABLE 9. The effect of SD-treatments of initially very young plants, 5 weeks old, during increasing periods, expressed as %% bolting in following LD and as average numbers of days in LD to bolting. Ten plants per treatment.

1	2	3
Duration of SD in weeks	% bolting	After ... days
4 w.	0	–
7 w.	0	–
10 w.	0	–
16 w.	10	67
22 w.	33	47
28 w.	90	68
34 w.	80	86

We see in column 2 of table 9 that the %% of bolting increase to high values as the duration of the SD-treatment increases. A 100% bolting is not reached, but this is seldom the case with SLD-induction, as mentioned before. The average numbers of days to bolting in column 3 do certainly not decrease regularly, but rather tend to increase. This is of secondary importance for the clear conclusion that juvenile plants after a prolonged SD-treatment reach the adult stage, or in other words: the juvenile phase is completed and the adult phase is reached during a SD-treatment. This is different from what we found for a prolonged cold treatment (see sub 3.2.2), but it should be kept in mind that the SD-treatment took place in high temperature. It is evident that in the cold the juvenile phase for SD is not completed, since this follows from the results which have been discussed sub 3.2.2.

Indications have been obtained that in LD the juvenile phase for SLD-induction is completed faster than in SD. Starting with 5 w. old plants, the following combinations gave similar results:

8 w. LD + 4 w. SD → 29% bolting
18 w. SD + 4 w. SD → 33% bolting

and:

16 w. LD + 4 w. SD → 92% bolting
24 w. SD + 4 w. SD → 90% bolting

3.3.4. The SLD-induction as such. – Flower formation in SD has never been observed, no matter whether or not LD preceded the SD-treatment, no matter how long the SD-treatment lasted. In one experiment the SD-treatment started with 12 w. old plants and lasted 33 weeks. In another experiment the SD-treatment started with 5 w. old plants and lasted 34 w., or with other plants almost 2 years. In none of these cases flowering occurred during the SD-treatment. In all cases bolting, followed by flower formation, took place in LD, following the SD, for 100%, 80% or 66% respectively after 62, 86 or 31 days respectively. Photo 7 gives an illustration.

The question arises whether after the SD indefinite LD is necessary, or whether after a limited number of LD the plants are day neutral. In order to answer this question, plants of 28 w. old received 6 w. of SD, followed by 0, 1, 2, ..., 7 w. of LD, followed by SD. Table 10 summarizes the results.

TABLE 10. The effect of different periods of LD following 6 weeks of SD and followed by SD, expressed as %% bolting and average numbers of days for bolting in SD. Eight plants per treatment.

1		2	3
Treatment		% bolting	After . . . days
6 w. SD. – 0 w. LD –	SD	0	–
6 w. SD. – 1 w. LD –	SD	0	–
6 w. SD. – 2 w. LD –	SD	50	30
6 w. SD. – 3 w. LD –	SD	67	9
6 w. SD. – 4 w. LD –	SD	67	13
6 w. SD. – 5 w. LD –	SD	60	10
6 w. SD. – 6 w. LD –	SD	86	4
6 w. SD. – 7 w. LD –	SD	86	7

It follows from column 2 in table 10 that already 2 w. of LD suffice to induce 50% bolting. Three or more weeks of LD increase the % of bolting, but the effects of 3, 4 or 5 w. of LD are almost identical, while 6 or 7 w. increase the % of bolting further. However, with 6 or 7 w. of LD some plants already showed the first symptoms of bolting *during* the LD and therefore may not be used in the present discussion. If we omit these plants, it remains quite clear that only a limited period of LD is sufficient for induction. A percentage of 100% induction has not been reached, but this is exceptional after SD-treatment, compare tables 7, 8 and 9. The duration for bolting has a tendency to decrease as more weeks of LD have been inserted.

In harmony with the above results is the observation that never during SD-treatment microscopical signs of flower bud formation have been found. Therefore, *Campanula medium* must be classified as an SLD-plant for induction, while after this induction it is day neutral.

3.4. *The relation between vernalization and SLD-induction*

The experimental results which have been dealt with in the foregoing provide us with many items to discuss the relation between vernalization and SLD-induction. This will be done in the next part. However, one peculiarity, already briefly mentioned in the preceding part, will be illustrated further on account of its interesting nature. It is the observation, mentioned in 3.1, that the juvenile phase for SD lasts shorter than the juvenile phase for cold. Hence plants reach an age upon which they already react to warm SD, but not yet to cold. Since furthermore a temperature of 5 °C is below the minimum for photoperiodical reaction, these plants do not react to SD at 5 °C and therefore are not brought to flower induction by cold, because they are cold-juvenile, neither by SD at 5 °C., because this temperature is too low for a photoperiodical reaction.

The above results have been obtained in three experimental series. In the last one plants of 20 w. old received 8 w .of cold SD or 8 w. of warm SD. Out of 12 plants none bolted during an LD-after-treatment of the cold SD-plants, 9 or 75% of the warm SD-plants after 52 days average. Photo's 8 and 9 illustrate these results.

4. Discussion

In the following an attempt will be made to build up a complete picture of the effects of the factors influencing flower initiation, in so far as the experimental results allow us to do so. Vernalization, SLD-induction, and their mutual relationships will be discussed separately, and some general remarks will follow.

4.1. *Vernalization*

Age of plants, duration of the cold treatment, and day length during the after-treatment are factors of primary importance. Since they closely interact, it is impossible to discuss them separately, but the duration of the cold treatment is the central factor and therefore the other two factors will be treated with the duration of the cold treatment as a starting-point.

Seed-vernalization is ineffective, neither can veiy young plants be vernalized. This points to the existence of a juvenile phase. The length of this juvenile phase is not a fixed characteristic, but is modifiable. Under ordinary greenhouse circumstances the juvenile phase lasts about 4–5 months, very roughly speaking. At 5 °C, however, the juvenile phase is fixed, at least under the experimental duration of 34 weeks. The question arises whether under a much longer duration of cold treatment, say several years, the juvenile phase is completed. This cannot be answered, but it does not seem improbable. It seems plausible that the transition from juvenile to adult is a growth process and that the adult phase is reached when a certain amount of energetic and building substances is available.

With adult plants and within certain limits the cold requirement of the plants decreases as they grow older. This finds its expression both in the percentage of bolting plants and in the duration for the bolting to start after the cold treatment. The higher the percentage of bolting plants, the lower is the average number of days for bolting during the after-treatment. The intensity of bolting seems to depend on the amount of substrate.

During a prolonged cold treatment of juvenile plants stem-elongation takes place, but the plants remain vegetative and upon transference to high temperature they form a rosette on the top of the elongated stem. Hence stem-elongation without flowering is brought about by prolonged cold. The same has been found in *Lunaria biennis* (11) and in *Cheiranthus allionii* (unpublished).

During a prolonged cold treatment of adult plants stem-elongation takes place also, but in this case the plants turn slowly into the generative phase. Complete flower formation respectively flowering, like in *Lunaria biennis* and *Cheiranthus allionii*, do not take place in the cold, however. The stem-elongation of adult plants during a prolonged cold treatment is quicker as the plants are older at the start of the vernalization. Again, this may be influenced by the amount of available substrate. It has recently been published (12) that gibberellic acid brings about stem-elongation without flowering in *Campanula medium* under high temperature. Therefore, in this case gibberellic acid replaces the influence of cold on stem-elongation, but not its vernalizing action and in *C. medium* stem-elongation and flower initiation are separate processes.

Usually LD is necessary after vernalization. However, with relatively much cold – not yet enough to induce stem-elongation – and with relatively old plants flower formation and complete flowering take place in SD also, be it much later than in LD. A similar situation was found in *Cheiranthus allionii* (unpublished).

4.2. *The SLD-induction*

As with vernalization, age of plants, duration of SD-treatment and daylength during the after-treatment are important factors, but the effect of the duration of SD-treatment is much less clear than with cold.

There is a juvenile phase for SD which lasts shorter than for cold, very roughly speaking about one month shorter. With regard to the modifiable nature of the juvenile phase in general, conclusions regarding the length of the juvenile phases for cold and for SD should be drawn only from strictly comparable material. The juvenile phase for SD is not completed in the cold, but is completed during a prolonged SD-treatment at high temperature. This is doubtlessly a growth process. In LD the juvenile phase for SD is completed much faster.

The minimum duration of SD-treatment applied in the described experiments is 4 weeks and this is often already optimal. In undescribed experiments a duration of 2 weeks had no effect. There is no straight relation between the duration of the treatment and the initial age of adult plants; nor does a relation exist between the % of bolting and the average number of days for bolting during the after-treatment.

In permanent SD bolting nor floral initiation take place. The longest treatment which has been applied, lasted almost 2 years. Bolting and flower initiation occur only when LD follows the SD. However, since a relatively short period of LD is sufficient, during which no bolting starts, whereafter the plants are day neutral, the induction depends on SD followed by LD and *C. medium* is an SLD-plant with regard to the induction.

4.3. *A comparison between vernalization and SLD-induction*

In several respects the reactions to cold and to SD differ. We shall compare the actions of these two factors:

1st. The juvenile phase for SD lasts shorter than for cold.

2nd. The juvenile phase for cold is not completed in the cold, neither is the juvenile phase for SD, but the latter is completed in SD at high temperature. Strictly speaking this is no consistent difference, but rather a remarkable fact.

3rd. Plants which are out of the juvenile phase for SD but still in the juvenile phase for cold, do not react to cold SD, because at 5°C. no photoperiodical reaction occurs.

4th. SD in itself never leads to bolting and floral initiation, but must be followed by LD, be it during a relatively short period. On the other hand cold, if applied prolonged, leads to bolting and to a beginning of flower initiation.

5th. SD in itself must always be followed by LD – compare 4th –, but LD after vernalization is only necessary with relatively young plants which have been vernalized during a relatively short period.

6th. Vernalization followed by LD may lead to a sure result of 100% bolting and flowering. SD followed by LD, no matter how long and with plants of what age, seldom leads to 100%. With vernalization a clear relation exists between the effect of the duration of the treatment and the age of the plants. For SD this relation is not clear.

7th. The period during the LD-after-treatment for bolting to start is usually shorter after cold treatment than after SD-treatment and often much shorter. Compare column 6 of table 1 (p. 5) with column 4 of table 7 (p. 10).

All this evidence justifies the conclusion that the mechanism of vernalization and the mechanism of SLD-induction in *C. medium* are completely different. Vernalization and SLD-induction have the same final effect, but this effect is reached by very different means. This conclusion is supported by the fact that cold is perceived by the growing-tip, day length by the leaves. In this respect it should be remarked that it would be incorrect to use the statement that SLD can *replace* cold, or the reverse. Cold and SLD are equivalent in having the same effect, but their actions are different.

4.4. *General remarks*

For more than one reason *Campanula medium* offers a remarkable, if not a unique case. Long-short day plants are known since the work of DOSTÁL (3) in 1950 with *Bryophyllum verticillatum*, of RESENDE (7) in 1952 with *Bryophyllum daigremontianum*, *Kalanchoë moçambicana*, *Kalanchoë rotundifolia* and *Aloe bulbifera*, of SACHS (8) in 1956 with *Cestrum nocturnum*. In his above cited paper RESENDE puts the question whether "short-long" day species might also exist. *Campanula medium* is the first case of this type and has remained the single case since its first publication (9) in 1949 until in 1957 CHOUARD (1) published the second case: *Scabiosa succisa*.

Furthermore, *C. medium* offers a remarkable case, because not only SLD induces flower formation, but also a cold treatment. Rye, the classical object of GREGORY and PURVIS, behaves similarly (see 4), but SD has not such a complete effect als cold; moreover, rye reacts only quantitatively to both SD and cold, whereas *C. medium* reacts completely qualitatively. CHOUARD's above cited *Scabiosa succisa* seems to be comparable with *C. medium* in all respects.

Not many SDP demonstrate the existence of a juvenile phase for SD so clearly as *C. medium.* For the rest the behaviour of *C. medium* finds many parallels in other plants. Although this behaviour has become fairly well known through the described experiments, the mechanisms of the action of cold and of SD remain obscure. As with most plants where attempts have been made to express the mechanism of floral initiation in schemes of reaction, the factual knowledge is far too meagre and therefore such an attempt will not be made. The experimental results give rise to three remarks of a theoretical nature, however.

First, considering the fact that in one and the same species (at least) two different mechanisms of floral initiation occur, it seems likely that in the vegetable kingdom as a whole several such mechanisms exist. This viewpoint opposes the existence of one general theory of flowering. It has been expressed earlier (10) and is in complete harmony with CHOUARD (2, p. 1801): "Je crois, pour ma part, ...que la vernalisation et le photopériodisme sont des comportements apparemment semblables chez des espèces différentes, mais qui peuvent présenter des mécanismes intimes fort différents".

Second, when we compare the action of cold during a limited period and followed by warm LD, with the action of a prolonged cold treatment, it is evident that the action of cold on flower induction consists at least of two parts. For the first part cold is absolutely essential. The second part may proceed during a prolonged cold treatment, but may also proceed in warm LD. A similar situation exists in the other two cases which have been mentioned before (p. 14): *Lunaria biennis* and *Cheiranthus allionii*, although the former is completely and the latter more or less day neutral. Although their cases are not strictly comparable to *C. medium*, several authors have split up the vernalization process in more than one process: LANG and MELCHERS (5) for biennial *Hyoscyamus niger*, GREGORY and PURVIS (see 4) for rye, NAPP-ZINN (6) for winter annual *Arabidopsis thaliana*. In so far as in *C. medium* SD would actually *replace* the cold, this would refer only to the first part of the action of cold and not to the second.

The third remark refers to the action of cold and of SD on stem-elongation which, as was demonstrated experimentally, has no direct relation with floral initiation in *C. medium*. The effect of cold on stem-elongation may be indirect (after a limited duration of the cold treatment) or direct (during a prolonged cold treatment). The effect of SD on stem-elongation is always indirect: stem-elongation never occurs in SD, but only in LD following the SD (or in SD following a SLD-treatment). This suggests that also for stem-elongation two different mecanisms occur.

5. SUMMARY

1. *General*

Campanula medium initiates flowers after each of the following treatments:

.1. Plant vernalization, usually to be followed by LD at high temperature.

.2. SD-treatment, always to be followed by LD, both at high temperature.

2. *Vernalization*

.1. There is a juvenile phase, normally lasting 4–5 months.

.2. Within certain limits the older the plants are, the less cold they need, ex-

pressing itself as a higher % of bolting and a shorter period for bolting to start.

.3. Juvenile plants, exposed to a prolonged cold treatment, do not form a rosette, but slowly elongate their stems, while they remain juvenile.

.4. Adult plants also elongate their stems during a prolonged cold treatment and moreover slowly turn generative, but do not reach a stage of complete flowerbuds.

.5. For flower initiation LD must follow a vernalizing treatment of limited duration, but rather old plants also flower in SD after a rather long vernalization.

3. *SLD-induction*

.1. Very young plants are in a juvenile phase for SD-action.

.2. The juvenile phase is not completed at 5 °C, but it is completed during a SD-treatment at high temperature, however more slowly than in LD.

.3. Even during a very prolonged SD-treatment no induction takes place. Induction only occurs when SD is followed by LD. Hence *C. medium* is an SLD-plant. The necessary duration of the LD is limited, so that after SLD-induction the plants are day neutral.

.4. Apart from the existence of a juvenile phase, there is no relation between age of plants and effect of duration of SD-treatment.

4. *A comparison between vernalization and SLD-induction*

.1. The juvenile phase for SD lasts shorter than the juvenile phase for cold, approximately one month.

.2. Plants which still are in the juvenile phase for cold, but which are out of the juvenile phase for SD, not only do not react to cold, but neither to cold *SD*, because 5 °C is below the minimum for photoperiodical action.

.3. During a prolonged cold treatment of adult plants stem-elongation and a beginning of flower formation occur. SD in itself never leads to stem-elongation and induction of flower formation.

.4. LD after vernalization is necessary with relatively young plants which have been vernalized during a relatively short period. LD is always necessary after SD.

.5. The period for bolting to start during the LD-after-treatment is usually shorter and often much shorter after vernalization than after SD.

.6. The above evidence, together with other differences of minor importance, justifies the conclusion that the mechanism of vernalization is completely different from the mechanism of SLD-induction.

5. *General remarks*

.1. Besides *Scabiosa succisa, Campanula medium* is the only SLD-plant known thusfar. Also, complete flower initiation by vernalization *or* by SLD is highly exceptional.

.2. The results with *C. medium* oppose the view that one general theory on flower initiation can be built up.

.3. The action of cold in *C. medium* seems to be at least twofold.

.4. For stem-elongation at least two mechanisms occur.

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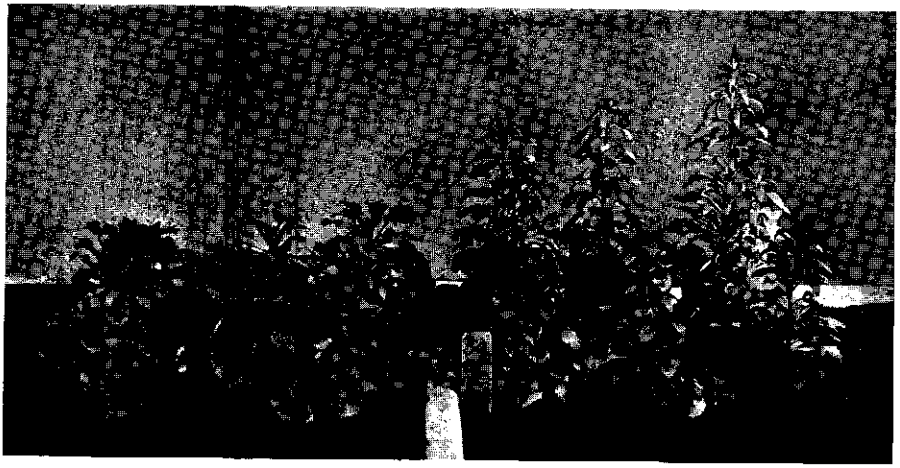

PHOTO 1. Plants of 28 weeks old, vernalized during 6 weeks (left) or 8 weeks (right), photographed in warm LD 35 days after vernalization. The plants at the right had bolted for 100% after 20 days. However, 50% of the left plants bolted after 62 days. The photograph was taken just in between the beginning of the bolting of both groups of plants.

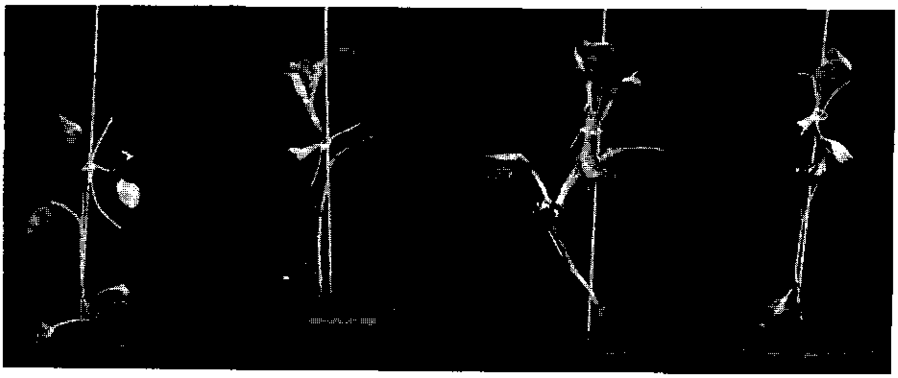

PHOTO 2. Representative plants which at the age of 5 weeks received cold LD during 34 weeks, photographed 2 weeks after this treatment. There is no rosette.

PHOTO 3. Three of the plants from photo 2 – one died prematurely –, photographed 26 weeks after the cold treatment. In warm LD a rosette was formed on the top of the elongated stem.

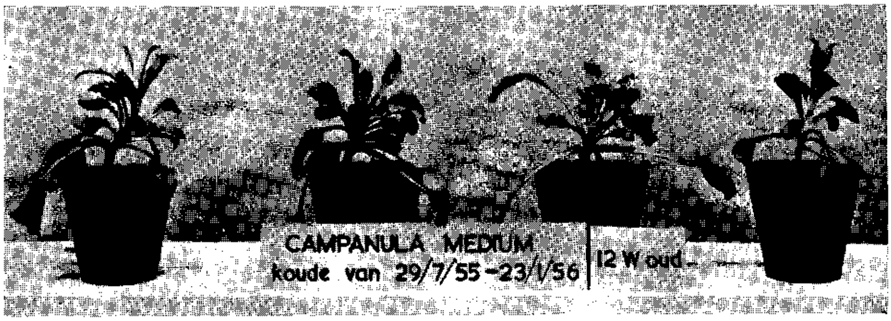

PHOTO 4. Plants of 12 weeks old when put at 5 °C., photographed after 178 days. Bolting has just started. Compare photo 5.

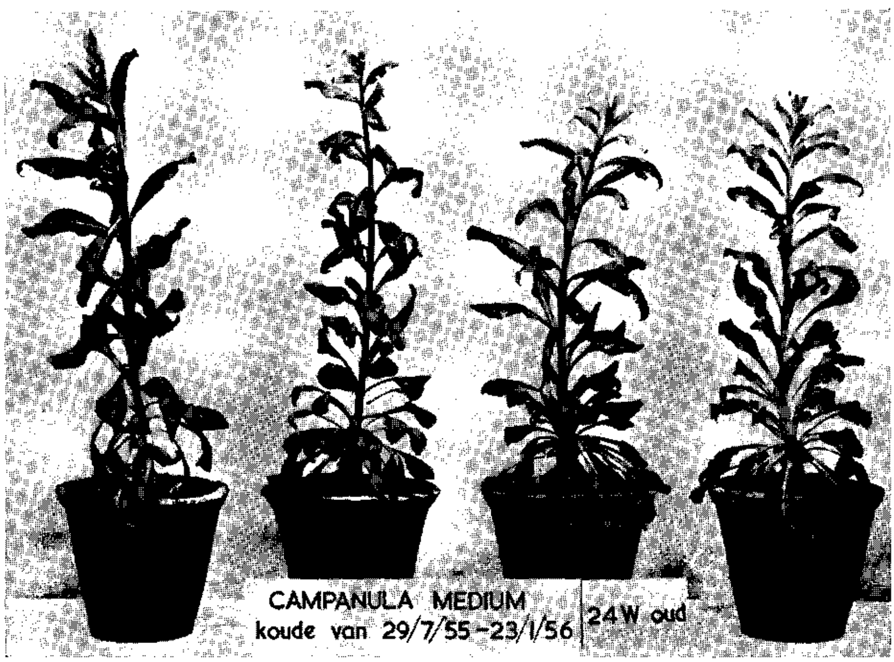

PHOTO 5. Plants of 24 weeks old when put at 5 °C., photographed after 178 days. Bolting in an advanced stage. Compare photo 4.

PHOTO 6. The effect of 6 weeks treatment of 9 months old plants with, from left to right, per day: 0 hrs 5° – 24 hrs 13°; 8 hrs 5° – 16 hrs 13°; 16 hrs 5° – 8 hrs 13°; 24 hrs 5°.

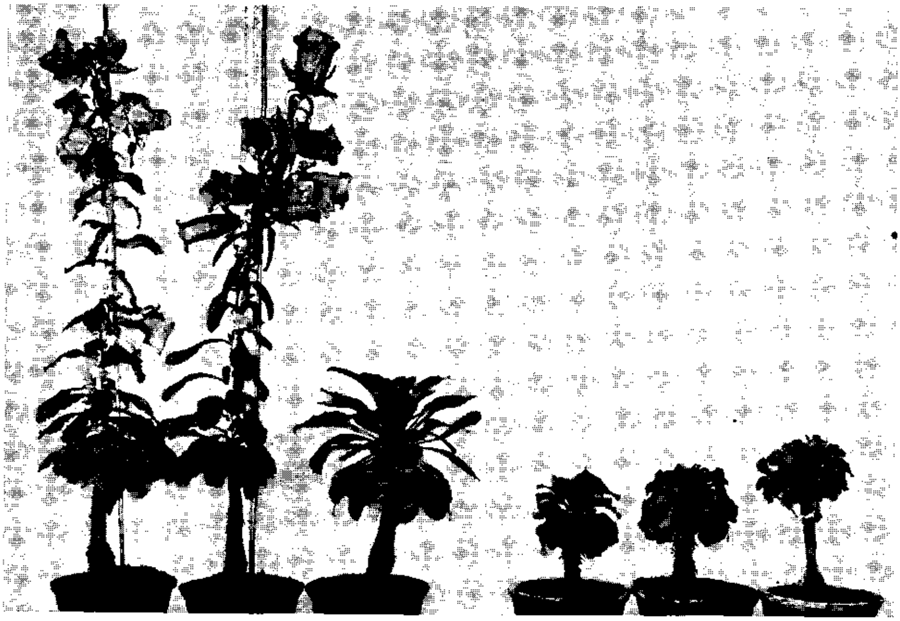

PHOTO 7. Plants of 5 weeks initial age when put into SD. The three plants at the right after over 2 years of uninterrupted SD. The three plants at the left received LD after SD-treatment during almost 2 years; 2 of the 3 have bolted and flowered. Note the difference in habit between the non bolted plant in LD and the 3 plants (at the right) in SD which difference arose in 67 days.

PHOTO 8. Plants treated at an age of 20 weeks with 8 weeks of cold SD, followed by warm LD. No bolting. Photograph taken 127 days after the cold treatment. Compare photo 9.

PHOTO 9. Plants treated at an age of 20 weeks with 8 weeks of warm SD, followed by warm LD. Bolting with 9 out of 12 plants, 6 of which have been photographed 127 days after the warm SD-treatment. Compare photo 8.